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Lower Extremity Muscle Activation Following a Previous Knee Injury: Implications for Post-Traumatic Knee Osteoarthritis

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Lower Extremity Muscle Activation Following a Previous Knee Injury: Implications for Post-Traumatic Knee Osteoarthritis

by

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Abstract

Individuals who sustain an intra-articular knee injury are at a high risk of developing post-traumatic knee osteoarthritis (PTOA) 10-20 years later. Compensatory activation patterns of knee muscles in response to the trauma may persist past the acute injury phase and result in abnormal mechanical loading and subsequent osteoarthritis of the knee. This dissertation aimed to use surface electromyography (sEMG) to explore abnormal leg muscle activation patterns and their possible involvement in PTOA development in individuals who suffered a previous knee injury 3-12 years ago. The first part of this thesis presents methodological investigations related to two sEMG-based assessments of knee muscle activation strategies. It was shown that 1) sEMG amplitude-based co-contraction indices during gait exhibit poor between-day reliability and 2) the magnitude of intermuscular coherence strongly depends on the configuration and alignment of the sEMG electrodes. Building on the methodological findings, the second part of this thesis investigated the association between a previous knee injury and leg muscle activation during walking and squatting while considering the influence of sex. Sex-specific abnormalities in quadricep and hamstring muscle activation patterns were present for the affected leg in individuals more than three years after a previous knee injury. Altered quadricep and hamstring muscle activation may result in abnormal movement and loading of the knee joint and thus be linked to mechanical risk factors for PTOA development. This dissertation could not provide evidence, however, that altered thigh muscle activation was associated with more self-reported knee pain or symptoms indicative of PTOA development. From a methodological perspective, the poor reliability of current sEMG-based markers for abnormal muscle activation may lower the sensitivity to detect associations with risk factors for PTOA. From a conceptual perspective, the development of PTOA is not solely based on joint mechanics but depends on the interplay

between mechanical, biological, and structural abnormalities of the joint following a knee injury. Therefore, the pathway to PTOA is likely unique to each individual such that a consistent association between abnormal muscle activation following a knee injury and PTOA risk may not exist.

Preface

Each of the following chapters is based on scientific manuscripts:

Chapter 3 is based on Mohr, M., Lorenzen, K., Palacios-Derflingher, L., Emery, C. A., & Nigg, B. M. (2018). Reliability of the knee muscle co-contraction index during gait in young adults with and without knee injury history. *Journal of Electromyography and Kinesiology*, 38, 17–27. doi: 10.1016/j.jelekin.2017.10.014

Statement of contribution: MM, KL, LP, CAE, BMN conceived the study design and protocol. MM and KL collected the data. MM and LP analyzed the data and wrote the first manuscript draft. MM, KL, LP, CAE, BMN edited the manuscript and approved the final manuscript version.

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Chapter 4 is based on Mohr, M., Schön, T., von Tscharner, V., & Nigg, B. M. (2018). Intermuscular Coherence Between Surface EMG Signals Is Higher for Monopolar Compared to Bipolar Electrode Configurations. *Frontiers in Physiology*, 9. doi: 10.3389/fphys.2018.00566

Statement of contribution: MM and VvT conceived and designed the experiments. TS performed the experiments. TS, MM, and VvT analyzed the data. VvT and BMN contributed reagents, materials, and analysis tools. BMN supervised. MM wrote the first manuscript draft. MM, TS, VvT, and BN reviewed, edited, and accepted final manuscript version.

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Chapter 5 is based on Mohr, M., von Tscharner, V., Whittaker, J. L., Emery, C. A., & Nigg, B. M. (2018). Quadriceps-hamstrings intermuscular coherence during single-leg squatting 3-12 years following a youth-sport related knee injury. *Submitted to Human Movement Science, October 2018.*

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Chapter 6 is based on Mohr, M., von Tscharner, V., Emery, C. A., & Nigg, B. M. (2018). Classification of gait muscle activation patterns according to knee injury history using a support vector machine approach. *Submitted to Human Movement Science, September 2018.*

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Ethics statement: All studies included in this thesis have been approved by the University of Calgary's Conjoint Health Research Ethics Board. Specifically, the studies presented in chapters three, five, and six have been approved as part of the project "Secondary prevention of osteoarthritis following joint injury in youth sport: A mixed methods study" (Ethics ID E-25075; Principal Investigator: Carolyn Emery). The study presented in chapter four has been approved as part of the project "Intermuscular synchronization during dynamic tasks – Comparison between EMG recording techniques" (Ethics ID REB17-0210; Principal Investigator: Benno M. Nigg).

Co-author permission statement: All co-authors of chapters three to six have agreed that the manuscripts contained in these chapters may be included in this dissertation (see Appendix B).

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To M.D.L.

Table of contents

List of Tables

List of Figures

List of Symbols, Abbreviations, and Nomenclature

α	alpha, referring to the statistical significance level
ACL	Anterior Cruciate Ligament
ANOVA	Analysis of variance
BF	Biceps Femoris
CCI	Co-contraction index
CI	Confidence interval
cm	centimeter
CNS	Central nervous system
DMP	Discriminatory multi-muscle pattern
EMG	Electromyography
FFT	Fast Fourier Transform
GL	Gastrocnemius Lateralis
GM	Gastrocnemius Medialis
Hz	Hertz
ICC	Intraclass correlation coefficient
kg	kilogram
KOOS	Knee Injury and Osteoarthritis Outcome Score
ln	Natural logarithm
LOO	Leave-one-out
MH	Medial hamstrings
MMP	Multi-muscle pattern
MU	Motor unit
MUAP	Motor unit action potential
MVC	Maximum voluntary contraction
MVIC	Maximum isometric voluntary contraction
N	Newton
OA	Osteoarthritis
Q-H	Quadriceps-Hamstrings
PCA	Principal component analysis
PrE-OA Study	Alberta Youth Prevention of Early Osteoarthritis Study
PTOA	Post-traumatic osteoarthritis
SD	Standard deviation
SE	Standard error
SEM	Standard error of measurement
sEMG	Surface EMG
SENIAM	Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles
SLS	Single-leg squat(ting)
SVM	Support Vector Machine
VL	Vastus Lateralis
VM	Vastus Medialis
V	Volt

Epigraph

Science is a way of trying to not fool yourself. The first principle is that you must not fool yourself, and you are the easiest person to fool.

Richard P. Feynman, 1974

Chapter One – **Introduction**

1.1 The burden of post-traumatic osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease that has been identified in human skeletons of all known historic and prehistoric time periods and presents a global health burden in our society (Cross et al., 2014; Straus & Cave, 1957). Symptoms of OA include chronic joint pain and stiffness and associated life-long disability that often requires total joint replacement surgery (Hunter, 2011; Weinstein et al., 2013). The knee and hip joint are most frequently affected by OA and are representing the 11th highest contributor to global disability in 2010 (Cross et al., 2014; Vos et al., 2012). Post-traumatic osteoarthritis (PTOA) is a subtype of OA, which develops as a result of a previous injury to the involved joint, including ligamentous, capsular, and meniscal injuries and injuries to the articular surface and subchondral bone (Anderson et al., 2011; Olson & Guilak, 2015). For a substantial fraction (~12%) of all patients who present with symptomatic OA, the disease arises secondary to a previous joint injury (Brown, Johnston, Saltzman, Marsh, & Buckwalter, 2006). In the US, this fraction corresponds to 5.6 million individuals affected by PTOA and an associated aggregate financial burden of over US$3 billion annually (Brown et al., 2006). The prevalence of PTOA is highest in the knee joint as the knee represents the most frequently injured joint in the wider community (Brown et al., 2006; Emery, Meeuwisse, & McAllister, 2006; Riordan, Little, & Hunter, 2014). While the association between joint injury and OA development is known and undisputed, the exact etiology of PTOA is currently unclear as it is highly complex and involves the consideration of multiple risk factors (Chu & Andriacchi, 2015). Despite the most advanced care and surgical treatments of knee joint injuries, the number of individuals who develop knee PTOA lies between 20-50% and has not decreased appreciably in the last 25 years (Øiestad, Engebretsen, Storheim, & Risberg, 2009;

Anderson et al., 2011). Considering the rising incidence of joint injuries during youth sport and recreation (Gage, McIlvain, Collins, Fields, & Dawn Comstock, 2012; Hootman, Dick, & Agel, 2007) as well as our aging population, the health burden of PTOA is projected to only increase over the next 25 years if the absence of effective treatment and/or prevention persists (Bombardier, Hawker, & Mosher, 2011). While the primary prevention of knee injuries could significantly reduce the incidence of knee OA (Felson & Zhang, 1998), it is impossible to prevent all joint injuries. Therefore, a long-lasting reduction of the global PTOA burden must focus on secondary prevention of disease progression once an injury has occurred.

The motivation for this thesis was to explore a possible neuromuscular pathway of knee PTOA. This pathway builds on the hypothesis that adaptations in neuromuscular control of the lower extremity muscles following a knee injury result in abnormal mechanical loading of the knee and subsequently to the accumulation of joint tissue damage leading to PTOA.

1.2 The neuromuscular pathway of post-traumatic osteoarthritis

The central nervous system (CNS), consisting of the brain and spinal cord, produces activation patterns in skeletal muscles that will result in muscle forces and subsequently in the necessary joint movement and stability to perform a given movement task at hand. In parallel, the CNS integrates afferent feedback from peripheral sensory organs pertaining to instantaneous muscle and joint dynamics to fine-tune muscle activation, protect from falls and/or injury, and to respond to unexpected perturbations (Scott, 2004). While muscles are moving and stabilizing joints, they are the main contributor to the loading of the articulating joint tissues (Herzog, Longino, & Clark, 2003). Thus, muscle forces influence joint tissue homeostasis, which is modulated by the mechanical as well as the biological environment within a joint (Griffin &

Guilak, 2005; Andriacchi, Koo, & Scanlan, 2009). Therefore, the activity of muscles surrounding a joint is ultimately linked to joint adaptation and degeneration (Herzog et al., 2003).

Following a knee injury, there is ubiquitous evidence that individuals demonstrate altered muscle activation patterns compared to a time point before injury or compared to individuals with no knee injury history (for a review, see: Ingersoll, Grindstaff, Pietrosimone, & Hart, 2008). These adapted activation patterns may serve the goals to compensate for the loss of joint stability due to damaged joint structures, avoid localized joint pain, or may be a compensation for deficient sensory information due to damaged sensory joint organs (Hodges & Tucker, 2011; Pietrosimone, McLeod, & Lepley, 2012). Whichever goal they serve, altered muscle activation patterns and the associated changes in the mechanical loading environment of the knee joint will affect the homeostasis of the tissues within the knee such as the cartilage, menisci, and subchondral bone (Herzog et al., 2003; Olson & Guilak, 2015; Turner, 1998). In consequence, the hypothesis has evolved that altered muscle activation patterns following a knee injury may be associated with knee joint tissue degradation, which over the course of 10-20 years will result in the development of post-traumatic knee osteoarthritis (PTOA) and explain the high prevalence (20-50%) of the degenerative joint disease following intra-articular knee injury (Hurley, 1999; Herzog et al., 2003; Andriacchi et al., 2004; Hurd & Snyder-Mackler, 2007; Bennell, Hunt, Wrigley, Lim, & Hinman, 2008; Palmieri-Smith & Thomas, 2009; Anderson et al., 2011; Roos, Herzog, Block, & Bennell, 2011; Krishnasamy, Hall, & Robbins, 2018).

If a neuromuscular risk factor for PTOA would be identified, targeted physical rehabilitation following a knee injury could be developed to prevent or slow PTOA progression, helping to mitigate the disease burden. To date, however, there is a lack of evidence to directly link abnormal muscle activation patterns following a knee injury to an increased risk of developing knee PTOA. There are at least three explanations for this missing link; a methodological explanation, a study design explanation, and a conceptual explanation.

1.3 Possible methodological explanation

The most common experimental methodology to investigate abnormal muscle activation patterns following a knee injury is surface electromyography (sEMG) – the study of muscle function based on the electrical signals that muscles emanate during contraction (Basmajian, 1985). Despite being non-invasive and easy to use, the derivation of muscle function and the underling neural control from sEMG signals during movement is challenging due to the complex interaction of physiological and non-physiological factors that influence these signals (De Luca, 1997; Farina, Merletti, & Enoka, 2004). While outcome variables to inform clinical practice must typically pass rigorous tests of reliability and validity, sEMG outcomes in clinical biomechanics studies are regularly given the benefit of the doubt. Reliability studies that determine the absolute measurement error associated with common sEMG outcome variables are scarce but the few existing studies suggest substantial between-session changes (Ball & Scurr, 2010; Murley, Menz, Landorf, & Bird, 2010). Therefore, poor reliability of sEMG-based analysis of muscle function following knee injuries may be one explanation for the difficulty to link abnormal muscle activation patterns to osteoarthritis development. Furthermore, the standard technology to acquire the sEMG signals has basically not advanced since Basmajian and de Luca published their classic EMG textbook ‘Muscles alive’ in the 1980s (Basmajian, 1985). Novel sEMG technologies have since evolved but the interest in their advantages to assess neuromuscular control has only recently grown in the clinical biomechanics community (Merletti & Farina, 2016; von Tscharner, Maurer, Ruf, & Nigg, 2013). Exploring the possibilities of novel EMG technologies may further enhance our understanding of neuromuscular control following a knee injury and its role in the development of PTOA.

The first aim of this thesis was to investigate methodological aspects related to sEMG data acquisition, sEMG signal processing, and the resulting reliability and sensitivity of sEMG-based outcome variables to assess muscle activation patterns during movement. The specific objectives were:

Objective 1a. To determine the reliability of a commonly used knee muscle co-contraction index during gait in young adults with and without knee injury history (Chapter 3, published in the Journal of Electromyography and Kinesiology (Mohr, Lorenzen, Palacios-Derflingher, Emery, & Nigg, 2018)).

Objective 1b. To elucidate the effect of different sEMG acquisition techniques on the magnitude, reliability, and sensitivity of a sEMG-based measure of intermuscular synchronization between knee extensor muscles during squatting exercises in healthy individuals (Chapter 4, published in Frontiers in Physiology (Mohr, Schön, von Tscharner, & Nigg, 2018)).

1.4 Possible study design explanation

The development of knee PTOA is a slow process. The first structural changes of the joint consistent with osteoarthritis may occur during the first year post-injury (Chu, Williams, Coyle, & Bowers, 2012; Culvenor et al., 2015). However, the clinical diagnosis of osteoarthritis according to radiographic changes and clinical knee symptoms is usually not made until ten years following the injury event (Lohmander, Östenberg, Englund, & Roos, 2004; Roos, 2005; Øiestad et al., 2009). According to the neuromuscular hypothesis, persistent changes in muscle activation patterns and corresponding joint mechanics following the injury result in a slow degradation of the joint tissues over time. If this hypothesis is true, such abnormal muscle activation patterns during movement should be present past the acute phase after the knee injury and before the clinical diagnosis of knee osteoarthritis, i.e. 3-12 years post-injury. Furthermore, individuals in which abnormal muscle activation patterns are most pronounced should show early signs of PTOA during this pre-osteoarthritis time period (Chu et al., 2012). Most studies examining neuromuscular abnormalities following a knee injury, however, have investigated the movement patterns of individuals during the first three years post-injury, typically with a focus on the acute phase after injury (for a review, see: Shanbehzadeh, Bandpei, & Ehsani, 2017). During the first year after the injury or surgical reconstruction, overwhelming evidence exists to demonstrate abnormal neuromuscular patterns of the muscles surrounding the knee during gait and other functional movement tasks (for a review, see: Ingersoll, Grindstaff, Pietrosimone, & Hart, 2008). Much fewer studies are available for the time frame beyond the first year post-injury and these studies convey conflicting findings regarding the persistence of abnormal neuromuscular patterns. Some authors reported that 'normal' knee muscle activation patterns during gait and functional tasks compared to a control group are re-established at one year after an ACL rupture or ACL reconstruction (Bulgheroni, Bulgheroni, Andrini, Guffanti, & Giughello, 1997; DeMont, Lephart, Giraldo, Swanik, & Fu, 1999; Swanik, Lephart, Giraldo, DeMont, & Fu, 1999; Knoll, Kiss, & Kocsis, 2004). Other authors have observed more persistent muscle activity adaptations during gait between 3-18 years following the injury event, specifically for the hamstring muscles (Limbird, Shiavi, Frazer, & Borra, 1988; Shiavi, Zhang, Limbird, & Edmondstone, 1992; Tsai, McLean, Colletti, & Powers, 2012; Hall, Stevermer, & Gillette, 2015). The reason for these contradictory observations is likely a combination of 1) differences in movement task difficulty (e.g. walking vs. single-leg landing), 2) varying EMG signal analysis techniques (amplitude, temporal, or pattern analysis), 3) a relatively small sample size (only one of the above studies investigated more than 20 injured individuals), and 4) a mixed analysis of male and female participants in some of the studies (Limbird et al., 1988; Knoll et al., 2004; Hall et al., 2015). Males and females may utilize sex-specific neuromuscular strategies when performing movement tasks following a knee injury and should thus be analyzed separately (Zeller, McCrory, Ben Kibler, & Uhl, 2003; Yamazaki, Muneta, Ju, & Sekiya, 2010). Further, none of the previous studies attempted to investigate whether neuromuscular abnormalities were associated with early symptoms of PTOA. This limits a judgement on the clinical relevance of previous findings for PTOA risk. Therefore, a comprehensive assessment of muscle activation patterns in individuals with a previous knee injury 3-12 years ago in comparison to a control population is warranted to better understand the persistence of abnormal neuromuscular control after knee trauma and their role in PTOA. Such an assessment should implement the methodological conclusions related to sEMG technology and analysis derived from the first part of this thesis and include a sex-specific, multi-faceted analysis of sEMG outcomes to provide a holistic view on neuromuscular control following a knee injury. Moreover, in the presence of persistent, abnormal muscle activation patterns, their association to early symptoms of PTOA development should be explored.

The second aim of this thesis was to compare activation patterns of muscles surrounding the knee joint between individuals with a previous knee injury 3-12 years prior and individuals with no injury history and explore a possible relationship between abnormal muscle activation and the presence of early symptoms of post-traumatic knee osteoarthritis. The specific objectives were:

Objective 2a. To investigate the influence of knee injury history, sex, and knee pain and symptoms on the degree of quadriceps-hamstrings co-contraction as quantified by an intermuscular coherence analysis between the respective sEMG signals during a single-leg squatting task. (Chapter 5, submitted to *Human Movement Science* in October 2018).

Objective 2b. To apply a pattern recognition and machine learning approach to compare the multi-muscle activation patterns of muscles surrounding the knee joint during gait between individuals with and without a knee injury history. (Chapter 6, submitted to *Human Movement Science* in September 2018).

1.5 Possible conceptual explanation

From a conceptual point of view, the null hypothesis must be considered, i.e. there is no consistent association between persistent, abnormal muscle activation patterns following a knee injury and the rate of progression to post-traumatic osteoarthritis. In this case, the hypothesized neuromuscular pathway for PTOA would have to be revised. Although knee OA has long been considered to be a 'mechanical disease', cumulative evidence from the last two decades has clearly demonstrated that the degeneration of joint structures in OA is most likely the result of a complex interaction between joint loading, joint structure, and biological processes, specifically inflammatory processes within the joint capsule (Griffin & Guilak, 2005; Anderson et al., 2011; Riordan et al., 2014; Chu & Andriacchi, 2015; Olson & Guilak, 2015). The contribution of each of these three factors to the development of knee PTOA may be unique to each patient (Chu & Andriacchi, 2015). In this case, it is unlikely to observe a consistent relationship between altered muscle activation patterns following a knee injury and osteoarthritis development.

An alternative hypothesis is that abnormal muscle activation and corresponding joint loading in the first two years after knee trauma cause early degenerative changes in the joint structures and are associated with future knee osteoarthritis risk even if ‘normal’ movement patterns are recovered following the acute knee injury phase (Wellsandt et al., 2016). In this case and similar to above, movement analyses in the time period of 3-12 years following a knee injury would fail to detect a consistent association between muscle activation patterns and the risk or rate of progression of OA.

The third aim of this thesis was to discuss abnormal muscle activation patterns following a knee injury observed in chapters five and six in the context of current evidence related to the hypothesized neuromuscular pathway for post-traumatic osteoarthritis. (Chapter Seven – Summary and Conclusions).

1.6 Overview of dissertation

In the second chapter, this thesis continues with a review of the most relevant literature related to the different neuromuscular and biomechanical processes that underlie the hypothesized neuromuscular pathway of knee PTOA as well as the experimental techniques for their assessment. The first aim is addressed in chapters three and four. The second aim is addressed in chapters five and six. Chapters three to six are written as separate manuscripts for publication in peer-reviewed journals. Therefore, the dissertation contains some repetition related to the introduction, methods, and discussion sections of these chapters. The final seventh chapter of this thesis contains a summary of the findings from each study and discusses them in the context of PTOA development to address the third aim.

Chapter Two – **Literature review**

The goal of this chapter is to provide a step-by-step review of the current evidence related the different systems and concepts involved in the hypothesized neuromuscular pathway of knee PTOA. The review will 1) summarize the function and properties of the motor unit as the most basic control unit of the neuromuscular system, 2) describe strategies of the CNS to coordinate the activity of motor units within and between muscles involved in a motor task, 3) critically evaluate the use of surface electromyography to assess neuromuscular control strategies, 4) assess the strengths and limitations of the existing literature related to the influence of knee injuries on the neuromuscular control of the lower extremities during movement, and finally 5) critically appraise the current evidence related to the role of abnormal lower extremity muscle activation following injury for the development of knee PTOA.

2.1 Motor unit – the basic neuromuscular control unit

2.1.1 Motor unit action potential

Muscle activity is governed by the central nervous system through motor neurons that relay information from the brain and/or the spinal cord to the individual muscles and their force-producing contractile elements. The spinal cord plays an important mediating role in inhibiting or facilitating muscle activity based on complex interneuron networks. These networks contain reflex arcs and process sensory feedback from mechano-sensors within the skin, muscles, and joints as well as other sensory information, e.g. from eyes or ears (Scott, 2004). One α – motor neuron originating in the brain or spinal cord innervates a group of muscle fibers within one muscle forming one motor unit (MU), the smallest contractile unit of the neuromuscular system. The excitation of a motor unit is achieved by the generation of a nerve fiber action potential within the brain or spinal cord, propagation of the action potential via the axon of the α – motor neuron and branching of the nerve to form connections with multiple muscle fibers, the neuromuscular junctions. The neuromuscular junctions are chemical synapses, which transmit the action potential from the motor neuron to the muscle fiber with the help of neurotransmitters, moving across the synaptic cleft to bind to receptors on the motor endplate integrated into the muscle fiber membrane. Receptor binding allows the exchange of sodium and potassium ions through the cell membrane and causes a muscle fiber action potential, i.e. the depolarization of the membrane resting potential from about -90 mV to 30-40 mV and subsequent repolarization to the resting potential during a time frame of 2-3 ms. If the local increase in the membrane potential is large enough, adjacent sites of the cell membrane will also depolarize and lead to a propagation of the action potential proximally and distally along the muscle fiber. The depolarization of the muscle cell membrane starts a cascade of events within the muscle cell, which eventually causes the serially organized contractile elements of the muscle fiber to contract and produce force (excitation-contraction coupling). Since one α – motor neuron innervates multiple muscle fibers belonging to the same motor unit, action potentials will be propagated along these fibers at almost the same time. The electrical potential measured near the motor unit is called the motor unit action potential (MUAP) and represents the temporal superposition of individual muscle fiber action potentials (for review of MUAPs, see Nigg & Herzog, 2007). The duration of one MUAP is 10-30 ms, depending on the proximity of the measurement, and therefore about ten times longer in duration compared to a single muscle fiber action potential (Merletti, Rainoldi, & Farina, 2001). This discrepancy is because the locations of the motor endplates of the muscle fibers are not well-localized, but they show some spatial scatter within an area called the innervation zone. For some muscles, e.g. vastus medialis, the innervation zones form a band that runs approximately medio-laterally through the muscle in an area half-way between the tendons (Gallina, Merletti, & Gazzoni, 2013). In contrast, motor endplates of muscle fibers from sartorius or tibialis anterior muscle are spread out along the entire length of the muscles (Aquilonius et al., 1984).

2.1.2 Motor unit properties

The ultimate goal of the neuromuscular control system is to excite motor units to produce muscle force and therefore generate movement and/or modulate the stiffness of human joints. On one hand, the amount of force that is produced by a single motor unit depends on 1) the rate at which motor neuron action potentials arrive at the neuromuscular junction, i.e. the motor unit firing rate, and 2) the size of the motor unit (Adrian & Bronk, 1929; Milner-Brown, Stein, & Yemm, 1973). The motor unit firing rate is defined by the number of times per second that the net synaptic input to the corresponding motor neuron surpasses the neuron's excitation threshold (De Luca, Roy, & Erim, 1993). The net synaptic input to motor neurons is composed of thousands of individual excitatory or inhibitory inputs from muscle spindle afferents, presynaptic neurons, as well as direct projections from upper motor neurons originating in the brain (Sears & Stagg, 1976). Depending on the strength of the synaptic input, human motor neurons typically fire in the range of 20-50 pulses per second during high-force muscle contractions but can reach up to 100 pulses per second during rapid or reflex contractions (Adrian & Bronk, 1929; Enoka & Fuglevand, 2001). Motor unit size generally depends on the function and size of the muscle. Leg muscles that act on large body masses such as gastrocnemius medialis may contain more than 1000 muscle fibers per motor unit while smaller muscles such as those in the fingers or eyes may contain less than ten fibers per motor unit (Buchthal, Erminio, & Rosenfalck, 1959; Enoka, 1995).

The summed force that muscle fibers of one motor unit exert during one impulse is small, e.g. a motor unit of the first dorsal interosseous finger muscle produces a peak force of about 7 mN and reaches its peak force about 45 ms after the occurrence of the motor unit action potential (Milner-Brown et al., 1973). In contrast, total calf muscle forces during maximal vertical jumps can exceed 2000 N – almost six orders of magnitude higher than the single motor unit force (Fukashiro, Komi, Järvinen, & Miyashita, 1995). In addition muscle activation events during fast movements may be as short as 30 ms (von Tscharner, Valderrabano, & Göpfert, 2010). It follows that the simultaneous activation of clusters of many motor units within multiple muscles at precisely the right time is essential for the CNS to generate the exact force vector that is needed to control the highly dynamic tasks that humans perform every day (Farina & Negro, 2015).

2.2 Neuromuscular control of movement

The pelvis and lower extremities combined contain close to 100 individual muscles many of which are active during locomotion (Winter, 1991). Lower extremity muscles such as gastrocnemius medialis or tibialis anterior may be comprised of more than 500 individual motor units (Enoka, 1995). Therefore, during walking the CNS must theoretically control tens of thousands of degrees of freedom. While walking may seem like a simple task to most healthy humans, the complexity of gait becomes apparent when observing a toddler who is learning to walk or when considering the difficulty of engineering robots that can generate a smooth walking motion. To master this computational challenge, the CNS makes use of motor control strategies aimed at simplifying the simultaneous control of multiple muscles and their motor units during movement, some of which are inherent to us and have evolved over time while others must be developed during motor learning processes throughout life.

Inherent motor control strategies involve reflex arcs where a sensory stimulus elicits reflex activations in one or more muscles without attention from the brain. A common example for a reflex arc is the patellar tendon reflex where a tap of the patellar tendon stretches muscle spindles in the quadriceps muscle eliciting a reflex contraction in the same muscle (Sherrington, 1892). In the context of knee injuries, it has been shown that a reflex arc exists between the anterior cruciate ligament (ACL) and the hamstring muscles where a stimulus of the ligament via anterior translation of the tibia or direct electrical stimulation elicits a reflex contraction of the hamstrings. This suggests that these muscles play a protective role in preventing sudden anterior translation of the tibia and a potential rupture of the ACL (Friemert, Bumann-Melnyk, et al., 2005; Friemert, Faist, et al., 2005). In addition to excitatory reflexes, the nervous system involves inhibitory mechanisms. For example, the muscle contraction of an agonist muscle is typically accompanied by an inhibition of the antagonistic muscle group, a mechanism called reciprocal inhibition (Sherrington, 1913). This control scheme facilitates the reciprocal muscle activation patterns that can be observed during many voluntary movements of humans (Nielsen & Kagamihara, 1992).

During gait, evolved neural circuitry is combined with learned motor programs, which allows the CNS to control the many degrees of freedom of the neural and musculoskeletal system and still produce a smooth walking trajectory. A full review of the motor control literature related to this topic is far beyond the scope of this dissertation. In general, however, a large body of evidence suggests that rather than controlling each muscle individually, the mammalian central nervous system regulates the activity of basic sets of muscles, often referred to as “muscle synergies”, that represent elementary building blocks for the generation of limb movement (Winter, 1991; Davis & Vaughan, 1993; Tresch, Saltiel, & Bizzi, 1999; d’Avella, Saltiel, & Bizzi, 2003;

Ivanenko, Poppele, & Lacquaniti, 2004; Bizzi, Cheung, d'Avella, Saltiel, & Tresch, 2008; Tresch & Jarc, 2009). This way, a simplified set of high-level commands allows the central nervous system to control complex multi-muscle activation patterns while integrating sensory information during a certain task (Ivanenko et al., 2004; Bizzi & Cheung, 2013; Laine, Martinez-Valdes, Falla, Mayer, & Farina, 2015). Such high-level commands likely originate from supraspinal centers such as the motor cortex, which descend to activate the spinal interneuron system where neural circuitries ultimately transform such commands into muscle activation patterns (Bizzi & Cheung, 2013).

2.2.1 Intermuscular synchronization

While the muscle synergy hypothesis offers insight into how the CNS may coordinate the timing and overall magnitude of activation of multiple muscles, it does not necessarily explain how the activity of individual motor neurons is regulated within and between multiple muscles. Early experiments by John V. Basmajian have demonstrated that under certain experimental conditions, humans can voluntarily control the activity of a single, individual MU revealing the astonishing precision and flexibility of the nervous system (Basmajian, 1963). Considering the thousands of MUs within the many muscles that must be controlled simultaneously during most functional tasks, however, individual MU control is difficult to reconcile. Instead, during most functional motor tasks, groups of MUs within and between the involved muscles have been shown to receive common neural inputs that result in correlated MU discharges both in the time and the frequency domain (Bigland & Lippold, 1954; Sears & Stagg, 1976; Farmer, Bremner, Halliday, Rosenberg, & Stephens, 1993). The tendency of groups of MUs within the same muscle or between separate muscles to show correlated discharges in the time or frequency domain will be referred to in the following as intramuscular and intermuscular synchronization, respectively. These topics of intra- and intermuscular synchronization have gained significant attention in the fields of neurophysiology, motor control, and biomechanics, since the classic experiments by Kirkwood, Sears, and Stagg in the 1970s and 1980s. This research group recorded and modeled the motor neuron input to the intercoastal muscles during breathing in anaesthetized cats (Sears & Stagg, 1976; Kirkwood, Sears, Stagg, & Westgaard, 1982; Kirkwood, Sears, Tuck, & Westgaard, 1982). It was shown that presynaptic neurons that branch and synapse onto multiple motor neurons cause synchronized firing of groups of MUs in the same and in different segments of the intercoastal musculature with an accuracy of ± 3 ms (Sears & Stagg, 1976). The synchronized firing of MUs within 3-5 ms was defined as short-term synchronization. The observations of short-term synchronization between cat intercoastal MUs were later reproduced from human recordings of individual MU activity during voluntary contraction of various upper and lower extremity muscles (Datta & Stephens, 1990; Bremner, Baker, & Stephens, 1991; De Luca, Roy, & Erim, 1993; Gibbs, Harrison, & Stephens, 1995). The branching of presynaptic neurons that carry neural information from different CNS sources allows multiple motor neurons of the same muscle or different muscles with a common function to receive a shared input, simplifying the simultaneous control of hundreds of MUs during a movement task (De Luca & Erim, 1994). Therefore, "intermuscular synchronization" in addition to "muscle synergies" represents a second perspective from which the neuromuscular control of movement can be studied. The following section will review the evidence related to intermuscular synchronization as a motor control scheme, particularly related to its significance for functional intermuscular coordination during movement.

Synchronized MU activity during voluntary muscle contraction has been proposed to originate primarily from common neural inputs of presynaptic neurons located in the corticospinal tract

(Farmer et al., 1993). This conclusion was drawn from MU recordings from intrinsic hand muscles in individuals with neurological disease, some with impairments of the corticospinal tract such as a history of stroke and others with severe deficiencies in the function of peripheral afferents. Compared to healthy individuals, the strength of MU synchronization in stroke patients was reduced, respectively while MU synchronization was largely unaltered by a lack of peripheral afferents (Farmer, Bremner, Halliday, Rosenberg, & Stephens, 1993; Farmer, Swash, Ingram, & Stephens, 1993). Such common inputs from the corticospinal tract to multiple muscles involved in a certain movement have two main effects on MU activity. In the time domain, common inputs raise the probability of the joint occurrence of firings of pairs of motor neurons and contraction of their associated muscle fibers within and between different muscles (Sears & Stagg, 1976; Bremner et al., 1991). This phenomenon will be referred to as 'motor unit synchronization' for the remainder of this dissertation. In addition, common inputs to motor neurons produce correlated oscillations in the cumulative MU activity within and between different muscles in frequency bands spanning from 1-100 Hz (De Luca & Erim, 1994; Grosse, Cassidy, & Brown, 2002; Semmler, 2002; Farina & Negro, 2015; Mohr, Nann, von Tscharner, Eskofier, & Nigg, 2015; Pizzamiglio, De Lillo, Naeem, Abdalla, & Turner, 2017). This phenomenon will be referred to as 'motor unit coupling' for the remainder of this dissertation.

The properties and strength of intermuscular synchronization can give insight into strategies of the CNS to coordinate the activity of these muscles during a given task. For example, significant intermuscular synchronization is present between antagonistic muscles if they are activated during a voluntary co-contraction (Nielsen & Kagamihara, 1994; Hansen, Hansen, Christensen, Petersen, & Nielsen, 2002) or to modulate the stiffness of a common joint (De Luca & Mambrito, 1987; Pizzamiglio et al., 2017). In contrast, antagonistic and synergistic muscles show no or weak intermuscular synchronization if they do not share a common function during a motor task and are thus activated more independently (Gibbs et al., 1995; Mohr et al., 2015; Reyes, Laine, Kutch, & Valero-Cuevas, 2017). Hansen and colleagues observed that the presence of intermuscular synchronization between antagonistic ankle muscles during co-contraction was accompanied by the presence of a significant coupling between ankle muscle activity and motor cortex activity (Hansen et al., 2002). Therefore, the authors concluded that during a voluntary co-contraction of antagonistic muscles, the motor cortex becomes actively involved in facilitating the simultaneous contraction of two antagonists, that would typically be supressed by reciprocal inhibition (Nielsen & Kagamihara, 1992; Hansen et al., 2002).

From a biomechanical perspective, the most important limitation of the existing literature related to intermuscular synchronization is the poor understanding of how MU synchronization and/or coupling between two or more muscles affects the resultant force output of these muscles. For example, it is unknown whether or how strongly the degree of intermuscular synchronization between the muscles surrounding the knee joint influences the pressure distribution within the tibio-femoral or patello-femoral joints. Answers to these questions would be highly relevant for the rehabilitation and/or prevention of musculoskeletal injury and disease such as patellofemoral pain or tibio-femoral osteoarthritis (Mellor & Hodges, 2005).

In a recent collection of publications, Farina and colleagues have advanced a model for the understanding of the functional significance of common inputs to motor neurons within and between different muscles in the context of muscle force control (Farina, Negro, & Dideriksen, 2014; Farina, Negro, Muceli, & Enoka, 2016; Farina & Negro, 2015). According to these authors, the motor neuron input may be modelled as a combination of independent inputs and a common input where the common input is comprised of one component associated with muscle force control and another component containing synaptic noise that is not associated with muscle force. Furthermore, Farina and colleagues have argued that the motor neuron pool functions as a low pass filter, filtering out all independent inputs to motor neurons and only conserving their common inputs, making the latter solely responsible for the effective neural drive to the muscle and the control of muscle force (Negro, Holobar, & Farina, 2009; Farina & Negro, 2015; Farina et al., 2016). Accordingly, oscillations in the neural drive to the muscle, i.e. the merged discharge times of all concurrently active MUs, can explain a large portion of the variability of muscle force during low-intensity isometric muscle contractions (Negro et al., 2009).

Only one simulation and one experimental study were identified that have investigated the relationship between common inputs to two separate muscles and the resultant force output of these muscles. These studies have suggested, that the between-muscles synchronization of MUs also influences muscle force and joint torque regulation. The simulation study demonstrated that increasing levels of synchronization between MUs of two different muscles resulted in stronger temporal similarities of their corresponding muscle force traces (Santello & Fuglevand, 2004). More recently, it has been shown that low-frequency coupling of MU activity between the vastus lateralis and medialis can explain fluctuations of the corresponding knee extension torque during isometric contractions (Laine et al., 2015).

It is important to emphasize, that all associations between MU synchronization and/or coupling and muscle forces reported above were limited to force oscillations in the low-frequency range, i.e. < 10 Hz. However, coupled MU activity within and between muscles is also present at higher frequencies, with many previous studies demonstrating coupled activity at frequencies between 15-60 Hz (Laine et al., 2015; Mohr et al., 2015; Reyes et al., 2017) or even up to 100 or 200 Hz (Pizzamiglio et al., 2017; von Tscharner, Ullrich, Mohr, Comaduran Marquez, & Nigg, 2018).

The functional significance of coupled MU activity between muscles at these higher frequencies remains largely unexplored. Farina and colleagues have hypothesized that coupled high-frequency oscillations are not correlated with the muscle force output but may represent a carrier frequency that is used by the CNS to transmit control signals from the corticospinal tract to the muscles (Farina et al., 2014). While this is one possibility, the current view on the significance of intermuscular synchronization for force control is limited to evidence from experiments involving isometric muscle contractions. Intermuscular synchronization at frequencies above 30 Hz, however, is specifically observed during movements, which involve dynamic muscle contractions that are restricted by the (often short) time course of the given motor task (von Tscharner, Ullrich, Mohr, Comaduran Marquez & Nigg, 2018). Therefore, a second possibility is that coupled MU activity between muscles at frequencies above 10 Hz become relevant for muscle force control during movements that require more precise and faster fluctuations in muscle forces compared to isometric contractions.

In summary, intermuscular synchronization reflects a strategy of the central nervous system to provide common neural inputs to groups of MUs with the goal to coordinate their activity within and between multiple muscles. The branching of presynaptic neurons primarily originating in the corticospinal tract provide the neural circuitry for the common drive and thus enable this neural control scheme. Consequently, shared neural input through branched presynaptic neurons has been suggested as the basis of "muscle synergies", i.e. a simplified set of high-level commands that results in a complex activation pattern of multiple muscles involved in a certain task (Tresch et al., 1999; Laine et al., 2015). While the functional role of common neural inputs to one or more muscles for muscle force control is not well understood, recent evidence has emerged to support an influence of intermuscular synchronization within and between muscles on their resultant force output and torque production. This opens the possibility that the topic of intermuscular synchronization may have implications for the pathogenesis of musculoskeletal injury and disease or for the performance during athletic movements (Semmler, 2002).

2.3 Electromyography – The assessment of neuromuscular control of movement

2.3.1 History and application in human movement science

Due to the complex interaction between the brain, the spinal cord, and the muscles in controlling human movement, the assessment of neuromuscular control mechanisms is challenging. Different research fields such as neuroscience, exercise physiology, and biomechanics have developed or adopted a variety of technologies to assess one or multiple aspects of neuromuscular control during movement. One of the most commonly used technologies to investigate neuromuscular control is electromyography (EMG). This is a technique to study the occurrence and properties of the electrical potentials that are generated by muscles for the purpose of muscle contraction (Basmajian, 1985). Although the first account of muscle-generated electricity dates to observations in electric ray fish by the Italian Francesco Redi in the 17th century, the relationship between electricity and muscle contraction was first discovered by the Italian scientist Luigi Galvani in 1791. However, it took another 60 years for the first detection of voluntarily elicited electrical signals from human muscles by the German physician and physiologist Emil du DuBois-Reymond in 1849 (Basmajian, 1985). Along with the development of more advanced technologies to record EMG signals, researchers started to deduce basic physiological mechanisms related to the neuromuscular control of muscle contraction. For example, Piper (1907) discovered rhythmic oscillations in the EMG signals of contracting human muscles, later termed ‘Piper rhythm’ and suggested to originate from parallel oscillatory activity in the motor cortex (Piper, 1907; Brown, Salenius, Rothwell, & Hari, 1998).

In fact, most of the evidence related to neuromuscular control during movement presented above has emerged from EMG recordings.

Generally, EMG technologies to study the neuromuscular system may be grouped into two categories; intramuscular EMG and surface EMG (sEMG). The advantage of intramuscular EMG is that the firing properties of individual motor units can be readily obtained using manual or automatic detection methods, which opens a window into basic neurophysiological processes during muscle contraction in both healthy individuals and in patients with neurological disease. Furthermore, intramuscular EMG 1) is not prone to cross-talk from neighbouring muscles due a relatively small detection volume of the needle or wire electrodes and 2) allows to record MU activity in deep muscles that cannot be accessed from the surface of the skin (Basmajian, 1985; Murley, Menz, Landorf, & Bird, 2010). The major disadvantages of intramuscular EMG are that 1) the invasive technique is often not feasible during dynamic muscle contractions, thus limiting its use for the analysis of movement, and 2) the high selectivity of indwelling electrodes may provide information on MU activity within a small area of a muscle that is not representative of other muscle compartments (Basmajian, 1985; De Luca & Merletti, 1988; Wakeling, 2009; Murley et al., 2010; Farina et al., 2016; Gallina et al., 2017). For these reasons, non-invasive surface EMG techniques, which provide more global information about the temporal and spatial characteristics of motor unit activity have become the preferred choice for biomechanical and neuromuscular studies in sport, rehabilitation, and clinical studies (De Luca & Merletti, 1988; De Luca, 1997; Farina et al., 2004; Vigotsky et al., 2018).

Despite the frequent use of sEMG in the analysis of human movement, the complex interaction between physiological and non-physiological factors that influence the sEMG recording results in many possible pitfalls during the interpretation of the information within in the signal (De

Luca, 1997). This was best described by Carlo de Luca, one of the most influential EMG scientists of the last decades: “Electromyography is a seductive muse because it provides easy access to physiological processes that cause the muscle to generate force […]. To its detriment, electromyography is too easy to use and consequently too easy to abuse” (De Luca, 1997). Nevertheless, useful information can be extracted from sEMG recordings if the many influencing factors on the signal are minimized by the experimental protocol and/or appropriately considered in the signal interpretation (Farina et al., 2004). The information that human movement scientists most commonly extract from the sEMG signal may be categorized into 1) the consequence of muscle activity, i.e. muscle force production (Buchanan, Moniz, Dewald, & Rymer, 1993; De Luca, 1997; Vigotsky et al., 2018), and 2) the origin of muscle activity, i.e. the organization of neural drive to the MUs by the CNS as well as the conducting properties of the muscle fibers that generate the potentials underlying EMG measurements (Merletti et al., 2001; Farina et al., 2004; von Tscharner & Barandun, 2010).

2.3.2 EMG signal amplitude

Two major factors that influence the amplitude of the sEMG signal are 1) the number of active (or detected) MUs and 2) their corresponding firing rates during a contraction. The spatial sum of motor unit action potentials in the detection volume of the surface electrodes then yields the time-dependent sEMG amplitude. In parallel, the combination of active MUs and their firing rates explains a large portion of the variability in isometric muscle force output (Farina & Negro, 2015). Therefore, sEMG amplitudes have been used as indirect measures for muscle force with the assumption of a linear relationship between these two quantities (Bigland & Lippold, 1954; Hof & van den Berg, 1977). The analytical steps for such amplitude measurements typically include band-pass filtering of the raw signal between 10-500 Hz, rectification, and smoothing with a low-pass filter to obtain a linear envelope of the signal (Merletti, 1999). Alternatively, the signal envelope can be extracted by means of a wavelet analysis, which has the advantage of yielding both temporal and spectral information of the signal amplitude (von Tscharner, 2000). The processed amplitude of a given muscle during a certain movement is then expressed relative to the maximum amplitude obtained from the same muscle during a maximum voluntary isometric contraction (MVIC) (Merletti, 1999). While the MVIC normalization is the most common and recommended technique to relate the EMG amplitude to muscle force (Burden, 2010), a body of evidence warns the EMG researcher of its limitations. For example, during isometric fatiguing contractions the sEMG amplitude increases despite a constant muscle force (Arendt-Nielsen & Mills, 1988; Merletti et al., 2001). Similarly, the relative timing of MU firings to one another and the corresponding superposition and cancellation of motor unit action potentials, e.g. in the presence of MU synchronization, affects the sEMG amplitude without changing the average muscle force (Yao, Fuglevand, & Enoka, 2000; Keenan, Farina, Maluf, Merletti, & Enoka, 2005; von Tscharner, 2010; Asmussen, Von Tscharner, & Nigg, 2018). These are only two examples demonstrating that in most cases, the relationship between sEMG amplitude and muscle force is in fact non-linear (Liu, Herzog, & Savelberg, 1999). Besides the influence of MU firing statistics, the non-linear relationship is further determined by the complex function that transforms the cumulative MU impulses into the total muscle force generated by the individual muscle fibers (Zajac, 1989). It is known that this function must include the contractile properties of the muscle fibers, such as their force-length, force-velocity, and history-dependent properties (Hill, 1938; Abbott & Aubert, 1952; Gordon, Huxley, & Julian, 1966). Therefore, particularly during movement when muscles undergo constant shortening or lengthening at varying speeds, the sEMG to muscle force relationship is highly non-linear and can most likely not be predicted from MVCs. Nevertheless, a plethora of clinical biomechanics studies have used this very approach; for example, to investigate changes in MVC-normalized EMG amplitudes of leg muscles during functional movements as a result of a previous knee injury and relate these changes to joint loading (Burden, 2010). In some instances, the predicted muscle forces are further used as an input to a musculoskeletal model, e.g. with the goal to estimate knee joint contact forces (Delp et al., 2007; Besier, Fredericson, Gold, Beaupré, & Delp, 2009). Due to the limitations of sEMG amplitude measurements described above, it is crucial to determine the measurement error and the associated reliability of such experimental approaches in order to avoid misleading conclusions and false recommendations to clinicians.

2.3.3 EMG-EMG intermuscular coherence

In contrast, analyzing the sEMG signal to extract the underlying neural strategies of the central nervous system does not typically require MVC amplitude normalization. For example, Stirling, Maurer, and colleagues applied time-frequency analyses to the sEMG signals of the medial gastrocnemius to investigate the presence and properties of the Piper rhythm during running (Stirling, von Tscharner, Kugler, & Nigg, 2011b; Maurer, von Tscharner, & Nigg, 2013). Further, the correlation of the Piper and other rhythms has been studied between sEMG signals from different muscles to infer strategies of the CNS to coordinate the activity of multiple muscles during motor tasks (Halliday et al., 2003; Kattla & Lowery, 2010; De Marchis, Severini, Castronovo, Schmid, & Conforto, 2015; Mohr et al., 2015; Laine & Valero-Cuevas, 2017; Pizzamiglio et al., 2017; Reyes et al., 2017; Castronovoa et al., 2018). These previous studies are most often based on an intermuscular coherence analysis between the sEMG signals of interest, a measure to quantify the degree of intermuscular synchronization discussed in section 2.2.1 (Semmler, 2002). The coherence analysis is an extension of the cross-correlation analysis from the time to the frequency domain and provides a bounded measure between 0 and 1 related to the correlation between the frequency components of two or more EMG signals of interest (Rosenberg, Amjad, Breeze, Brillinger, & Halliday, 1989). More specifically, the coherence value between two signals at a given frequency indicates the degree to which the phase relationship of these signals at this frequency is constant. It follows that for a coherence equal to one, the phase of one signal can be linearly predicted from the phase of the other signal.

For an example, let us imagine a scenario where two microphones are recording a piano concert at a distance of 10m (S1) and 20m (S2) from the stage in an otherwise quiet concert hall. Due to the known distance between the two microphones, the phases of S2 at each frequency could be perfectly predicted from the phases of S1, i.e. the coherence spectrum between S1 and S2 would have a constant value of one. In a second scenario, the concert hall is filled with an audience, whispering, clapping, and thus, adding additional frequency components that are represented to varying degrees in the two recordings. In this case, the coherence would drop and obtain a value between 0 and 1 since the phases of S2 can only partially be predicted from S1. In the third scenario, the second microphone is in the lobby, recording people talking and laughing far away from any music in the concert hall. Here, the two recordings represent two linearly independent processes and their coherence would approach zero (given a sufficiently long signal duration for the analysis). The analysis of coherence between two sEMG signals typically yields a result similar to the second scenario, where the signals contain some correlated frequency components, e.g. due to common neural inputs to these muscles from the central nervous system, and some independent frequency components, e.g. due to a more independent control strategy of these muscles (Mohr et al., 2015; Reyes et al., 2017).

Since the intermuscular coherence analysis determines the phase relationship between two EMG signals, the coherence result is more strongly dependent on the spectral properties of the sEMG signals rather than their absolute amplitudes. The physiological factors that most strongly influence the sEMG power spectrum are 1) the shape and duration of the detected MUAPs and 2) their corresponding recruitment pattern (De Luca, 1997; Farina et al., 2004; von Tscharner & Nigg, 2008). Two underlying anatomical factors that affect the MUAP shape and duration are 1) the average muscle fiber conduction velocity, which is dependent on the muscle fiber type, and 2) the spatial distribution of motor endplates (Wakeling & Syme, 2002; Farina et al., 2004). During ramp isometric contractions, the general recruitment pattern of MUs is governed by the size principle, an inverse relationship between the size of newly recruited MUs and their force-dependent recruitment threshold (Henneman, Somjen, & Carpenter, 1965). Superimposed on the general recruitment pattern, MU firing is modulated by different oscillatory processes, e.g. the Piper rhythm, that will affect the sEMG power spectrum (Weytjens & Steenberghe, 1984; Asmussen et al., 2018). If such oscillations are correlated or synchronized between two different muscles, a significant intermuscular coherence can be obtained. Many previous studies have applied an EMG-EMG coherence analysis to detect correlated oscillations in activity between muscles and have reported significant coupling of MU activity in different frequency bands. Grosse and colleagues (2002) have summarized these bands into the following categories: 1) the 'common drive' band at frequencies below 5 Hz with debated neural origin (De Luca & Erim, 2002; Laine et al., 2015; Farina et al., 2016), 2) the 6-12 Hz band, which is associated with physiological tremor and may originate from common oscillations in the monosynaptic stretch-reflex loop (Lippold, 1970; Christakos, Papadimitriou, & Erimaki, 2006), 3) the 15-30 Hz beta-band, originating from common neural inputs of supraspinal centers during isometric contractions (Farmer, Bremner, et al., 1993; Conway et al., 1995; Salenius, Portin, Kajola, Salmelin, & Hari, 1997; Hansen et al., 2002; Kattla & Lowery, 2010), and 4) the 30-60 Hz gamma- or Piper-band, which is also thought to reflect cortical inputs to the muscles but is more commonly observed during stronger or dynamic muscle contractions (Brown, 2000; Clark, Kautz, Bauer, Chen, & Christou, 2013; Mohr et al., 2015; Castronovoa et al., 2018).

While the analysis of intermuscular coherence in different frequency bands offers an exciting window into the neural control of multiple muscles during motor tasks, many non-physiological factors related to EMG data acquisition and signal processing have been shown to influence the intermuscular coherence spectrum (Farina et al., 2004; Farina, Negro, & Jiang, 2013; Keenan, Massey, Walters, & Collins, 2012; Boonstra & Breakspear, 2012; Dideriksen et al., 2018). For example, there is an ongoing debate on whether raw or rectified sEMG signals are more suitable to extract common neural inputs from the CNS to different muscles (Neto & Christou, 2009; Boonstra & Breakspear, 2012; Farina et al., 2013). Furthermore, an effect of electrode location on intermuscular coherence has been reported by Keenan and colleagues who showed that coherence estimates are reduced if sEMG signals are recorded in proximity to the muscles' innervation zone (Keenan et al., 2012). In contrast, the influence of the sEMG detection system, i.e. the amplifier technology and the electrode configuration, on intermuscular coherence is much less understood. Currently, most investigators use traditional bipolar potential amplifiers to investigate EMG-EMG coherence with the advantage that bipolar sEMG signals are less susceptible to electrical noise as well as to cross-talk from neighbouring muscles due to increased spatial filtering (De Luca & Merletti, 1988; De Marchis et al., 2015; Laine & Valero-Cuevas, 2017; Reyes et al., 2017; Castronovoa et al., 2018). In contrast, other authors have used monopolar current amplifiers to determine synchronized MU activity with the motivation that the monopolar EMG signal is the most direct representation of the underlying motor unit activity of a muscle and thus, better suited to resolve common neural inputs between muscles (von Tscharner, Maurer, Ruf, & Nigg, 2013; von Tscharner, Maurer, & Nigg, 2014; Mohr et al., 2015). To date, a systematic comparison between the two systems to quantify intermuscular coherence is lacking.

In summary, the many physiological and non-physiological factors that can influence the sEMG signal makes the interpretation of sEMG-based assessments of muscle force or neural strategies difficult. Regardless, sEMG remains one of the best non-invasive technologies available to neuroscientists and biomechanists who are interested in neuromuscular strategies during movement and how these strategies are adapted in response to changing intrinsic or extrinsic factors such as injury or fatigue. The methodological shortcomings and influencing factors related to sEMG-based markers of neuromuscular control are too often overlooked or not sufficiently described in the current literature. Two methodological aspects of sEMG-based assessments were identified that warrant further investigation, i.e. the reliability of sEMG amplitude-based markers of neuromuscular control as well as the influence of the recording system and electrode configuration on EMG-EMG coherence as a neuromuscular marker. Further insight into such methodological aspects ensures a valid interpretation of sEMG signals and helps to advance our understanding of neuromuscular strategies in the healthy and pathological state, e.g. following a knee injury. In this context, the following paragraph will review the most relevant findings related to adaptations in neuromuscular control that are observed in individuals with a history of knee injury.

2.4 The influence of knee injury on neuromuscular control

A knee injury results in neuromuscular changes at all levels of the central and peripheral nervous systems. These include physical damage to sensory organs in muscles and ligaments related to the joint as well as changes in spinal and cortical neural pathways. In combination, these changes result in altered muscle activation patterns during movement (for reviews on this topic, see: Ingersoll, Grindstaff, Pietrosimone, & Hart, 2008; Pietrosimone, McLeod, & Lepley, 2012; An, 2018).

2.4.1 Quadriceps weakness and reduced activation

An unequivocal finding in previous studies (for a review, see: Hart, Pietrosimone, Hertel, & Ingersoll, 2010) is a deficit in the ability to voluntarily activate the quadriceps muscle following a knee injury, referred to as arthrogenic muscle inhibition (Hopkins & Ingersoll, 2000). Quadriceps weakness has been observed after a rupture of the Anterior Cruciate Ligament (ACL), ACL reconstruction, meniscectomy, and in patients with anterior knee pain (Glatthorn, Berendts, Bizzini, Munzinger, & Maffiuletti, 2010; Hart et al., 2010; Whittaker, Toomey, Nettel-Aguirre, et al., 2018). The primary origin of arthrogenic muscle inhibition is altered afferent information from joint mechanosensors to the spinal cord due to soft tissue damage, joint effusion, or joint pain, that results in inhibition of quadriceps motor neurons via the interneuron system (Hopkins & Ingersoll, 2000). Quadriceps inhibition manifests in lower maximum isometric knee extension force as a consequence of lower voluntary activation (Hart et al., 2010). Despite surgical treatment and rehabilitation, quadriceps weakness may persist for ten or more years post-injury (Hart et al., 2010; Whittaker, Toomey, Nettel-Aguirre, et al., 2018). Many authors suggest, that quadriceps activation deficits following a knee injury are an important contributor to the development of knee PTOA since reduced quadriceps strength has been identified as an independent risk factor for the incidence of idiopathic knee OA (Slemenda et al., 1998; Palmieri-Smith & Thomas, 2009; Segal et al., 2009; Roos, Herzog, Block, & Bennell, 2011).

Other authors have used the internal knee extension moment to infer the amount of quadriceps activation after an ACL rupture. The finding of a lower knee extension moment during gait in ACL-deficient patients has been referred to as a 'quadriceps avoidance' gait (Berchuck, Andriacchi, Bach, & Reider, 1990). The avoidance of quadriceps muscle activity when the knee operates close to full extension during gait is thought to avoid an anterior displacement of the tibia, a movement which may not be desired by ACL-deficient patients who are missing the mechanical resistance of the ACL to that motion. This argument, however, was solely based on an analysis of external knee moments during the respective movements. Subsequent sEMG-based investigations of quadriceps muscle activity during walking and jogging resulted in conflicting evidence for 'quadriceps avoidance' following ACL-deficiency or reconstruction and controversy remains regarding the prevalence and persistence of this neuromuscular adaptation (Beard, Soundarapandian, O'connor, & Dodd, 1996; Roberts, Rash, Honaker, Wachowiak, & Shaw, 1999; Rudolph, Axe, Buchanan, Scholz, & Snyder-Mackler, 2001; Knoll, Kiss, & Kocsis, 2004; Hurd & Snyder-Mackler, 2007; Hall, Stevermer, & Gillette, 2015; Shanbehzadeh, Bandpei, & Ehsani, 2017).

Knoll and colleagues (2004) evaluated sEMG-based muscle activation patterns during gait in individuals during the acute phase following ACL rupture (average time since injury = 12 days), and at 6 weeks, 4 months, 8 months, and 12 months following ACL reconstruction. While a 'quadriceps avoidance' gait pattern was observed in the acute injury phase and 4 months post-surgery, individuals returned to a gait pattern that was similar to control participants at 8-12 months post-surgery. These findings would suggest that reduced quadriceps activity during gait is a neuromuscular adaptation that is most characteristic for the acute phase and early rehabilitation period after ACL injury while normal levels quadriceps activity are recovered at more than one year post-injury. The two hypothesized reasons for reduced quadriceps activity, however, following a knee injury during are 1) arthrogenic muscle inhibition and/or 2) an attempt by the individual to avoid anterior-posterior instability of the knee. Both muscle inhibition and sagittal plane knee instability have been shown to persist past the first year post-knee injury and despite ACL reconstruction (Barrett, 1991; Kvist & Gillquist, 2001; Hart et al., 2010) and thus, a persistent 'quadriceps avoidance' gait may be expected. In line with these arguments, Hall and colleagues (2015) examined individuals who had undergone ACL reconstruction at an average of five years post-surgery during a stair descent task and demonstrated a reduced activity of the rectus femoris – one of the four quadriceps femoris muscles – compared to uninjured controls. This finding suggests that reduced quadriceps activity during gait may be a persistent adaptation in individuals with a previous knee injury and thus may be involved in the pathogenesis of knee PTOA.

The controversy between previous studies investigating quadriceps activity during gait in individuals with a previous ACL injury may stem from either different gait protocols (e.g. stair descent, normal walking, running) and/or different sEMG-based outcome variables of quadriceps activation. For example, gait tasks that require the generation of higher quadriceps muscle forces such as descending stairs or running may exacerbate a 'quadriceps avoidance' strategy compared to walking at a preferred speed. Furthermore, a sEMG analysis that is limited to the comparison of quadriceps activity on- and offset times (Knoll et al., 2004) cannot capture a reduction in the quadriceps sEMG amplitude as was observed by Hall and colleagues (Hall et al., 2015). In consequence, a comprehensive sEMG-based analysis of quadriceps activity during gait that includes both amplitude and timing aspects of muscle activation is warranted to resolve the controversy whether this neuromuscular adaptation to a knee injury persists past the acute injury phase.

2.4.2 Increased hamstring activity

Berchuck and colleagues attributed lower internal knee extension moments during gait in ACL-deficiency to a reduction in quadriceps muscle force (Berchuck et al., 1990). Alternatively, the observed reduction in the knee extension moment could have originated from an increase in hamstring force or a change in lower extremity kinematics and kinetics (Beard et al., 1996). Increased hamstring muscle activity during gait and functional movements is a second widely-reported neuromuscular adaptation to a knee injury and has been consistently shown in ACL-deficient and ACL-reconstructed individuals as well as in individuals with previous meniscectomies (Limbird, Shiavi, Frazer, & Borra, 1988; DeMont, Lephart, Giraldo, Swanik, & Fu, 1999; Swanik, Lephart, Giraldo, DeMont, & Fu, 1999; Chmielewski, Hurd, Rudolph, Axe, & Snyder-Mackler, 2005; Hurd & Snyder-Mackler, 2007; Sturnieks, Besier, & Lloyd, 2011; Tsai, McLean, Colletti, & Powers, 2012; Hall et al., 2015; Willy, Bigelow, Kolesar, Willson, & Thomas, 2017). The hamstring muscle complex, consisting of the biceps femoris, semitendinosus, and semimembranosus muscles, has insertion points spread across medial aspects of the proximal tibia and the fibula and can thus resist tibia translation and rotation (Koulouris & Connell, 2005). Therefore, individuals with a previous knee injury may use a 'hamstrings facilitation' strategy to prevent rotational and translational instability of the knee joint (Liu & Maitland, 2000; Rudolph et al., 2001; Shelburne, Torry, & Pandy, 2005; Andriacchi & Dyrby, 2005). Although beneficial for knee joint stability, a large increase in hamstring muscle activity and a corresponding larger knee flexion moment may alter the locations of contact between the tibial and femoral articular surfaces during gait, which has been suggested as a risk factor for degenerative changes of the cartilage following a knee injury (Andriacchi & Dyrby, 2005). The evidence for a 'hamstrings facilitation' strategy during normal gait, however, is primarily based on studies involving patients with acute ACL rupture or chronic ACL-deficiency. It is currently unknown whether an increase in hamstrings activity during gait persists past the first year after a knee injury in individuals who underwent reconstructive surgery.

2.4.3 Increased quadriceps-hamstrings co-contraction

A stronger simultaneous activation of the antagonistic quadricep and hamstring muscles groups, i.e. increased quadriceps-hamstrings (Q-H) co-contraction, is a third neuromuscular strategy observed in individuals with previous ACL and/or meniscal injury (Rudolph et al., 2001; Sturnieks et al., 2011; Tsai et al., 2012). During their simultaneous activation, the quadriceps and hamstrings produce opposing torques about the knee joint resulting in increased knee joint stiffness and reduced joint laxity (Baratta et al., 1988). While beneficial for joint stability, an increase in Q-H co-contraction alter the mechanics of the knee joint by shifting the load bearing regions on the articulating joint surfaces and increasing the joint contact force (Victor, Labey, Wong, Innocenti, & Bellemans, 2010). Abnormal joint kinematics in combination with an increase in knee joint contact forces during weight-bearing movements may represent a risk factor for the degeneration of the cartilage as a hallmark of PTOA development (Chaudhari, Briant, Bevill, Koo, & Andriacchi, 2008; Tsai et al., 2012). The following findings or the lack thereof, however, make it difficult to reconcile the suggested association between Q-H co-contraction and PTOA risk. First, the most common outcome variable for the assessment of Q-H co-contraction following knee injuries (the muscle co-contraction index (Rudolph et al., 2001)) show a poor correlation with estimates of the knee joint contact force (Winby, Gerus, Kirk, & Lloyd, 2013; Wellsandt et al., 2017). This observation challenges the importance of such indices for the mechanical loading of the knee joint. Q-H co-contraction indices are typically derived from sEMG analyses that include amplitude normalization to a maximum voluntary contraction. Based on limitations of sEMG amplitude measurement explained above, a poor reliability and validity of the normalized sEMG amplitude as a surrogate measure of muscle force may be the origin of the poor correlation with joint contact forces. Second, there are some studies where a persistent increase in Q-H co-contraction greater than one year following a previous knee injury was not observed during gait, a step-up task, and a knee shear loading task (Knoll et al., 2004; Aalbersberg, Kingma, Blankevoort, & van Dieen, 2005; Ortiz et al., 2008; Wellsandt et al., 2017). Third, there is lack of studies to show a quantitative association between increased Q-H co-contraction following a knee injury and markers of early PTOA development such as joint degeneration observed via medical imaging or self-reported knee complaints (Luyten et al., 2018). In consequence, the presence and relevance of a pronounced Q-H co-contraction strategy in individuals who have sustained a knee injury more than one year ago should be re-evaluated. This re-evaluation should apply sEMG techniques that do not rely on amplitude normalization to a maximum voluntary contraction and quantify the association with potential markers of PTOA development.

2.4.4 Quadriceps-hamstrings intermuscular coherence

An alternative approach to investigating the co-contraction between the quadriceps and hamstrings muscles may be to determine the correlation between their EMG signals in the frequency domain, i.e. intermuscular EMG-EMG coherence. While there is some evidence related to changes of the EMG frequency content of individual leg muscles as a result of a knee injury (McNair & Wood, 1993; Drechsler, Cramp, & Scott, 2006), the correlation between EMG signals from different muscles surrounding the knee is a novel field of study in individuals with previous knee trauma. Significant intermuscular coherence has previously been observed between antagonistic muscles if they are activated during a voluntary, isometric co-contraction (Hansen et al., 2002; Geertsen et al., 2013). If individuals with a previous knee injury in fact use a neuromuscular strategy to active the quadricep and hamstring muscle groups as one functional unit to increase joint stiffness, this strategy should become visible in an intermuscular coherence analysis. Specifically, previously injured individuals should demonstrate stronger, common neural inputs to the quadricep and hamstring muscles resulting in a higher intermuscular coherence between their sEMG signals as compared to a control condition. Conveniently, the application of EMG-EMG coherence does not require amplitude normalization, which circumvents the methodological limitations associated with co-contraction indices that are based on the sEMG amplitude. To date, the use of EMG-EMG coherence to assess abnormalities in intermuscular coordination following a knee injury has remained unexplored.

Besides the insight that intermuscular coherence can provide into the functional coordination of multiple muscles during a movement, the coupling of MU activity between muscles may influence the resultant torque of the joint that the respective muscles act on (Santello & Fuglevand, 2004; Laine et al., 2015; Pizzamiglio et al., 2017) (see section 2.2.1). During a movement, the effect of intermuscular synchronization of MU activity on joint loading is certainly much smaller compared to the effect of the overall force profiles of the involved muscles and is likely negligible if considered for only a few movement cycles. Knee PTOA, however, develops over the course of at least a decade (Lohmander, Englund, Dahl, & Roos, 2007), involving millions of cycles of weight-bearing movements. If a previous knee injury has caused abnormal levels of intermuscular synchronization between the quadricep and hamstring muscles as suggested before, the corresponding differences in resultant forces acting on the tibio-femoral and patella-femoral joints in combination with millions of movement cycle repetitions may be a contributing factor to the slow post-traumatic degeneration of joint tissues. A possible relationship between intermuscular synchronization surrounding the injured knee and development of PTOA has not been mentioned in the clinical biomechanics literature but may help to further understand the link between the debilitating disease and abnormal muscle activation patterns post-knee injury.

In summary, the existing literature is pointing towards three possible adaptations in lower extremity muscle activation patterns during movement following a knee injury: 1) reduced quadriceps activation, 2) increased hamstring activation, and 3) increased Q-H co-contraction. The evidence for the presence of these neuromuscular adaptations is strong when they are examined in the acute phase post-knee injury or during more strenuous tasks than walking such as running or drop-landings. The main limitation of the current literature is the controversy about the long-term persistence of these neuromuscular adaptations during everyday activities, i.e. whether abnormal quadriceps and hamstrings activity can be observed past the acute injury and rehabilitation phase into the time period when individuals have returned to sports (> three years post-injury). Further, if persistent abnormal muscle activation patterns in previously injured participants were identified in previous studies, there is a lack of evidence that these abnormalities are associated with the risk or progression of PTOA development. To resolve this controversy, quadricep and hamstring muscle activation as quantified by sEMG during non-strenuous movements should be re-investigated in individuals with a previous knee injury that was sustained at least three years ago. This re-investigation should include the analysis of timing, amplitude, as well as frequency aspects of multiple sEMG signals to provide a comprehensive assessment of lower extremity muscle activation patterns following a previous knee joint injury. In the presence of abnormal muscle activation patterns in previously injured individuals, their relationship with markers for early knee PTOA development should be explored.

2.5 PTOA – the role of abnormal muscle activity following a knee injury

The physiological basis underlying the hypothesized neuromuscular pathway of knee PTOA is that tissues within the knee joint adapt and change in response to the mechanical and biological joint environment (Taber, 1995; Nigg & Herzog, 2007). The mechanical joint environment is given by the resultant shear and compressive forces that act on a joint. These resultant forces are determined by the sum of external forces (e.g. the ground reaction force) and more importantly internal forces, which are produced by the muscles and ligaments that have a moment arm about the joint. Resultant forces in combination with the joint surface geometry and tissue material properties will define the local stress-strain states experienced by the tissues covering the articulating joint surfaces, e.g. cartilage or menisci, as well as the underlying subchondral bone. While it is known that biological tissues such as bone or cartilage depend on regular loading in order to maintain a healthy, homeostatic state of adaptation and regeneration, loading conditions outside the ‘normal’ range may contribute to tissue remodelling and possibly degeneration (Andriacchi, Koo, & Scanlan, 2009). The line of arguments above forms the theoretical basis that abnormal muscle activation patterns following a knee injury could result in abnormal mechanical loading of the joint, associated changes in the stress and strain experienced by the knee joint tissues and ultimately knee joint degeneration.

The possible relationship between abnormal knee joint loading following a knee injury and PTOA development has been investigated by many research groups using different experimental models and approaches. The following sections will review the findings from three widely discussed approaches in the biomechanical PTOA literature. The specific focus of the review is to critically evaluate the evidence from the three approaches and how it relates to the involvement of abnormal muscle activity in PTOA development.

2.5.1 Approach 1 – Evidence from traditional 3D gait analyses

Tom Andriacchi's group from the University of Stanford has made considerable efforts to understand the role of knee joint kinematics and kinetics obtained from traditional 3D gait analyses on knee joint tissue adaptation. Specifically, they investigated the effect of knee joint mechanics on cartilage morphology in the healthy state and following an ACL injury. One of the most important and repeatedly observed results was that cartilage adapts to the typical kinematic and kinetic patterns within the knee joint associated with gait by increasing its thickness in the contact areas that bear the highest loads (Koo & Andriacchi, 2007; Koo, Rylander, & Andriacchi, 2011; Scanlan, Favre, & Andriacchi, 2013). Similarly for bone, dynamic knee loading as measured by the external knee adduction moment during gait was associated with the medio-lateral distribution of bone mineral content of the proximal tibia (Hurwitz, Sumner, Andriacchi, & Sugar, 1998). A second result was that the load bearing areas may move to new locations on the articulating surfaces following ACL rupture due to abnormal knee joint kinematics during gait (Andriacchi & Dyrby, 2005; Scanlan, Chaudhari, Dyrby, & Andriacchi, 2010; Scanlan et al., 2013; Erhart-Hledik, Chu, Asay, & Andriacchi, 2018). Specifically, abnormal knee joint kinematics after ACL rupture were characterized by altered flexion-extension and internal-external rotation angles and associated changes in the contact locations between tibia and femur during gait. Such altered knee joint kinematics following ACL injury were confirmed by other research groups and were observed even after ACL reconstruction, independent of ACL graft type (patellar tendon vs. hamstring) and at time points greater than two years post-injury (Stergiou, Ristanis, Moraiti, & Georgoulis, 2007). Therefore, the argument was made that altered loading of the cartilage surfaces following an ACL injury may cause degeneration over time as the tissue cannot adapt to these new loading conditions and may degenerate over time (Andriacchi et al., 2004; Stergiou et al., 2007; Chaudhari et al., 2008; Andriacchi et al., 2009). In support of this argument, abnormal knee kinematics during gait were associated with the local variation of cartilage thickness in experimental and simulation studies of ACL deficiency (Andriacchi, Briant, Bevill, & Koo, 2006; Koo, Dyrby, & Andriacchi, 2007). In consequence, Andriacchi and colleagues have advanced the hypothesis that altered knee joint kinematics following ACL injury and the associated shift of load bearing regions on the articular cartilage are an important risk factor for the development of knee PTOA.

The influence of muscle activity on the relationship between altered kinematic and kinetic patterns of the knee joint and cartilage morphology following a knee injury has not received much attention from Andriacchi's research group. This is even though one of their earlier studies suggests that the offset in knee joint rotation and translation observed in ACL-injured individuals can largely be explained by the magnitude of the external knee flexion moment during the early stance phase of gait (Andriacchi & Dyrby, 2005). The external knee flexion moment during the early stance phase of gait is significantly influenced by muscle forces produced by the quadriceps and hamstrings (Schipplein & Andriacchi, 1991). This indicates that altered quadricep and hamstring muscle activation patterns as described in section 2.4 were likely responsible for abnormal knee joint kinematics following ACL injury observed by Andriacchi and colleagues.

A strength of the approach of Andriacchi's group is that traditional 3D gait analyses were combined with cartilage measurements obtained from magnetic resonance imaging to show an association between abnormal kinematic patterns of the knee joint following ACL injury and local variations in cartilage thickness. However, the derived conclusion that altered kinematics following ACL injury are a risk factor for PTOA is limited by the uncertainty whether the observed variations in cartilage thickness indeed predict a clinical diagnosis of knee OA later in life. Besides a degeneration of the articular cartilage, symptomatic knee OA is characterized by other structural joint defects such as the formation of osteophytes, meniscal degeneration, subchondral bone remodelling and most importantly knee pain (Hunter, 2011). Therefore, it remains unknown to what extent altered knee joint kinematics following ACL injury can explain the risk of knee PTOA as a painful, whole-joint disease.

2.5.2 Approach 2 – Evidence from animal models

The idea that abnormal mechanical loading of the knee joint may precede osteoarthritic changes has also been investigated by other research groups. Herzog and colleagues used an animal model to study muscle activation and knee joint contact mechanics during gait as well as corresponding articular cartilage properties in ACL-transected cats (Herzog, Adams, Matyas, & Brooks, 1993; Herzog, Longino, & Clark, 2003; Hasler, Herzog, Leonard, Stano, & Nguyen, 1997; Hasler & Herzog, 1997; Maitland, Leonard, Frank, Shrive, & Herzog, 1998; Suter, Herzog, Leonard, & Nguyen, 1998). Similar to findings in human studies, ACL-transected cats showed changes in the knee flexion-extension angle during walking and showed reduced resistance of the tibia to passive translation and rotation with respect to the femur (Hasler et al., 1997; Maitland et al., 1998; Suter et al., 1998). Further, *in vivo* measurements of patellofemoral joint contact forces indicated a 30% reduction of peak patellofemoral joint loading following

ACL transection (Hasler & Herzog, 1997). Interestingly in the context of this thesis, the reduction of mechanical load was caused primarily by a diminished patellar tendon force, which is determined by the sum of individual quadricep muscle forces (Hasler et al., 1997; Hasler & Herzog, 1997). Although patellofemoral contact pressures were also lower after ACL transection, the magnitude of reduction in pressure was not proportional to the reduction in contact forces. Herzog and colleagues demonstrated that this was due to changed cartilage material properties following the ACL surgery, specifically increased cartilage thickness and reduced cartilage stiffness leading to a larger joint contact area and thus reduced pressures (Herzog et al., 2003). Most importantly, the ACL-transected knees developed early signs of joint degeneration over the first year that were consistent with structural signs of knee OA in humans, e.g. the formation of osteophytes and meniscal degradation (Herzog et al., 1993; Suter et al., 1998). This was even though hindlimb kinematics, kinetics, and EMG patterns at 6 months post-ACL transection were almost identical to the pre-injury condition. Therefore, it was suggested that reduced muscle forces following ACL transection and the resulting reduction in pressure exerted on the articular surfaces are an important risk factor for joint degeneration and the development of PTOA. Even if the external loading variables return to pre-injury levels, the observed change in material properties of the articular cartilage may permanently alter the local stress and strain behaviour of the joint tissues.

The findings from the cat ACL-transection model that reduced muscle forces may be a PTOA risk factor agree well with the evidence from human studies demonstrating quadriceps weakness and inhibition following knee injury as described in section 2.4. While animal models are not suitable to assess deficits in voluntary muscle activation (Herzog et al., 2003), arthrogenic muscle inhibition may have been one of the reasons for the observed reduction of patellar tendon forces in the ACL-transected cats and thus represent a risk factor for PTOA development as suggested by other authors (Palmieri-Smith & Thomas, 2009). In fact, more recent evidence from a muscle weakness model in rabbits led Herzog and colleagues to suggest that quadriceps muscle weakness is a risk factor for both PTOA and idiopathic OA of the knee (Herzog & Longino, 2007; Rehan Youssef, Longino, Seerattan, Leonard, & Herzog, 2009; Egloff et al., 2014).

One strength of the animal model approach by Herzog and colleagues is that the mechanical loading at the tissue level, e.g. muscle forces or local joint contact pressure, could be measured *in vivo*. The knowledge of the local pressure distributions within a joint provides insight into the stress-strain behaviour of the intra-articular joint tissues, which will ultimately influence their remodelling responses at the cell-level and possibly explain why intra-articular tissues degenerate following a knee injury. The second strength is that the use of animal models has allowed Herzog's research group to better isolate the effects of different interventions such as ACL-transection or muscle weakness on the development of knee OA as a whole-joint disease (Mahmoudian, Assche, Herzog, & Luyten, 2018). Due to this advantage, muscle weakness could be identified as an independent risk factor for knee OA.

The question that remains unclear is whether the relationship between ACL injury, muscle weakness and knee OA is driven by the weakness-induced reduction in mechanical joint loading, the loss of muscular control, a combination of the two, or other effects (Herzog et al., 2003). Therefore, the investigation of adaptations in intermuscular coordination following knee injuries in either animal and human studies as suggested in section 2.4.4 may help to provide further insight into this question. An inherent limitation of animal models is the uncertainty about the generalizability of findings to human populations. Herzog and colleagues reported that limb kinematics and kinetics as well as leg muscle activation patterns in the ACL-transected cats return to pre-injury levels at 6 months post-ACL transection (Suter et al., 1998; Herzog et al., 2003). As described in section 2.4, there is evidence that neuromuscular and biomechanical adaptations to a previous knee injury may be present in humans for years after the knee injury event. Therefore, neuromuscular abnormalities following a knee injury may play a more significant role for the development of PTOA in humans compared to cats.

2.5.3 Approach 3 – Evidence from musculoskeletal models

Besides traditional gait analyses and animal models, more recent experimental protocols in humans have incorporated musculoskeletal models to estimate knee joint contact forces during gait and their association with early structural signs of knee OA development following ACL and meniscal injuries (Saxby et al., 2016; Wellsandt et al., 2016; Saxby et al., 2017).

Collectively, these studies have generally corroborated the findings of Herzog and colleagues that reduced mechanical loading of the knee joint following a knee injury may be a risk factor for PTOA development. In a large-scale biomechanical study (n > 150), forces of the lower extremity muscles and the resulting tibio-femoral contact forces during walking, running, and side-stepping were estimated using an EMG-driven musculoskeletal model (Saxby et al., 2016). Study participants included 104 adults with a history of ACL reconstruction (average time from surgery 2.5 years) and 60 individuals with no history of knee injury. During all tasks, participants with a previous reconstructive knee surgery showed lower knee joint contact forces relative to their body weight compared to the control group indicative of a general reduction of joint loading as observed in animal studies. In a follow-up study, Saxby and colleagues showed that the healthy response to build up cartilage thickness in areas of high tibio-femoral contact forces may be disturbed in individuals with a previous ACL reconstruction (Saxby et al., 2017).

Wellsandt and colleagues followed a group of individuals after an ACL injury with subsequent reconstructive surgery and estimated knee joint moments and contact forces during gait before and after the surgery as well as one and two years following the surgery (Wellsandt et al., 2016). In addition, the incidence of radiographic knee OA was determined at a five-year follow-up. Individuals who showed early knee PTOA walked with reduced medial compartment tibio-femoral contact forces as well as reduced knee adduction and knee flexion moments at six months post-surgery compared to the individuals who did not develop early knee OA. Due to the small sample size of this study (n = 22), the results should be interpreted with caution. The findings, however, do support the argument that joint unloading following a knee injury may contribute to the slow degeneration of the joint tissues that characterize knee OA.

Although not specifically discussed in the above studies, quadriceps muscle forces determine up to 50% of the total load experienced by the knee and thus are the main contributor to tibio-femoral contact force during gait (Winby, Lloyd, Besier, & Kirk, 2009). Therefore, reductions in tibio-femoral contact forces post-knee injury were most likely associated with reductions in quadriceps forces, which would agree with the animal model findings presented above. A strength of the musculoskeletal approach is that such models enable to quantify the *in vivo* joint contact forces of the medial and lateral knee joint during human gait. Joint contact forces are more indicative of the actual mechanical loading experienced by the knee joint tissues as compared to global external knee moments and may thus provide a better insight into possible mechanisms of knee PTOA. Extending the joint contact force model to the analysis of cartilage stresses and strains as demonstrated by Adouni and colleagues (2012; 2014) may further enhance the understanding of such mechanisms. An important limitation of most musculoskeletal modelling studies is that the accuracy of estimated loading variables is unknown since there is currently no direct, non-invasive method to validate joint contact or muscle forces. Medial and lateral knee joint contact forces have been shown to be highly sensitive to estimates of frontal plane knee alignment and joint contact locations (Saliba, Brandon, & Deluzio, 2017) while model-based estimates of muscle forces can deviate drastically from experimentally measured forces in animal preparations (Herzog & Leonard, 1991). Therefore, the usefulness of current musculoskeletal models to estimate tissue-level loading of the knee joint for individual patients with the goal of predicting knee PTOA development remains unknown.

In summary, the structures of the knee joint depend on mechanical loading for sustaining a healthy joint environment. Cyclic mechanical loading is provided by the external ground reaction force and the internal forces due to muscle contraction during locomotion or other weight-bearing exercises. Evidence from different experimental approaches suggests that following a knee injury, abnormal mechanical loading of the joint during locomotion may not be sustained by the joint structures and contribute to tissue degeneration and knee PTOA. Abnormal mechanical loading may be characterized by 1) a significant reduction in the magnitude of mechanical loading as a result of muscle weakness or inhibition and/or 2) a shift of the load bearing regions within the joint as a result of altered joint kinematics. Either way, the activation patterns of the muscles surrounding the knee joint are intimately connected to this mechanical pathway of PTOA as they primarily regulate joint movement and loading. While findings from animal models have clearly pointed at abnormal muscular activity following knee trauma as a risk factor for PTOA development, human PTOA studies have been mostly focused on the role of external joint loading or joint movement. Therefore, an investigation of abnormal lower extremity muscle activity following knee injury during human movement and its possible relationship with knee PTOA is needed, specifically from the perspective of possible therapeutic interventions aimed at PTOA prevention.

A limitation that is common to all three experimental approaches described above is the current lack of biomarkers with a good ability to predict whether an individual (human or animal) will develop symptomatic knee osteoarthritis in the future (Carbone & Rodeo, 2017). Particularly in humans, biomechanical and neuromuscular investigations related to PTOA are typically lacking long-term follow-up data that could confirm whether the suggested risk factors indeed resulted in a clinical diagnosis of knee OA. Furthermore, common indicators of early knee PTOA development that are based on medical imaging such as MRI-defined knee OA may have little or no association with self-reported knee pain, symptoms, and function (Whittaker et al., 2018). Progressively worse knee symptoms and increased pain, however, are central symptoms of knee PTOA development (L. Stefan Lohmander et al., 2007; Luyten et al., 2018; Whittaker, Toomey, Nettel-Aguirre, et al., 2018). Therefore, studies to address neuromuscular and biomechanical risk factors for PTOA should ideally consider associations with both structural markers of the disease and self-reported knee symptoms and pain.

2.6 Summary and rationale

Post-traumatic knee osteoarthritis is common 10-20 years following a knee joint injury and leads to disability and a reduced quality of life. An extensive body of literature is available that describes adaptations in neuromuscular control of the lower extremity during movement in the acute time period after a knee injury. Such abnormal control strategies are typically investigated using muscle activity recordings based on surface electromyography (sEMG). Adaptations in neuromuscular control strategies result from the physical tissue damage, pain, and joint instability associated with the injury, which in combination cause changes at all levels of the central nervous system. Since muscle forces regulate joint loading, the hypothesis has advanced that abnormal muscle activation patterns following a knee injury and corresponding abnormal knee joint loading patterns lead to a degeneration of joint structures over time and eventually to the development of knee osteoarthritis. Evidence for this neuromuscular pathway of post-traumatic osteoarthritis, however, is scarce. The factors underlying this lack of evidence may include that 1) common muscle activity outcome variables determined from sEMG exhibit poor reliability, 2) neuromuscular strategies following knee injuries have not been explored beyond amplitude and temporal features of muscle activity, 3) persistent abnormalities in muscle activation patterns past the acute phase after knee injuries are not well understood, and/or 4) the pathogenesis of post-traumatic knee osteoarthritis is primarily driven by biological, and structural changes initiated by the injury and to a lesser degree by abnormal muscle activation and joint movement.

This doctoral thesis aims to address each of these factors by first examining how methodological aspects related to sEMG techniques affect the reliability and sensitivity of neuromuscular markers and to then implement the conclusions in a comprehensive assessment of persistent, abnormal neuromuscular control strategies in individuals who have suffered a previous knee injury more than three years prior. The findings from this assessment will be discussed in the context of the hypothesized neuromuscular and alternative pathways for the development of post-traumatic knee osteoarthritis. The ultimate goal is to improve our understanding of modifiable risk factors that contribute to the degenerative disease and thereby inform the design of rehabilitation interventions following knee injuries that reduce the risk and rate of osteoarthritis progression.

Chapter Three – **Reliability of the knee muscle co-contraction index during gait in young adults with and without knee injury history**

3.1 Introduction

The simultaneous activation of antagonistic muscles is often referred to as muscle co-contraction and is frequently studied as a compensation mechanism for joint instability in young adults who have sustained a previous knee injury (Rudolph, Axe, & Snyder-Mackler, 2000; Rudolph, Axe, Buchanan, Scholz, & Snyder-Mackler, 2001; Tsai, McLean, Colletti, & Powers, 2012; Hall, Stevermer, & Gillette, 2015). All of the above studies use surface electromyography (EMG) to evaluate the activity of selected muscles of the lower leg during gait and infer muscle co-contraction strategies. One commonly used measure of muscle co-contraction in clinical biomechanics research is the muscle co-contraction index (CCI) (Rudolph et al., 2000). The CCI represents a weighted ratio of the EMG signal intensities obtained from two antagonistic or synergistic muscles with reference to the maximum EMG signal intensity achieved during maximum voluntary muscle contractions (MVCs). This ratio is typically determined for knee extensor – flexor or medial – lateral muscle pairs and then compared between post-knee injury and control groups (Hurd & Snyder-Mackler, 2007; Sturnieks, Besier, & Lloyd, 2011; Hall et al., 2015). In other studies, changes in the CCI are investigated that occur in response to interventions such as knee bracing or perturbation training (Chmielewski, Hurd, Rudolph, Axe, & Snyder-Mackler, 2005; Ramsey, Briem, Axe, & Snyder-Mackler, 2007).

Conclusions from such investigations to inform clinical practices can only be drawn if the reliability of the CCI for repeated measurements in the population of interest is established. To the best knowledge of the authors, the reliability of the CCI during gait in young post-knee injury and control populations is currently unknown. Previous investigators have reported substantial random error of greater than 200% and poor typical errors of measurements of up to 25% when repeatedly measuring the amplitude of surface EMG signals normalized to MVCs (Ball & Scurr, 2010; Murley, Menz, Landorf, & Bird, 2010). Therefore, one may speculate that the CCI, which relies on such amplitude measurements, would also demonstrate large random error and consequently exhibit low test-retest reliability. Only one study was identified that evaluated the relative reliability of CCIs in a moderate knee OA population (Hubley-Kozey, Robbins, Rutherford, & Stanish, 2013) using the intra-class correlation coefficient. However, these findings may not be generalized to the younger, more active, and less symptomatic populations that are often examined in post-knee injury studies (Whittaker, Woodhouse, Nettel-Aguirre, & Emery, 2015a). Furthermore, previous pathologies may influence the reliability of biomechanical outcomes in gait analyses (Steinwender et al., 2000; McGinley, Baker, Wolfe, & Morris, 2009). Therefore, reliability studies should include individuals with and without the pathology of interest, in this case a previous knee injury, and the pathology group should reflect a continuum of severity from mild to severe (Lijmer et al., 1999; McGinley et al., 2009).

The primary objective of this study was to quantify the absolute and relative intra-rater reliability (within day and between days measured 7-13 days apart) of the knee muscle co-contraction index during gait in young adults with varying knee injury history. A secondary objective was to explore if differences in intra-rater reliability exist between individuals with and without a previous knee injury.

3.2 Methods

3.2.1 Study design

This is a reliability study that was conducted in the context of a larger longitudinal cohort study with the goal to investigate outcomes associated with early post-traumatic osteoarthritis 3-10 years following knee joint injury in youth (Whittaker et al., 2015, 2018). For this specific cohort study and for researchers investigating similar longitudinal designs, it is essential to determine the reliability of outcome measures such as the muscle co-contraction index. The description of this study complies with the guidelines for reporting reliability and agreement studies proposed by Kottner and colleagues (Kottner et al., 2011).

3.2.2 Participants

A convenience sampling method was used to recruit twenty young adults (ages 16-31; 10 males, 10 females) who volunteered and provided written informed consent to participate in this study; ten had sustained a previous knee injury (5 males, 5 females) and ten controls had no history of knee injury. The sample size of n = 20 was selected based on feasibility while exceeding the sample size of most previous studies investigating the within or between-day reliability of EMG signal amplitudes (see Burden (2010) for a comprehensive review). Inclusion and exclusion criteria and the knee injury definition were selected to closely resemble the participant characteristics of the Pre-OA cohort study. Specifically, previously injured participants had to 1) be between 18-30 years old, 2) have sustained the primary knee injury two to ten years prior to the study, and 3) have not sustained a secondary or contralateral knee injury since then. A knee injury was defined as a self-reported injury to the cruciate or collateral ligaments and/or an injury to the menisci that resulted in both medical consultation as well as disruption of sport participation (Whittaker et al., 2015). Control participants had to be between 18-30 years old and exclusion criteria were 1) a history of previous knee injury as defined above or 2) the presence of any other lower extremity injury within six months prior to study participation. Ethical approval for research involving human participants was obtained from the University of Calgary's Conjoint Health Research Ethics Board (Ethics ID E-25075).

3.2.3 Reliability protocol

Participants visited the Human Performance Laboratory at the University of Calgary on two separate days, about a week (median (range), 7 (7-13) days) apart. The time of day when the testing sessions were performed was kept constant for both visits. During the first visit, participants completed one round of isometric dynamometry followed by two rounds of the same gait protocol. These two rounds of gait testing were separated by about 30 minutes and were used to determine within-day reliability. During the second visit, participants completed one round of isometric dynamometry and one round of gait measurements. The first rounds from each testing day were used to determine between-day reliability. On both days, all electromyography procedures were carried out by the same investigator (MM, doctoral student, four years of training and experience in electromyography and motion analysis). Motion analysis procedures were performed by two investigators (MM; KL, graduate student, three years of training and experience in motion analysis) according to internally standardized marker placement protocols and participant instructions.

3.2.4 Data Collection

All procedures and assessments were carried out on the side of the previous knee injury in the injury group and on the right side (preferred leg to kick a soccer ball for all participants) in the control group. Upon arrival, participants first filled out an initial questionnaire related to their demographics, injury history, and knee-related function as assessed through the Knee Injury and Osteoarthritis Outcome (KOOS) questionnaire (Roos, Roos, Lohmander, Ekdahl, & Beynnon, 1998). Afterwards, participants were prepared for EMG measurements according to widely used SENIAM guidelines including skin preparation (shaving, light abrasion, and cleaning with alcohol wipes) and identification of standardized landmarks for the measurement of surface

EMG signals from lower extremity muscles (Hermens, Freriks, & Merletti, 1999). Bipolar surface electrodes (Ag/AgCl, 10 mm diameter, 20 mm inter-electrode distance, Norotrode Myotronics-Noromed Inc., US) were placed in the muscle fibre direction on six lower extremity muscles: vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), medial hamstrings (MH, semitendinosus/semimembranosus), gastrocnemius medialis (GM), and gastrocnemius lateralis (GL). In order to validate the placement of each electrode, manual muscle testing for knee extension and flexion and ankle plantarflexion were performed. EMG signals were recorded with reference to a ground electrode on the tibial tuberosity at 2400 Hz, pre-amplified and bandpass-filtered between 10-500 Hz (Biovision, Wehrheim, Germany) via a 12-bit A/D converter (National Instruments, Austin, TX). In addition to EMG recordings, a 1D-accelerometer was taped to the lateral aspect of the heel for heel strike detection during walking trials.

Following the EMG set-up, participants completed a series of standardized submaximal warm-up contractions followed by two trials of five second isometric maximum voluntary contractions (MVCs) for each muscle group (knee extensors and flexors, and ankle plantarflexors). All contractions were completed while seated in a Biodex 3 System Pro (Biodex Medical System Inc, New York, NY, USA) according to the MVC protocol described by Albertus-Kajee and colleagues (Albertus-Kajee, Tucker, Derman, Lamberts, & Lambert, 2011). The investigator gave verbal encouragement during all maximum effort trials. The subject’s position in the dynamometer was documented on day 1 and kept consistent on day 2.

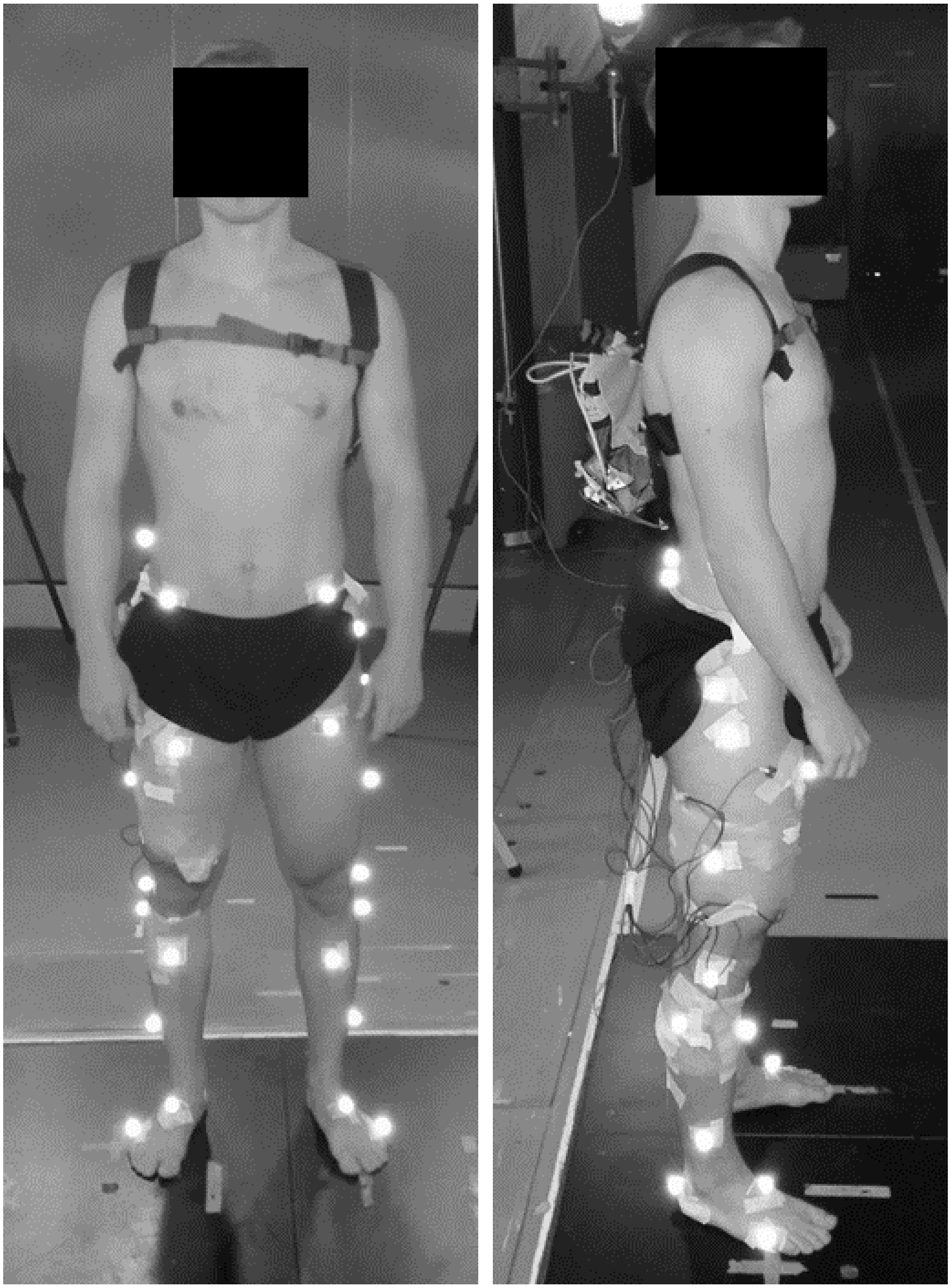

Figure 3.1. Reflective marker and EMG testing set-up.

Prior to the gait measurements, participants were equipped with retroreflective markers that were mounted on the thigh and shank segments to track three-dimensional lower limb kinematics (Figure 3.1). In addition, initial static recordings of the participants in an upright standing position were obtained after mounting additional markers over the greater trochanter, medial and lateral knee and ankle joints to define joint center locations. Next, participants completed seven successful trials of barefoot over-ground walking at their preferred speed along a 20 m walkway. The preferred speed was self-selected by the participants while walking on a treadmill before performing the over-ground walking trials. A minimum of six walking trials and a self-selected speed has been recommended to obtain reliable electromyographic data during gait (Kadaba, Wootten, Gainey, & Cochran, 1985; Shiavi, Frigo, & Pedotti, 1998). During each over-ground walking trial, lower-extremity EMG as well as kinematics and synchronized ground reaction forces were recorded for one gait cycle using a high-speed motion analysis system (8 cameras; Motion Analysis Corp, Santa Rosa, CA, USA) and a floor-embedded force plate at sampling rates of 240 Hz and 2400 Hz, respectively. Over-ground walking speed was monitored in real-time using photoelectric timing gates and trials were repeated if the walking speed fell outside of 10% of the preferred walking speed set on the treadmill (Hubley-Kozey et al., 2013). The self-selected speed defined in the first testing session was kept constant during the second testing session.

3.2.5 Data processing

The EMG and motion data were synchronized at the instant of heel strike according to a peak in the heel accelerometer signal (EMG system) and the onset of the vertical ground reaction force (20 N threshold) provided by the force plate.

All EMG analysis was performed using a filter bank of 13 nonlinearly scaled wavelets specifically designed for the analysis of electromyographic recordings (von Tscharner, 2000). The wavelet transform results in the decomposition of the raw EMG signal into the time-dependent signal power in each of the frequency bands (wavelets). For this analysis, powers from nine wavelets with center frequencies between 38 Hz and 395 Hz were summed to derive the total power. Wavelets with center frequencies below and above this range were excluded to avoid signal power originating from motion artefacts or high frequency noise, respectively (Conforto, D'Alessio, & Pignatelli, 1999). The square root of the total power yields the total intensity, which is a measure of the time-dependent overall EMG intensity and a close approximation of the frequently used EMG root mean square (von Tscharner, 2000). The EMG total intensity recorded from each muscle during walking was amplitude-normalized to the highest EMG total intensities that were achieved during the corresponding MVC exercises (e.g. knee extension for vastus lateralis EMG normalization) (Figure 3.2). EMG total intensities obtained during either session on day 1 were normalized to MVCs from day 1 while EMG intensities on day 2 were normalized to MVCs from day 2.

The raw marker trajectories obtained from motion analysis were reconstructed using Cortex software (Motion Analysis Corp., Santa Rosa, CA, USA) and imported into Visual3D software (C-Motion, Inc., Germantown, MD, USA). Visual3D was used to low-pass filter the marker trajectories (fourth-order Butterworth, 20 Hz cut-off), build a lower limb six degree of freedom model, and determine the sagittal knee joint angle as the relative rotation between thigh and shank segments according to a Cardan sequence of rotations (x – flexion/extension axis, y – abduction/adduction axis, z – internal/external rotation axis) (Nigg et al., 2017).

3.2.6 Co-contraction index

The normalized EMG total intensity waveforms were analyzed for three specific phases of the gait cycle that were defined according to heel strike and the sagittal plane knee angle: Pre-heel strike (150ms window before heel strike), early stance (heel strike to peak knee flexion), and mid stance (peak knee flexion to peak knee extension) (Figure 3.2). During these phases, the periarticular knee muscles fulfill different functional roles as they shorten and lengthen under different loads, which may evoke muscle co-contraction strategies that are unique to each phase (Chmielewski et al., 2005; Hurd & Snyder-Mackler, 2007).

For each phase, the level of co-contraction for a given knee extensor – knee flexor muscle pair was evaluated using the co-contraction index (CCI) according to equation 3.1 (Rudolph et al., 2000):

$$\boldsymbol{CCI = \frac{\sum_{t=1}^{n}[\frac{LowerEMG(t)}{HigherEMG(t)} \times (LowerEMG(t) + HigherEMG(t)) \times 100]}{n}}$$

Equation 3.1

where *n* represents the number of samples of the investigated gait phase, *LowerEMG(t)* is the normalized total intensity of the less active muscle and *HigherEMG(t)* the normalized total intensity of the more active muscle for a certain sample *t*. For each participant and gait phase, CCIs were calculated for co-contraction between VL-BF, VM-MH, VL-GL, and VM-GM, and averaged across seven walking trials to obtain the final CCI outcome variables. The gastrocnemius muscle pairs were included due to their moment arms about the knee joint in the frontal and sagittal planes and their potential to stabilize the joint via general co-contraction strategies (Lloyd & Buchanan, 2001; Heiden, Lloyd, & Ackland, 2009).

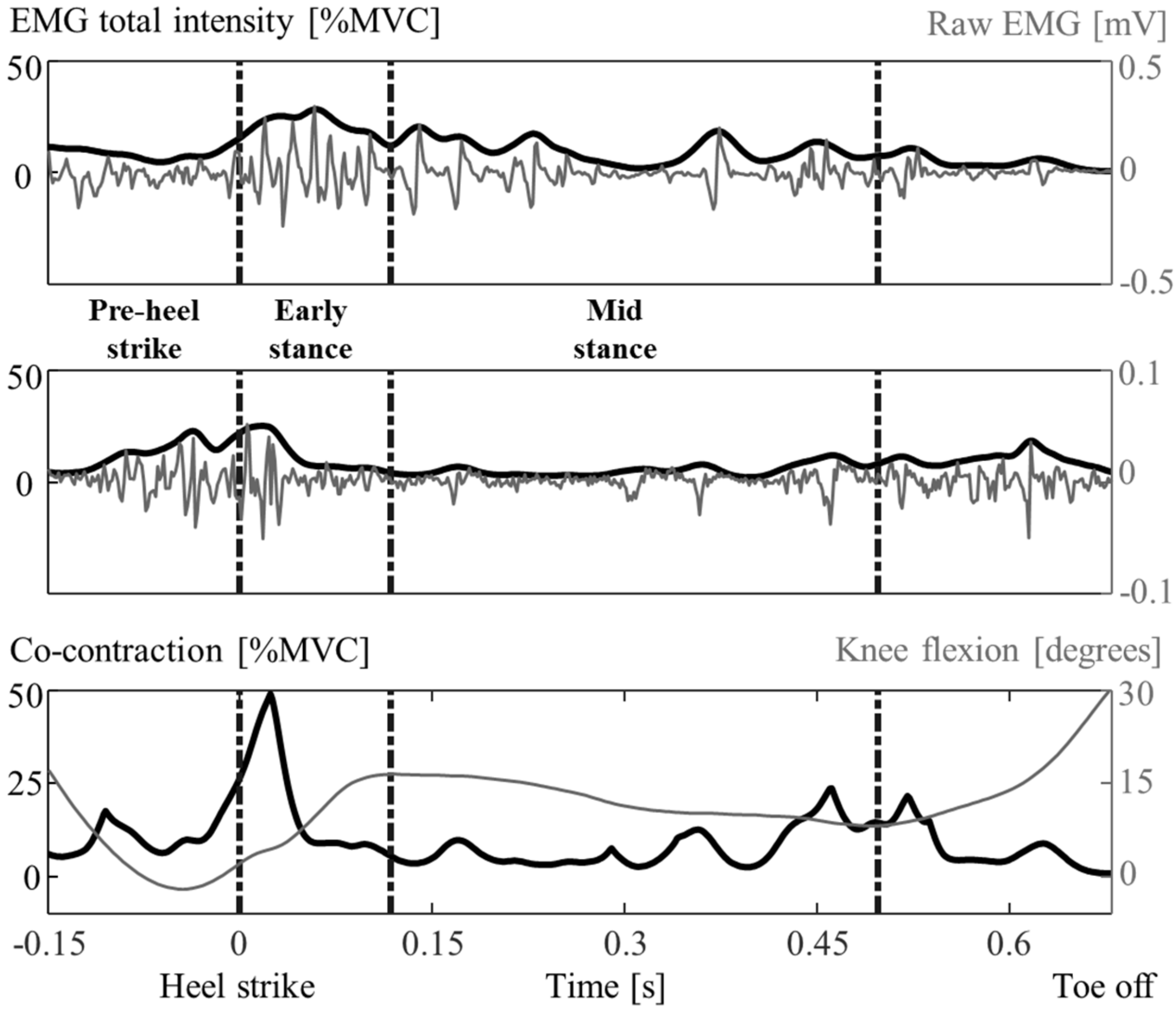

Figure 3.2. Example for the calculation of the Vastus Lateralis (VL) – Biceps Femoris (BF) co-contraction index. Raw EMG (grey) and EMG total intensity (black) as a percentage of maximum activation during MVC for the VL (top) and BF (middle) for one stance phase; resulting co-contraction waveform (black, bottom).

3.2.7 Reliability analysis

Primary data analyses were performed for the whole group of participants due to small sample size as in previous reliability studies (Juul, Langberg, Enoch, & Søgaard, 2013). In addition, given the high function and low symptomology in young adults following recovery from knee injuries (Ardern, Webster, Taylor, & Feller, 2011), a difference in the reliability of the knee muscle co-contraction index between individuals with and without a history of knee injury was not expected. Descriptive statistics included medians and ranges of CCIs across all participants during each testing session and for each of the three gait phases and four antagonist muscle pairs (12 total CCI outcomes for each session = 24 CCI outcomes). In addition, medians and ranges of peak torques were determined for knee extension, knee flexion, and ankle plantarflexion MVCs.

Absolute and relative intra-rater reliability were determined for each CCI outcome between the two sessions on day 1 (within-day reliability) and between the first sessions on day 1 and day 2 (between-day reliability). Absolute reliability was calculated according to Bland-Altman statistics including the mean ($\bar{d}$) and standard deviation (s) of the within-subject differences (measurement errors) in CCIs between two sessions and the corresponding 95% limits of agreement ($LoA = \bar{d} \pm 1.96 \times s$) (Bland & Altman, 1986). The limits of agreement are expected to contain 95% of the between-session differences and represent the random measurement error. According to Nevill & Atkinson (Nevill & Atkinson, 1997), it is common for measurement errors in sport and exercise research to exhibit heteroscedasticity, which occurs if the measurement errors are associated with the magnitude of the measurement. For this case, the limits of agreement may not accurately represent the degree of measurement error. In the presence of heteroscedastic errors, it is recommended to log-transform the measurements before calculating the systematic bias and limits of agreement and then to perform the antilog transformation of the bias and limits (Bland & Altman, 1986). The resulting dimensionless ratio limits of agreement include one ratio describing the measurement bias (bias ratio of 1 equals no bias), multiplied or divided by a second ratio that describes the level of agreement (agreement ratio of 1 equals perfect agreement). The presence of heteroscedastic errors was examined for all EMG and torque outcomes using a plot of the absolute errors against the measurement mean. If the correlation coefficient (Kendall's tau) between the two axes exceeds 0.2, it can be assumed that at least slight heteroscedasticity is present and it is beneficial to analyze ratio limits of agreement (Atkinson & Nevill, 1998; Brehm, Scholtes, Dallmeijer, Twisk, & Harlaar, 2012).

Within- and between-day relative reliability was assessed using the intra-class correlation coefficient (ICC, model 3 (two-way mixed effects, absolute agreement), type k = 7 trials) and 95% confidence intervals. Relative reliability was interpreted based on the ICC 95% confidence intervals according to the following guidelines: Values less than 0.5 indicate poor reliability, values between 0.5-0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.9 indicate excellent reliability (Portney, 2009; Koo & Li, 2016). Reliability increases with higher ICCs, smaller random error and corresponding narrower limits of agreement (Atkinson & Nevill, 1998).

The reliability of outcomes from MVC testing were analyzed in two different ways. First, the between-day reliability of the peak isometric torque was evaluated as explained above. Second, the trial-to-trial reliability of the peak EMG total intensities obtained during the first and second MVC for each muscle was analyzed as explained above for the first and second day (2 days x 6 muscles = 12 peak MVC EMG outcomes). These additional analyses provide insight into how consistently the MVCs were produced within and across days and can be used for discussion of the co-contraction reliability outcomes.

To address the second, exploratory objective of this study, the Bland-Altman plots were color coded according to injury status and visually analyzed regarding any obvious differences in the co-contraction measurement errors between previously injured and healthy individuals. A simple qualitative analysis was performed since the sample sizes of the individual groups (n=10) were not large enough to perform quantitative comparisons.

3.3 Results

Participant age, height, and weight were similar between the control and injury group (Table 3.1). Medians and ranges of KOOS subscale scores did not indicate obvious differences in knee-related pain, symptoms or function. The median KOOS score on the quality of life subscale was reduced by 16 units in previously injured compared to uninjured individuals (Table 3.1). The median time since injury in the injury group was 42 months with a range of 23 to 82 months.

Table 3.1. Participant characteristics

Variable	**No history of knee injury** *n = 10*	**Previous knee injury 2-10 years ago** *n = 10*
Sex (% female)	50	50
Age (yrs; median, range)	24.5 (21-27)	22.5 (16-31)
Height (cm; median, range)	174 (157-191)	172 (163-200)
Weight (kg; median, range)	63.6 (52-94)	67.5 (56.5-99.3)
Age at injury (years; median, range)	n/a	18.5 (13-19)
Time since injury (months; median, range)	n/a	42 (23-82)
Ligamentous injuries	n/a	9*
Additional meniscal injury	n/a	6
Isolated meniscal injury	n/a	1†
KOOS Pain (median, range)	97 (86-100)	94 (86-100)
KOOS Symptoms (median, range)	96 (86-100)	93 (68-100)
KOOS Function, daily living	100 (60-100)	100 (96-100)
KOOS Function, sports and recreational activities (median, range)	95 (80-100)	93 (80-100)
KOOS Quality of life	100 (81-100)	84 (69-100)

*4 of these were surgically treated †Surgically treated
KOOS, Knee Injury and Osteoarthritis Outcome Score

3.3.1 Heteroscedastic errors

Heteroscedastic errors were present for 34 out of 39 reliability outcomes (24 CCI outcomes, 3 peak torque outcomes, 12 peak MVC EMG outcomes). In order to enable the comparison of reliability estimates between different outcomes, ratio limits of agreement were computed for all

CCI, peak torque, and peak MVC EMG variables. After log-transformation, the number of variables that showed a correlation of more than 0.2 was successfully reduced to 10 out of 39 variables demonstrating the absence or much weaker relationship between measurement errors and the measurement mean for most variables. Figure 3.3 demonstrates two representative examples for the presence of heteroscedastic errors in the raw data (Figure 3.3 a,b,d,e) and the absence of heteroscedasticity after the log-transformation (Figure 3.3 c,f). Interestingly, heteroscedasticity was present for both the previously injured and control participants (Figure 3.3 b,e). This indicates that independent of the knee injury history, higher co-contraction indices were associated with higher measurement errors.

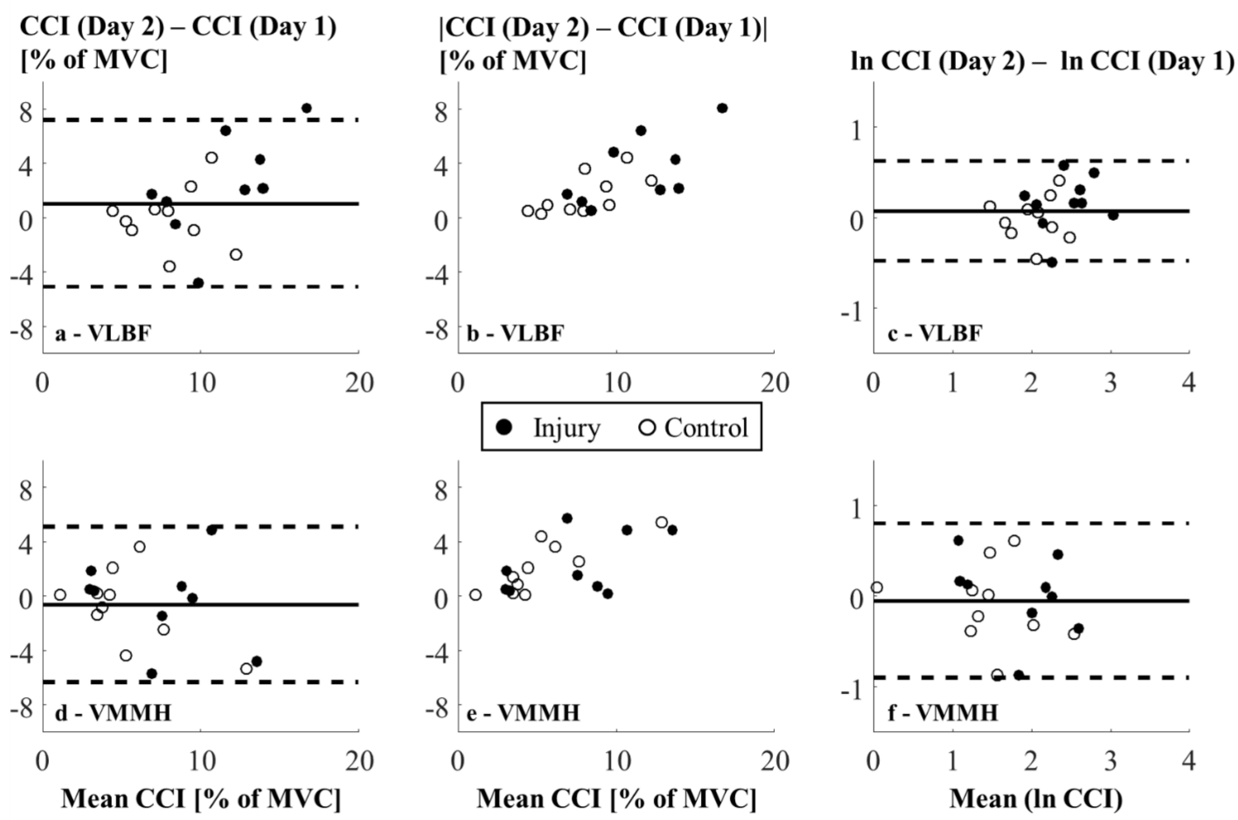

Figure 3.3. Bland-Altman plots for the example of VL-BF (a) and VM-MH CCI (d) during pre-heel strike; Absolute CCI differences vs. mean CCI (b,e); Difference of log-transformed CCIs vs. mean of log-transformed CCIs (c,f). Solid line and dashed lines correspond to mean day difference and 95% limits of agreement, respectively.

Table 3.2. Absolute and relative within-day reliability of investigated co-contraction index outcomes

Muscle pair	*Gait phase*	Descriptives: Visit 1 - Round 1* Median	Visit 1 - Round 1* Range (min, max)	Visit 1 - Round 2* Median	Visit 1 - Round 2* Range (min, max)	Within-day reliability: Absolute reliability Bias ratio (*/÷ Agreement ratio)	Relative reliability ICC	Relative reliability 95% CI
VLBF	*Pre-heel strike*	8.61	(4.16, 20.46)	8.36	(3.82, 18.21)	0.91 (*/÷ 1.37)	0.96	(0.91 0.99)
	Early stance	8.72	(1.93, 28.30)	8.44	(2.42, 26.49)	0.97 (*/÷ 1.38)	0.98	(0.96 0.99)
	Mid stance	1.85	(0.68, 12.03)	1.71	(0.64, 6.13)	0.87 (*/÷ 1.98)	0.88	(0.69 0.95)
VMMH	*Pre-heel strike*	4.34	(1.00, 15.99)	4.57	(0.65, 14.90)	0.96 (*/÷ 1.56)	0.96	(0.90 0.99)
	Early stance	7.10	(1.63, 28.44)	7.36	(1.26, 29.41)	0.99 (*/÷ 1.77)	0.97	(0.91 0.99)
	Mid stance	1.38	(0.36, 5.14)	1.68	(0.36, 5.60)	1.10 (*/÷ 1.90)	0.95	(0.87 0.98)
VLGL	*Pre-heel strike*	4.89	(0.90, 15.17)	3.90	(0.76, 14.73)	0.93 (*/÷ 1.36)	0.99	(0.98 1.00)
	Early stance	6.26	(2.19, 33.05)	6.82	(2.25, 27.26)	1.03 (*/÷ 1.63)	0.98	(0.96 0.99)
	Mid stance	3.64	(1.20, 12.43)	3.68	(0.83, 10.56)	0.97 (*/÷ 1.67)	0.96	(0.89 0.98)
VMGM	*Pre-heel strike*	2.93	(0.97, 11.68)	3.00	(0.80, 11.91)	0.94 (*/÷ 1.75)	0.98	(0.96 0.99)
	Early stance	5.30	(1.46, 15.90)	5.19	(1.48, 13.63)	1.00 (*/÷ 1.31)	0.99	(0.96 0.99)
	Mid stance	1.83	(0.30, 7.04)	2.03	(0.27, 6.29)	1.03 (*/÷ 1.59)	0.99	(0.96 0.99)

*Medians and ranges have the unit of [%MVC]

3.3.2 Within-day reliability

Table 3.2 shows the absolute and relative within-day reliability for all co-contraction outcomes. Agreement ratios ranged between the best agreement of 1.31 for VM-GM co-contraction during early stance to the poorest agreement of 1.98 for VL-BF co-contraction during mid stance. For the example of VM-GM co-contraction during early stance, the lower and upper ratio limits of agreement can be determined as the bias ratio divided and multiplied by the agreement ratio, which yields 0.76 (1.00 ÷ 1.31) to 1.31 (1.00 * 1.31). The interpretation of these limits in the original units of measurement is as follows: If the VM-GM co-contraction index during early stance of an individual is 8 %MVC during one testing session, the CCI during a second session on the same day will fall between 6.08 %MVC (8 * 0.76) and 10.48 %MVC (8 * 1.31) for 95% of the cases. In other words, the CCI during a second session may be up to 1.31 times larger or smaller compared to the first session. Any value outside of these limits would represent a true change in the co-contraction index.

The relative within-day reliability in CCIs was excellent for most gait phases and muscle pairs with lower bounds of the ICC 95% confidence intervals above 0.9. Moderate to excellent and good to excellent relative reliability was found for VL-BF, VM-MH, and VL-GL co-contraction during mid stance.

3.3.3 Between-day reliability

Table 3.3 displays the absolute and relative between-day reliability for all CCI and peak torque outcomes. The absolute reliability for CCIs obtained on two separate testing days was considerably lower compared to within-day reliability demonstrated by agreement ratios of up to 3.04 for VL-GL co-contraction during early stance.

Table 3.3. Absolute and relative between-day reliability of investigated co-contraction index and peak torque outcomes

Muscle pair	*Gait phase*	Descriptives: Visit 1 - Round 1* Median	Range (min, max)	Descriptives: Visit 2* Median	Range (min, max)	Between-day reliability: Absolute reliability Bias ratio (*/÷ Agreement ratio)	Relative reliability ICC	95% CI
VLBF	*Pre-heel strike*	8.61	(4.16, 20.46)	8.75	(4.68, 21.13)	1.08 (*/÷ 1.73)	0.85	(0.62 0.94)
	Early stance	8.72	(1.93, 28.30)	9.59	(1.98, 27.57)	1.13 (*/÷ 1.96)	0.86	(0.65 0.95)
	Mid stance	1.85	(0.68, 12.03)	1.86	(0.71, 8.36)	1.06 (*/÷ 2.13)	0.86	(0.65 0.95)
VMMH	*Pre-heel strike*	4.34	(1.00, 15.99)	4.28	(1.09, 13.11)	0.95 (*/÷ 2.34)	0.83	(0.56 0.93)
	Early stance	7.10	(1.63, 28.44)	8.82	(1.64, 41.47)	1.17 (*/÷ 2.70)	0.89	(0.71 0.96)
	Mid stance	1.38	(0.36, 5.14)	1.59	(0.58, 8.28)	1.29 (*/÷ 2.18)	0.90	(0.74 0.96)
VLGL	*Pre-heel strike*	4.89	(0.90, 15.17)	3.92	(1.21, 17.89)	1.04 (*/÷ 2.56)	0.87	(0.66 0.95)
	Early stance	6.26	(2.19, 33.05)	7.56	(2.99, 38.52)	1.09 (*/÷ 3.04)	0.82	(0.55 0.93)
	Mid stance	3.64	(1.20, 12.43)	3.77	(1.42, 12.41)	1.07 (*/÷ 2.33)	0.78	(0.43 0.91)
VMGM	*Pre-heel strike*	2.93	(0.97, 11.68)	2.80	(1.14, 15.79)	1.12 (*/÷ 2.53)	0.77	(0.40 0.91)
	Early stance	5.30	(1.46, 15.90)	6.57	(1.64, 28.50)	1.21 (*/÷ 2.20)	0.79	(0.45 0.92)
	Mid stance	1.83	(0.30, 7.04)	2.04	(0.63, 5.90)	1.19 (*/÷ 2.72)	0.74	(0.33 0.90)
Peak torque	*Extension*	203.39	(111.71, 371.00)	192.43	(103.90, 341.08)	0.96 (*/÷ 1.35)	0.96	(0.91 0.99)
	Flexion	86.45	(45.07, 144.55)	75.40	(44.22, 148.36)	0.96 (*/÷ 1.22)	0.95	(0.94 0.99)
	Plantarflexion	93.53	(40.12, 158.39)	97.92	(35.63, 157.87)	0.94 (*/÷ 1.44)	0.90	(0.74 0.96)

*Medians and ranges have the unit of [%MVC] for the co-contraction indices and the unit of [Nm] for peak torques.

This corresponds to ratio limits of agreement of 0.36 – 3.31, which means that for an individual with a CCI of 8 %MVC on day 1, any day 2 CCI above 2.88 %MVC or below 24.32 %MVC would fall within the random error.

Relative reliability was poor and moderate to excellent for all investigated co-contraction outcomes as all lower bounds of the ICC 95% confidence intervals were below 0.75. Specifically, VL-GL co-contraction during mid stance and VM-GM co-contraction during all phases, lower bounds of the confidence intervals fell below 0.5, which demonstrates that the study sample included several measurements with poor reliability.

Both knee extension and flexion torque showed acceptable absolute and excellent relative reliability with ICCs of 0.96 (0.91, 0.99) and 0.95 (0.94, 0.99). Peak plantarflexion torque exhibited lower relative and absolute reliability compared to knee extension / flexion with moderate to excellent relative reliability and agreement ratios of 1.44.

3.3.4 Peak EMG trial-to-trial reliability

The relative reliability of the peak EMG total intensities between the first and second MVC trials was excellent for all muscles except for the VL on day 2 (good to excellent) (Table 3.4). However, agreement ratios demonstrate that the peak EMG intensities between the MVC trials still varied by a factor of 1.3 to 1.5 for the thigh muscles and a factor of up to two for the medial and lateral gastrocnemius.

Table 3.4. Absolute and relative trial-to-trial reliability of peak MVC EMG total intensities on Day 1 and 2

Muscles	**Trial-to-trial reliability Day 1**						**Trial-to-trial reliability Day 2**					
	Absolute reliability		Relative reliability				Absolute reliability		Relative reliability			
	Bias ratio (*/÷ Agreement ratio)		ICC	95% CI			Bias ratio (*/÷ Agreement ratio)		ICC	95% CI		
Vastus Lateralis	0.95	(*/÷1.26)	0.99	(0.96	0.99)		0.97	(*/÷1.46)	0.93	(0.83	0.97)	
Vastus Medialis	0.95	(*/÷1.36)	0.99	(0.96	0.99)		0.94	(*/÷1.50)	0.96	(0.90	0.98)	
Biceps Femoris	0.91	(*/÷1.23)	0.99	(0.97	1.00)		1.00	(*/÷1.34)	0.98	(0.96	0.99)	
Medial Hamstrings	0.91	(*/÷1.33)	0.99	(0.97	1.00)		0.93	(*/÷1.45)	0.97	(0.91	0.99)	
Gastroc. Medialis	1.02	(*/÷1.82)	0.98	(0.95	0.99)		0.94	(*/÷1.39)	0.97	(0.93	0.99)	
Gastroc, Lateralis	1.02	(*/÷1.96)	0.98	(0.94	0.99)		0.98	(*/÷1.93)	0.99	(0.97	0.99)	

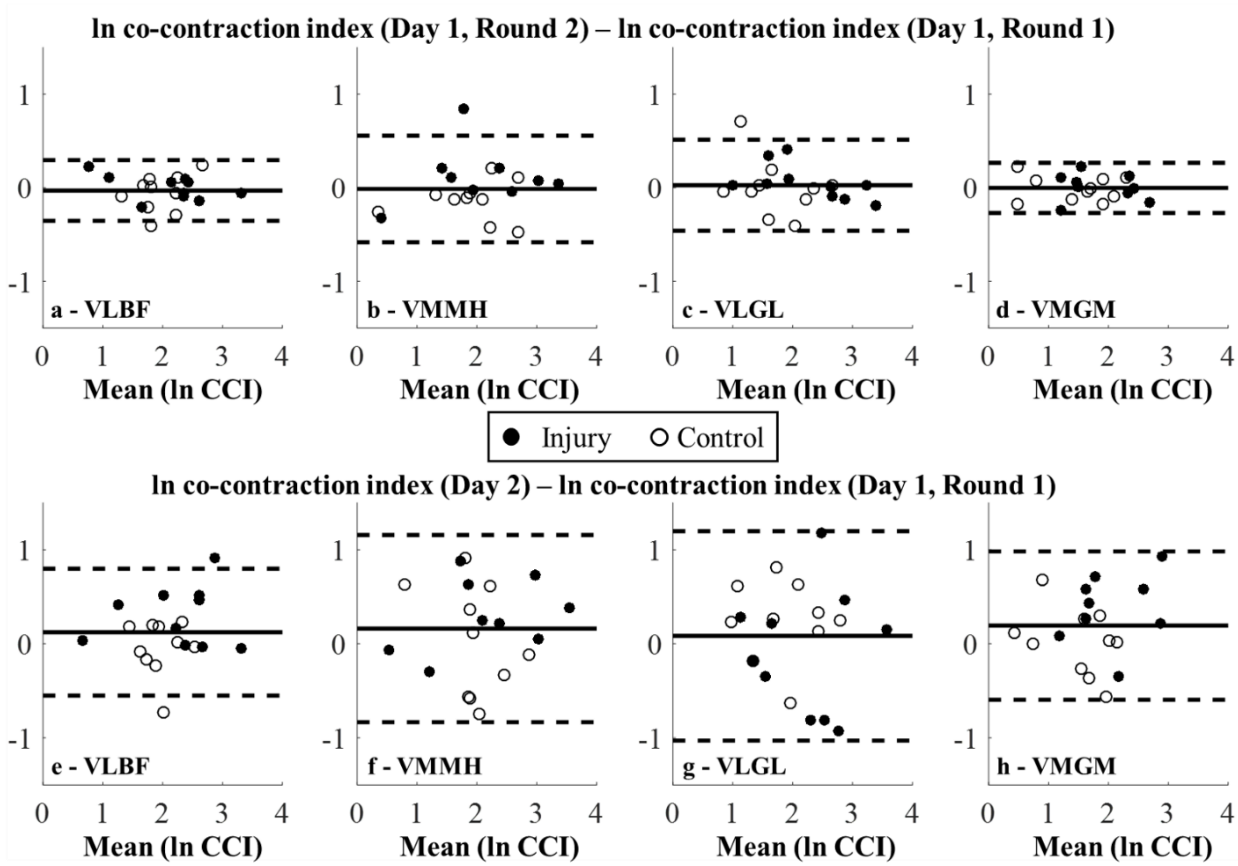

Figure 3.4. Bland-Altman plots of the difference of log-transformed CCI vs. mean of log-transformed CCI for within-day (top) and between-day (bottom) comparisons of VL-BF (a,e), VM-MH (b,f), VL-GL (c,g), and VM-GM (d,h) co-contraction during the early stance phase

3.3.5 Injury vs. control

Figure 3.4 shows the log-transformed Bland-Altman plots and ratio limits of agreement for the within-day and between-day reliability of all co-contraction indices during the early stance phase, which was representative of the other two phases. The color coding for participants from the injury or control group did not reveal any systematic patterns that would indicate a difference in the measurement errors between the groups. The Bland-Altman plots corresponding to the other two gait phases and for peak torque also showed a lack of differences in reliability between study groups. Furthermore, the visual analysis of the mean co-contraction indices on the x-axis of the Bland-Altman plots in Figure 3.3 and 3.4 shows that previously injured participants generally produced the highest co-contraction indices for most muscle pairs and gait phases compared to the control group.

3.4 Discussion

The objectives of this study were to determine the within-day and between-day reliability of the co-contraction index during different gait phases and to explore differences in reliability between individuals with and without a previous knee injury. This study's findings suggest that 1) the within-day reliability of co-contraction indices is acceptable for most analyzed CCI outcomes, 2) the between-day reliability is lower than within-day reliability and is unacceptable for most analyzed CCI outcomes, and 3) the reliability is not affected by the participant's injury history.

3.4.1 Acceptable within-day reliability

The relative within-day reliability of the CCIs was good to excellent for all investigated gait phases and muscle pairs, shown by ICCs greater than 0.9. Whether or not the absolute reliability according to the observed ratio limits of agreement for CCIs is acceptable can only be evaluated in the context of previous studies that reported changes in muscle co-contraction following an intervention. If the typically reported changes following clinically relevant interventions fall outside of the observed limits of agreement, the absolute reliability can be deemed acceptable (Bland & Altman, 1986). For example, Horsak and colleagues investigated the effect of wearing unstable shoes on CCIs during gait in young, healthy individuals with similar demographics to the control group of this study (Horsak, Heller, & Baca, 2015). Compared to stable shoes, walking in unstable shoes led to an average increase in VM-GM and VL-GM co-contraction during the first half of stance of up to 5.9 units, respectively. The ratio limits of agreement from the present study showed that for an individual with a VM-GM CCI of 8 units during early stance, subsequent measurements on the same day will fall between 6.08 – 10.48 units, indicating an error of about 2 units. Since the observed error is smaller than the reported clinically relevant change in CCIs, the within-day reliability is acceptable. However, this conclusion is specific to the VM-GM CCI during early stance and may not be generalizable to all other CCI outcomes or gait phases. The reliability of each individual CCI outcome has to be interpreted in the context of their respective clinically relevant changes.

The majority of within-day measurement errors in the co-contraction index can be attributed to natural intra-subject variability in the muscle activation patterns during walking rather than the influence of the investigator (Kadaba et al., 1985). This can be assumed because extrinsic factors influencing the EMG signal amplitude such as EMG electrode position and orientation, as well as the EMG signal normalization factor from MVC exercises did not change between the two measurements (De Luca, 1997). Consequently, the present findings may be used to inform the design and interpretation of future studies investigating the within-day effects of interventions on the CCI during gait in young adults. In general, this study suggests that the CCI should only be used for intervention studies if an increase or decrease in the co-contraction index of at least 1.5-fold is expected.

3.4.2 Poor between-day reliability

Between two sessions performed on separate days, the reliability was considerably poorer compared to the within-day results, which corroborates early results for signal amplitudes of individual muscles during gait (Kadaba et al., 1985, 1989). All investigated CCI outcomes showed large random errors with agreement ratios of up to 3, corresponding to expected errors of up to 300% between two days. In light of the typically reported changes of CCIs described above, such large random errors are not acceptable and suggest that the CCI should not be used as a measurement for co-contraction between repeated testing sessions on different days. To the best knowledge of the authors, Murley and colleagues were the only investigators to include Bland-Altman statistics when determining the reliability of surface EMG outcomes. Similar to this study, they found that the random error between testing days in EMG peak amplitudes of tibialis anterior and medial gastrocnemius during gait may be up to 240%, deeming these variables unusable for repeated measurements on separate days. The relative between-day reliability of the co-contraction indices (ICCs between 0.74 – 0.9) was similar to the results reported by Hubley-Kozey and colleagues (2013). However, the relative reliability of *Quadriceps – Gastrocnemius* co-contraction indices was generally lower compared to *Quadriceps – Hamstrings* muscle pairs. A similar result was found for the between-day reliability of the peak torque and the trial-to-trial reliability of the peak EMG intensities during the MVCs where the plantarflexion torque and the medial / lateral gastrocnemius EMG intensities exhibited the lowest absolute reliability compared to the other exercises and muscles. This is in agreement with previous research showing lower reliability for plantarflexion compared to knee extension exercises, which may be related to 1) the more complex, bi-articular musculature producing plantarflexion and 2) the higher difficulty for individuals to maximally activate the plantar flexors while minimizing force contributions from other leg muscles (Sleivert & Wenger, 1994).

While the between-day measurement error in the co-contraction index can also be partially explained by the intra-subject variability in the execution of the walking task, the higher agreement ratios compared to the within-day results suggest that additional error sources are present when performing measurements on different days. The additional error likely arises from differences in the execution of the MVCs leading to variability in the normalization factors as well as from deviations in electrode placement (Kadaba et al., 1985; Mathur, Eng, & MacIntyre, 2005). Despite the isometric MVC being recommended as the most appropriate EMG normalization procedure (Knutson, Soderberg, Ballantyne, & Clarke, 1994; Burden, 2010), there is conflicting evidence for the degree of reliability of peak EMG amplitudes recorded from leg muscles during isometric MVCs (Yang & Winter, 1983; Knutson et al., 1994; Pincivero, Green, Mark, & Campy, 2000; Burden, 2010). Although the present study shows good to excellent between-day reliability for peak isometric torque, the maximum EMG signal amplitude obtained from each individual muscle can still vary substantially as shown by measurement errors of up to 200% between the repeated MVC trials. This indicates that individuals may have used inconsistent muscle activation patterns to produce similar peak torques, e.g. varying number and firing patterns of recruited motor units or varying synergistic muscle contributions (Pincivero et al., 2000; Mathur et al., 2005). Furthermore, the lowest trial-to-trial reliability in peak EMG amplitudes of the medial and lateral gastrocnemius coincided with the lowest reliability for plantarflexion torque and co-contraction indices between muscle pairs involving the gastrocnemius muscles (VL-GL, VM-GM), suggesting a propagation of error from the execution of MVC exercises to the co-contraction index.

The second potential source of error, electrode position, was minimized by using standardized recommended procedures for surface EMG measurements (Hermens et al., 1999). Therefore, the influence of the investigator on the determined between-day measurement errors in the present study is likely minimal and it is assumed that the present findings should be reproducible by other investigators (raters) who follow the same procedures. However, this study did not determine the reliability of the CCI between multiple raters. Therefore, future studies of the CCI during gait that include multiple measurements with varying raters have to additionally determine the inter-rater reliability of the CCI outcomes before interpreting changes in CCIs over time.

3.4.3 Effect of injury history

The exploratory evaluation of the log-transformed Bland-Altman plots did not suggest a difference in reliability between individuals with and without a history of knee injury. Consequently, the observed between-day and within-day measurement error in co-contraction indices can be considered non-differential and therefore, knee injury and control groups may be treated similarly when interpreting co-contraction indices. Nevertheless, non-differential measurement error and low reliability may reduce the accuracy of diagnostic tests and bias studies towards detecting no differences between groups (Portney, 2009). Therefore, future investigations of group differences in co-contraction indices should ensure high enough sample sizes to achieve sufficient statistical power and compensate for the large non-differential measurement error. The result of similar reliability between study groups corroborates an earlier report showing that the between-day reliability of a one legged hop task is not affected by a previous knee injury (Paterno & Greenberger, 1996). This is likely due to the fact that individuals 2-10 years following a knee injury are highly functional and only show few knee-related symptoms and pain as demonstrated by high KOOS scores in this and other studies (Whittaker et al., 2015, 2018). The KOOS knee-related quality of life subscale was the only scale that indicated a possible reduction in scores in previously injured compared to control participants. While this study was not designed to investigate this reduction in detail, a lower quality of life following a previous injury has been reported frequently in the literature (Frobell et al., 2013; Whittaker et al., 2018).

Despite a lack of difference in reliability, it is interesting to note that for both previously injured and control participants, almost all muscle pairs exhibited a positive relationship between the magnitude of the co-contraction index and the measurement error, i.e. heteroscedasticity. Previous research has demonstrated that co-contraction indices in individuals with ACL-deficiency, previous ACL-reconstruction as well as previous partial meniscectomy produce higher knee muscle co-contraction compared to healthy controls (Hurd & Snyder-Mackler, 2007; Sturnieks et al., 2011; Hall et al., 2015). The Bland-Altman plots for VL-BF and VL-GL co-contraction during the pre-heel strike and early stance phase in this study support this observation (see Figures 3.3 and 3.4). Consequently, in absolute terms a previously injured group may show a larger error in the co-contraction index simply due to the increased measurement error at higher levels of co-contraction.

3.4.4 Limitations

Previous authors have recommended to use a sample size of n = 30 or more when investigating reliability (Morrow Jr & Jackson, 1993; Koo & Li, 2016). The limitation of the smaller sample size of n = 20 used in this study is that the uncertainty of the reliability estimates increases. Therefore, the ICCs presented in this study should always be interpreted in the context of their 95% confidence interval. Furthermore, the smaller sample size and sample heterogeneity did not allow to perform statistical comparisons of reliability estimates or absolute co-contraction indices between previously injured and healthy individuals. The comparison of reliability between these two groups was considered exploratory and the log-transformed measurement errors and means were only compared visually using the Bland-Altman plots. The observation that a previous knee injury 3-10 years ago does not affect the log-transformed measurement errors has to be confirmed in future studies with a larger sample size and a more homogeneous injury group.

3.5 Conclusions

The frequently studied knee extensor-flexor co-contraction index during different gait phases shows acceptable within-day reliability but poor between-day reliability in young adults with and without a history of knee injury. While there was no apparent difference in the reliability of co-contraction indices between individuals with and without previous knee injuries, absolute measurement errors generally increase for higher co-contraction indices. Consequently, the authors recommend that the co-contraction index may be used to investigate within-day changes in the co-contraction index in response to interventions if electrode placement and EMG amplitude normalization value are kept constant. Repeated measurements of the co-contraction index on separate days should be avoided and group comparisons may only be performed if the sample sizes are large enough to compensate for low reliability.

Chapter Four – **Intermuscular coherence between surface EMG signals is higher for monopolar compared to bipolar electrode configurations**

4.1 Introduction

The vasti muscles have to work in concert to control knee joint motion and maintain balance of the body during movements such as walking, running, and squatting. Coherence analysis between surface EMG signals from synergistic muscles is a common technique to study intermuscular synchronization and gain insight into strategies of the central nervous system to control the execution of such motor tasks (Farmer, Bremner, Halliday, Rosenberg, & Stephens, 1993; Semmler, 2002). Specifically, previous researchers have used EMG-EMG coherence analyses to elucidate the functional role of intermuscular synchronization, e.g. by investigating its task-dependent property for different motor tasks (Gibbs, Harrison, & Stephens, 1995; Huesler, Hepp-Reymond, & Dietz, 1998; Kilner et al., 1999; Asseldonk, Campfens, Verwer, Putten, & Stegeman, 2014; von Tscharner, Maurer, & Nigg, 2014; Mohr, Nann, von Tscharner, Eskofier, & Nigg, 2015; Reyes, Laine, Kutch, & Valero-Cuevas, 2017) or changes in coherence during fatiguing exercises (Boonstra et al., 2008; Kattla & Lowery, 2010; Chang et al., 2012; Clark, Kautz, Bauer, Chen, & Christou, 2013; McManus, Hu, Rymer, Suresh, & Lowery, 2016). These studies suggest that the neuromuscular system adjusts the degree of intermuscular synchronization based on the physical and possibly psychological demands of the movement task. However, some disagreement exists regarding the direction of change in intermuscular synchronization between different movement tasks. For example, higher and lower coherence has been reported to be necessary for balancing movements, which require individual muscle control compared to movements that are stable and require synergistic muscle control (Gibbs et al., 1995; Mohr et al., 2015; Reyes et al., 2017).

When comparing the observed EMG-EMG coherence between multiple studies, it is obvious that the magnitude of coherence as well as the frequency bands where coherence is present can be vastly different. Conceptually, there are three reasons for why previous studies show a large variation in coherence outcomes: First, different EMG recording systems were used (e.g. monopolar vs. bipolar EMG), second, different EMG signal processing techniques were applied, and/or third, the investigated motor tasks and involved muscles were governed by different neuromuscular control strategies leading to different levels of intermuscular synchronization. While many discrepancies between studies can likely be explained by the second and/or third aspect, some studies show considerable differences in coherence despite using the same analysis approaches and despite investigating the same muscles during similar tasks. Therefore, this study will address the first aspect – the influence of the EMG recording system on intermuscular coherence.

For example, Chang and coauthors (2012) showed that the intermuscular coherence between the vastus medialis and lateralis during a single-leg step-up task is generally lower than 0.5 across frequencies and muscle pairs. In contrast, Mohr and colleagues (2015) reported EMG-EMG coherence between the vasti muscles during a single-leg squat of generally higher than 0.5 and for a wider range of frequencies up to 80-100 Hz. The major difference between these studies is the use of bipolar and monopolar EMG recording systems, respectively. The rationale for the use of a monopolar over a bipolar EMG amplifier is twofold: First, monopolar EMG avoids the inherent limitation of bipolar EMG systems that the bipolar electrodes must be aligned with the muscle fiber direction. Second, due to differential amplification, bipolar EMG leads to a higher spatial selectivity while monopolar surface EMG provides a more ‘global’ view on the activity of a muscle (De Luca & Merletti, 1988). Although high spatial selectivity of bipolar EMG may be beneficial when trying to investigate the behaviour of individual motor units (Reucher, Rau, & Silny, 1987), global information on the activity of two muscles may be desired when investigating intermuscular synchronization at a whole muscle level.

The underlying concept of the bipolar technique is to detect the same motor unit action potentials twice but spatially shifted along the muscle. Then, differential amplification of these two signals leads to a reduction of noise that is common to both electrodes while the signal of interest is retained, i.e. the differential of the summed motor unit action potentials (Basmajian, 1985). This concept relies on the assumption that bipolar electrodes can in fact be aligned with the muscle fiber direction. However, most muscles fibers are oriented at a three-dimensional pennation angle with respect to the aponeurosis and the skin surface, which may change as a function of joint position and muscle force (Wickiewicz, Roy, Powell, & Edgerton, 1983; Friederich & Brand, 1990; Rutherford & Jones, 1992; Merletti et al., 2001; Rainoldi, Bullock-Saxton, Cavarretta, & Hogan, 2001). Even if the investigator can achieve a good alignment of the electrodes before the measurement, the assumption that the bipolar electrodes remain aligned with the muscle fiber direction during movements that involve muscle length and force changes does not hold. Bipolar electrode alignment error can alter the amplitude and frequency content of the differential EMG signal in an unknown and unpredictable way (von Tscharner, 2014), which may reduce the ability of this technology to detect intra- and intermuscular coherence.

In contrast, monopolar EMG measurements do not require electrode alignment, represent the entire information about motor unit activity near the measurement point and may thus be more suitable to study intermuscular synchronization. Accordingly, monopolar EMG has been successfully applied to resolve the task-dependent property of intermuscular synchronization between isometric and dynamic squats with a high sensitivity (Mohr et al., 2015). To the best knowledge of the authors, the effect of monopolar vs. bipolar EMG measurements on the analysis of EMG-EMG coherence is currently unknown. Based on the above argument, however, it is speculated that the disruption of information in bipolar EMG recordings may lead to a lower resolution to detect high EMG-EMG coherence between muscles and explain the discrepancies between previous studies. Despite these possible advantages of monopolar surface EMG, the technique is not commonly used in biomechanical and neuromuscular investigations. This is due to the susceptibility of monopolar surface EMG to noise from stray-potentials, movement artefacts, and possibly cross-talk due to low spatial selectivity (De Luca & Merletti, 1988; von Tscharner, Maurer, Ruf, & Nigg, 2013), which may compromise the reliability of a monopolar system.

In addition to using a monopolar electrode configuration, Mohr and colleagues obtained EMG signals via a recently developed current amplifier in contrast to the classic EMG potential amplifier (von Tscharner et al., 2013). The main difference is that the current amplifier injects or withdraws charges at the skin surface above the active muscle to keep all measurement points at ground potential while the potential amplifier relies on a potential at the skin surface with respect to the ground electrodes. The concept of the current amplifier has the advantage that inter-electrode currents are avoided, which enables EMG measurements during conditions when the inter-electrode impedance is largely reduced, e.g. when sweat builds up on the skin or even during extreme conditions such as during underwater measurements (Whitting & von Tscharner, 2014). Furthermore, current measurements may be more sensitive to the EMG signals at higher frequencies compared to measurements from potential amplifiers and may therefore be more suitable to explore coordinated motor unit activity at frequencies beyond the typically investigated beta and gamma bands (> 60 Hz) (von Tscharner et al., 2013).

In summary, there are well-founded arguments for the use of monopolar amplifiers and current measurements instead of the traditional bipolar potential measurements when investigating EMG-EMG coherence. However, the effect of monopolar vs. bipolar electrode configurations and potential vs. current EMG recording techniques on the magnitude and frequency of intermuscular coherence has not been systematically investigated. Similarly, it is unknown whether one of these EMG recording techniques can more reliably detect intermuscular coherence or if one is more sensitive to detecting a change in intermuscular coherence between different movement tasks.

Therefore, the first objective of this study was to compare intermuscular coherence of vastus lateralis and medialis surface EMG signals during a dynamic, bipedal squatting task between three different EMG recording techniques: Bipolar potentials, monopolar potentials, and monopolar currents.

The second objective was to compare these three techniques regarding their reliability when repeatedly assessing a stable squatting task and their sensitivity to detecting a change in intermuscular coherence between squatting on a stable vs unstable surface.

It was hypothesized that:

1) VL-VM intermuscular coherence would be higher for monopolar EMG signals compared to bipolar signals, and

2) All three recording techniques would be sensing a lower VL-VM intermuscular coherence during unstable compared to stable squatting although monopolar systems would show a reduced reliability between similar squatting trials.

4.2 Materials and Methods

4.2.1 Participants

Eighteen healthy, male (n=14) and female (n=4) participants (mean ± SD; age 26 ± 5 y; height 175 ± 6 cm; mass 69 ± 7 kg) volunteered to participate in this study. This study was carried out in accordance with the guidelines of the University of Calgary's Conjoint Health Research Ethics Board. The protocol was approved by the University of Calgary's Conjoint Health Research Ethics Board (#REB17-0210). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

4.2.2 Study design

Each participant completed a total of six squatting trials, three trials were recorded with the monopolar current amplifier system and three trials were recorded with the monopolar potential amplifier system (Table 4.1). The bipolar potential signals were computed from the monopolar signals following data acquisition (see section 'EMG potential measurements'). The order of recording systems was balanced randomized. The order of trials was kept constant and consisted of two trials of squatting on a stable surface and one trial of squatting on an unstable surface. The protocol included two trials of stable squatting to determine the between-trial reliability of intermuscular coherence and one trial of unstable squatting to determine the sensitivity of the three systems.

Table 4.1. Design of experimental procedures

Amplifier	Configuration	Trial 1	Trial 2	Trial 3
Potential	Monopolar Bipolar	Stable	Stable	Unstable
Current	Monopolar	Stable	Stable	Unstable

4.2.3 Squatting tasks

During each trial, participants performed a series of squats down to a knee flexion angle of 70 degrees (0 degrees represents full extension) for a duration of 90 seconds. The distance between the participants' feet was self-selected and kept constant throughout all trials but had to be at least shoulder wide apart (Figure 4.1). Stable squatting trials were performed on the laboratory floor while unstable squatting trials were completed on the flat side of a BOSU ball (Figure 4.1). For all trials, the squatting speed was set to 20 squats per minute and controlled for by using a metronome at 40 bpm yielding a total of 30 squats per trial that were used for data analysis. In order to ensure consistent knee flexion angles at the lowest squat position, participants were given visual real-time feedback from a one-dimensional electro-goniometer (Biometrics Ltd., UK) taped across the anterior side of their knee joint. Each participant was given one initial practice trial to familiarize with the equipment and squatting speed. For each following trial, the EMG recording system was started once the participant had found the correct squatting rhythm.

4.2.4 EMG data acquisition

EMG electrode placement

In order to obtain surface EMG signals from VM and VL, the skin surface above the muscles was shaved, slightly abraded with sand paper and cleaned with alcohol wipes to ensure high signal conductivity. Two Ag-AgCl electrodes (10 mm diameter, 20 mm inter-electrode distance, Norotrode Myotronics-Noromed Inc., US) in a bipolar configuration were placed over the muscle bellies of VM and VL using the following procedure. First, the electrode positions and orientations on VM and VL were located and marked according to EMG sensor locations described in SENIAM guidelines (Hermens, Freriks, & Merletti, 1999). Next, an ultrasound machine was used to verify that the marked electrode locations were within the proximal-distal and medio-lateral boundaries of the muscles while the participants where performing a static squat at 45 degrees of knee flexion.

EMG recording systems

EMG recordings of each muscle were obtained using two separate recording systems with separate ground electrodes, data acquisition cards (12-bit A/D converter, National Instruments, Austin, TX), and battery powered laptops. Thus, the systems consisted of two electronically separated circuits to avoid hardware-based crosstalk (Mohr et al., 2015). In system 1, EMG signals of VL were recorded with reference to two ground electrodes placed side by side on the right anterior superior iliac spine. In system 2, EMG signals of VM were recorded with reference to two ground electrodes placed on the medial and lateral malleoli (Figure 4.1). Two ground electrodes were used in each system to improve the stability of the ground potential and to further reduce the resistivity to the returning currents. Each electrode was connected to an extension lead and then fixed in place using adhesive stretch tape. This step was necessary to ensure that the electrode-skin connection was kept constant throughout the protocol when switching between the current and potential measurements. The two recording systems of VL and VM were synchronized using a custom-built device that simultaneously transmitted a pulse to both systems upon pressing a button at the beginning and end of each measurement.

EMG potential measurements

Two monopolar EMG potentials were recorded from each muscle using a total of four differential amplifiers at a sampling frequency of 2400 Hz with a hardware-based bandpass filter between 10-500 Hz (Biovision, Wehrheim, Germany). The positive input of the amplifiers was connected to one of the two electrodes placed on each muscle and the negative input was connected to the respective ground.

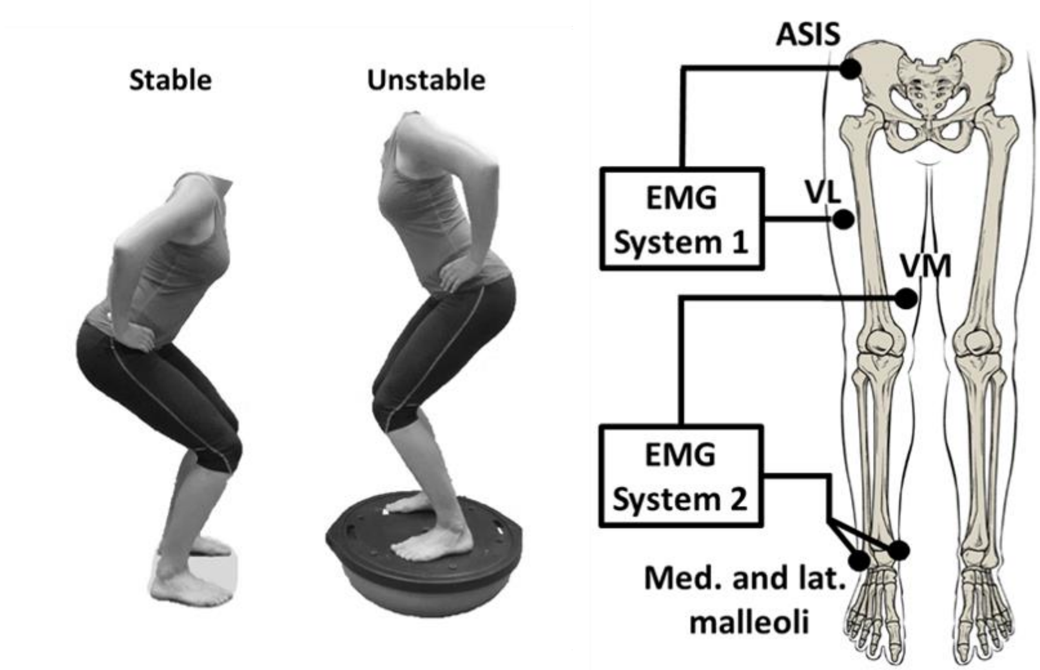

Figure 4.1. Stable vs. unstable squat (left); conceptual EMG recording set-up (right).

In this configuration, one can use a differential amplifier to record monopolar EMG potentials. Bipolar EMG potentials for VM and VL were computed following data acquisition by calculating the difference between the two monopolar EMG potentials obtained from each muscle (proximal – distal electrode). This approach was selected to compare intermuscular coherence between monopolar and bipolar EMG potential measurements that were obtained from the same squatting trial. A pilot experiment was conducted where bipolar EMG potentials directly recorded from the VL with a single differential amplifier were compared with the computed bipolar EMG potentials as explained above. The power spectra of a 60 second isometric squat were virtually identical between the two methods, thus verifying the validity of the approach.

EMG current measurements

Monopolar EMG currents were recorded from the proximal electrode on each muscle using a previously described and validated current amplifier at a sampling frequency of 2400 Hz and a hardware-based bandpass filter between 10-500 Hz (von Tscharner et al., 2013; Mohr et al., 2015).

4.2.5 EMG signal analysis

Filtering

Goniometer data were low-pass filtered (cut-off frequency of 1 Hz) using a wavelet-based filter method. The 60 Hz line-frequency contamination was removed from all monopolar EMG signals by applying a line-frequency averaging method and a line filter. In short, this procedure allows to subtract the average line-frequency contamination from the EMG signal without inducing a notch in the EMG power spectrum at 60 Hz (see von Tscharner et al., 2013) for further details). Removing the line-frequency from the signals avoided an artificial intermuscular coherence at 60Hz. The lowest frequency that was considered for this analysis was 10 Hz, which is given by the 10-500 Hz bandpass filter of the EMG amplifiers and by the notion that the power density function of the surface EMG signal has negligible contributions below 10 Hz (Merletti, 1999).

Sequencing

For each squatting trial, the signals were separated into 30 sequences of 4096 samples (1.7 s) according to peaks in the goniometer signal that represented the time points of highest knee flexion, i.e. the deepest positions during the squats. While these sequences contained the majority of the EMG power during the squats (Figure 4.2), the exact sequence size facilitated using a Fast Fourier Transform (FFT) during the analysis.

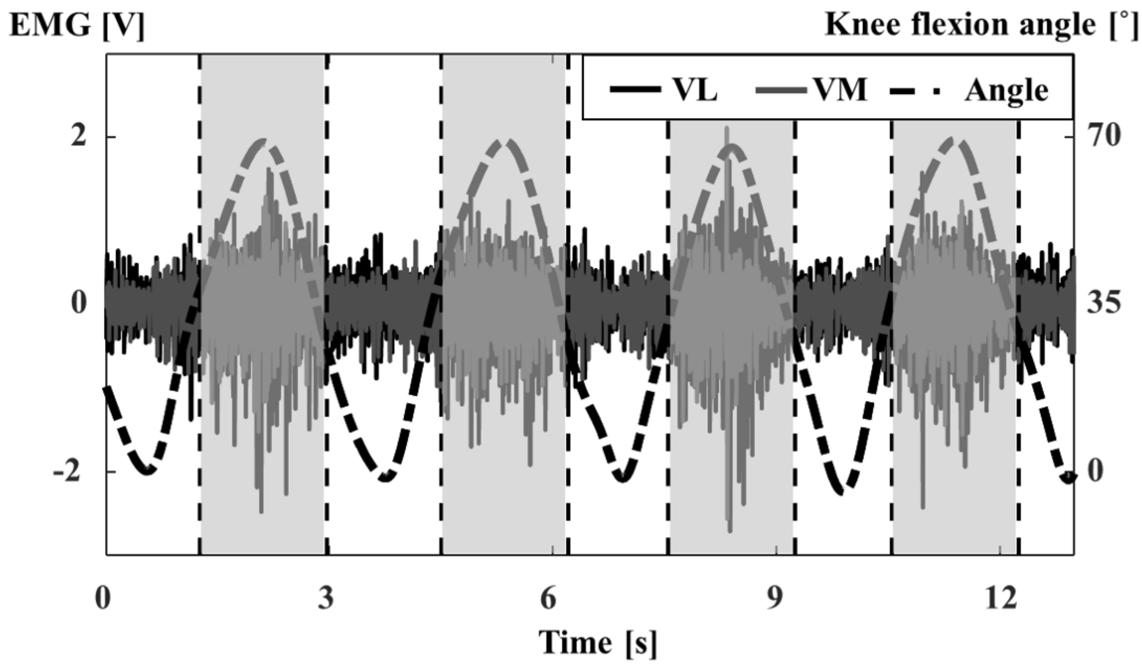

Figure 4.2. Procedure to separate individual data sequences according to peaks in knee flexion angle. VL (black, solid line) and VM (grey, solid line) filtered EMG signals recorded with the monopolar potential system and corresponding knee flexion angle (black, dash-dot line). Dashed vertical lines to the left and right of peaks in the knee flexion angle trace indicate the boundaries of individual data sequences (shaded) that were used for further analysis.

4.2.6 Power and coherence

The FFT of the unrectified EMG signals was computed for each data sequence, leading to a frequency resolution of 0.6 Hz. The power spectra for each muscle and trial were determined by multiplying the FFT of each sequence with its complex conjugate and averaging across all data sequences. Intermuscular coherence as a function of frequency λ (coherence spectrum) between VL and VM EMG signals for one given squatting trial was computed from the average cross-spectra normalized by the corresponding power spectra across s = 30 data sequences (Rosenberg et al., 1989):

$$\boldsymbol{coherence}(\boldsymbol{\lambda}) = \frac{\left|\overline{\boldsymbol{F}_{\boldsymbol{VL}_s}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VM}_s}(\boldsymbol{\lambda})^*}\right|^2}{\overline{(\boldsymbol{F}_{\boldsymbol{VL}_s}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VL}_s}(\boldsymbol{\lambda})^*)} \cdot \overline{(\boldsymbol{F}_{\boldsymbol{VM}_s}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VM}_s}(\boldsymbol{\lambda})^*)}}$$

Equation 4.1

For each trial and participant, the average coherence was computed as the mean of the coherence spectrum between 10-60 Hz. The frequency range of 10-60 Hz was chosen since the coherence in this range was highest across all trials and participants and since it spans frequencies in the beta (15-30 Hz) and gamma (30-60 Hz) bands, at which intermuscular coherence is typically reported in the literature (Clark et al., 2013; De Marchis, Severini, Castronovo, Schmid, & Conforto, 2015; Pizzamiglio, De Lillo, Naeem, Abdalla, & Turner, 2017).

To assess the possible influence of cross-talk between the vasti muscles on the level of intermuscular coherence measured with different recording systems, a simple simulation was performed. From previous studies it was estimated that in the monopolar electrode configuration, there may be an additional 10% of cross-talk compared to the bipolar configuration due to the absence of spatial filtering (Reucher et al., 1987; Farina, Merletti, Indino, Nazzaro, & Pozzo, 2002). Therefore, a pair of simulated monopolar VL and VM signals was computed from the respective bipolar EMG signals by adding the VL signal multiplied by a factor of 0.1 to the VM signal and vice versa. The coherence analysis as described above was then repeated for these computed signals with simulated cross-talk.

4.2.7 EMG Intensity

In order to determine whether the level of VL and VM muscle excitation changed between the stable and unstable squatting condition, the overall EMG intensity was determined using a wavelet transform. In short, a filter bank of 30 non-linearly scaled wavelets specifically designed for EMG analysis was used to decompose the raw EMG signals into the time-dependent power in each of the frequency bands (wavelets) (von Tscharner, 2000). For this analysis, powers from twenty wavelets with center frequencies between 10-300 Hz were summed to derive the total power. The square root of the total power yields the total EMG intensity, a close approximation of the frequently used EMG root mean square (von Tscharner, 2000). The overall EMG intensity of VL and VM representing the average level of muscle excitation was calculated as the sum of the total EMG intensity for each individual squat. For each recording system separately, the overall EMG intensities were normalized to the maximum overall EMG intensity obtained across all 90 squats (3 trials of 30 squats). Finally, the normalized EMG intensities were averaged across the 30 squats for each trial to derive one normalized, mean overall EMG intensity for each trial, system, muscle and participant.

4.2.8 Statistical analysis

For each trial (stable 1, stable 2, unstable) and recording technique (bipolar potential, monopolar potential, monopolar current), the mean and standard deviation of the power and coherence spectra, average coherence values, and normalized overall EMG intensities were computed across 16 participants. In addition, the mean and standard deviation of the average coherence values for the simulated monopolar signals were determined to investigate a possible influence of cross-talk. Two male participants had to be excluded from the analysis as they were not able to perform the unstable squatting trials without help from the investigator. A two-way repeated measures ANOVA with the within-subject factors ‘trial’ and ‘recording technique’ was performed to detect significant main and interaction effects on the average coherence. Mauchly’s test of sphericity was used to test the assumption of sphericity. If the assumption of sphericity was violated, the Huynh-Feldt correction was used and reported. Bonferroni-corrected post-hoc tests were carried out to determine pairwise comparisons of coherence between individual trials and recording techniques. To investigate a possible effect of squatting technique on the level of muscle excitation, separate two-way repeated measures ANOVAs with the within-subject factors ‘trial’ and ‘recording technique’ were performed for the overall EMG intensities of the two muscles VL and VM. All statistical tests were carried out at a significance level of 0.05 using IBM SPSS statistics (v. 24; SPSS Inc., Chicago, IL).

The reliability of the average VL-VM coherence was determined for each recording technique using the first and second stable squatting trial. Relative reliability was computed using the intra-class correlation coefficient (ICC, model 3 (two-way mixed effects, absolute agreement), type 1) and the corresponding 95% confidence intervals (Shrout & Fleiss, 1979; McGraw & Wong, 1996; Koo & Li, 2016). Absolute reliability was determined using the standard error of measurement (SEM) according to equation 4.2:

$$\boldsymbol{SEM = \sqrt{MS_E}}$$

Equation 4.2

where MS_E is the error term obtained from the ANOVA table of the ICC calculations (Eliasziw, Young, Woodbury, & Fryday-Field, 1994). The SEM represents the random error of the obtained scores in comparison to the ‘true’ scores in the original units of measurement with the assumption that there is no systematic bias between the measurements. To decide whether an observed change in the obtained scores can be considered ‘true’ change, the SEM can be used to derive the minimal detectable change (MDC), according to equation 4.3:

$$\boldsymbol{MDC = 1.96 \cdot \sqrt{2} \cdot SEM}$$

Equation 4.3

The sensitivity of the three recording systems to a change in the average VL-VM coherence when changing from the stable to the unstable squatting condition was assessed according to Cohen's *d* as a measure of effect size. Specifically, the effect size for each system was determined as the mean of the differences between the two conditions (unstable – stable) divided by the standard deviation of the differences. Values for Cohen's *d* of greater than 0.8 represent large effects (Cohen, 1992).

4.3 Results

4.3.1 Mean power and coherence spectra

Figure 4.3 displays the average power spectra and coherence spectra for all recording techniques and the stable and unstable squatting condition. The average VL-VM coherence spectra are clearly reduced when obtained from bipolar compared to monopolar recordings (Figure 4.3, G-I). Although the coherence spectra of monopolar potentials and currents generally show a similar shape, the spectra obtained from monopolar currents demonstrate a higher coherence for frequencies above 80 Hz. In addition, it can be observed that the coherence during unstable squatting is larger compared to stable squatting, particularly for frequencies below 40 Hz.

While the power spectra for VL and VM show a similar pattern, the spectra show a different shape when comparing the bipolar and monopolar recording techniques. Specifically, for monopolar recordings the power spectra demonstrate a pronounced peak in the frequency range of 30-50 Hz, which is much less visible in spectra obtained from bipolar recordings. It is also within this frequency band, that a high coherence was observed in the coherence spectra. Similarly, the magnitude of this 30-50 Hz peak is reduced during the unstable compared to the stable squatting condition (Figure 4.3, A-F).

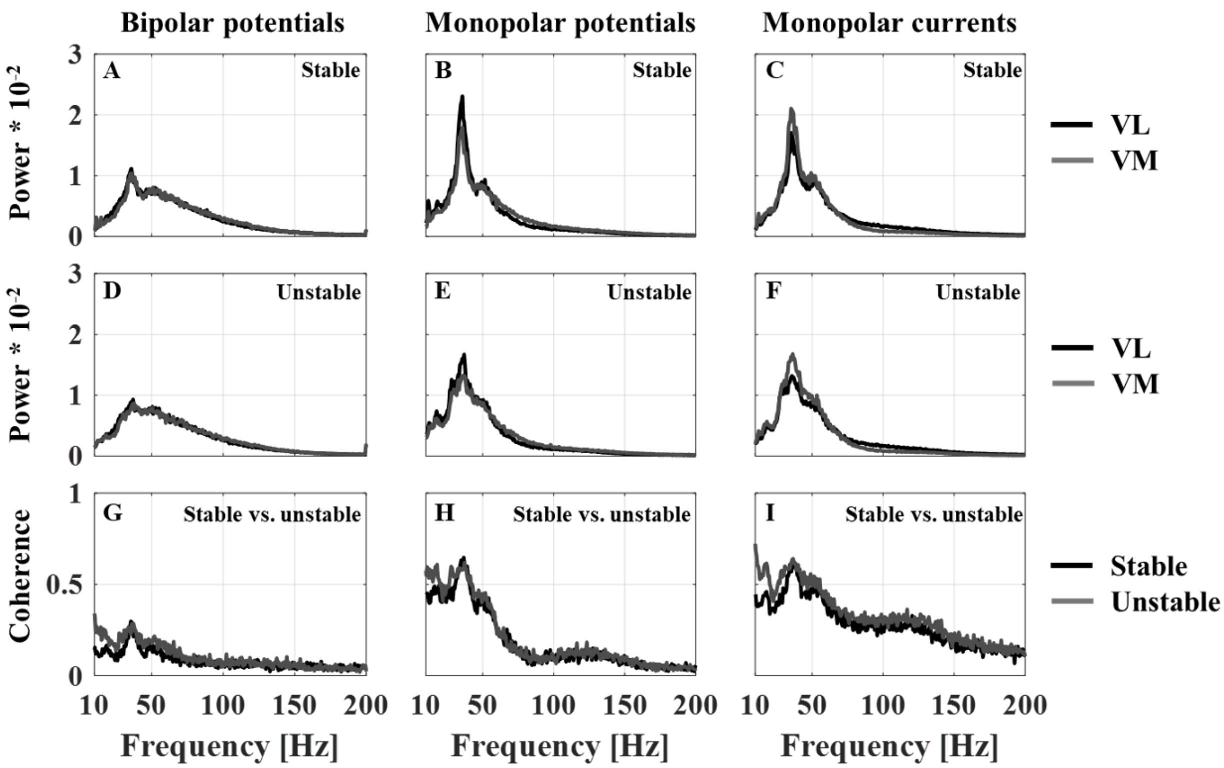

Figure 4.3. Average of the normalized power spectra across all subjects (n = 16) for vastus lateralis and medialis for each recording technique (BP – bipolar potentials, MP – monopolar potentials, MC – monopolar currents) for the stable squat (A,B,C) and unstable squat (D,E,F); Average of the coherence spectra across all subjects (n = 16) between vastus lateralis and medialis for each recording technique and squatting condition (G,H,I).

4.3.2 Average coherence

There were significant main effects of 'trial' (F(1.44,21.59) = 18.24, p<0.001) and 'recording technique' (F(3,45) = 61.3, p<0.001) on the average coherence. There was no significant interaction term between 'trial' and 'recording technique' (F(3.86,57.9) = 1.79, p=0.144).

Regarding the first study objective, the post-hoc comparisons indicated that VL-VM intermuscular coherence was significantly reduced by more than 50% for bipolar potential measurements compared to monopolar potential and monopolar current measurements for each individual squatting trial. There were no significant differences in average coherence between the monopolar potential and current measurements. Further, there were no significant differences in average coherence between the bipolar measurements and the simulated monopolar signals with added cross-talk (Figure 4.4A).

Coherence was significantly higher during the unstable squatting trial compared to both stable squatting trials for the bipolar potential and monopolar current measurements. Despite a similar trend, there were no statistically significant differences in the average coherence between squatting trials for the monopolar potential measurements (Figure 4.4B).

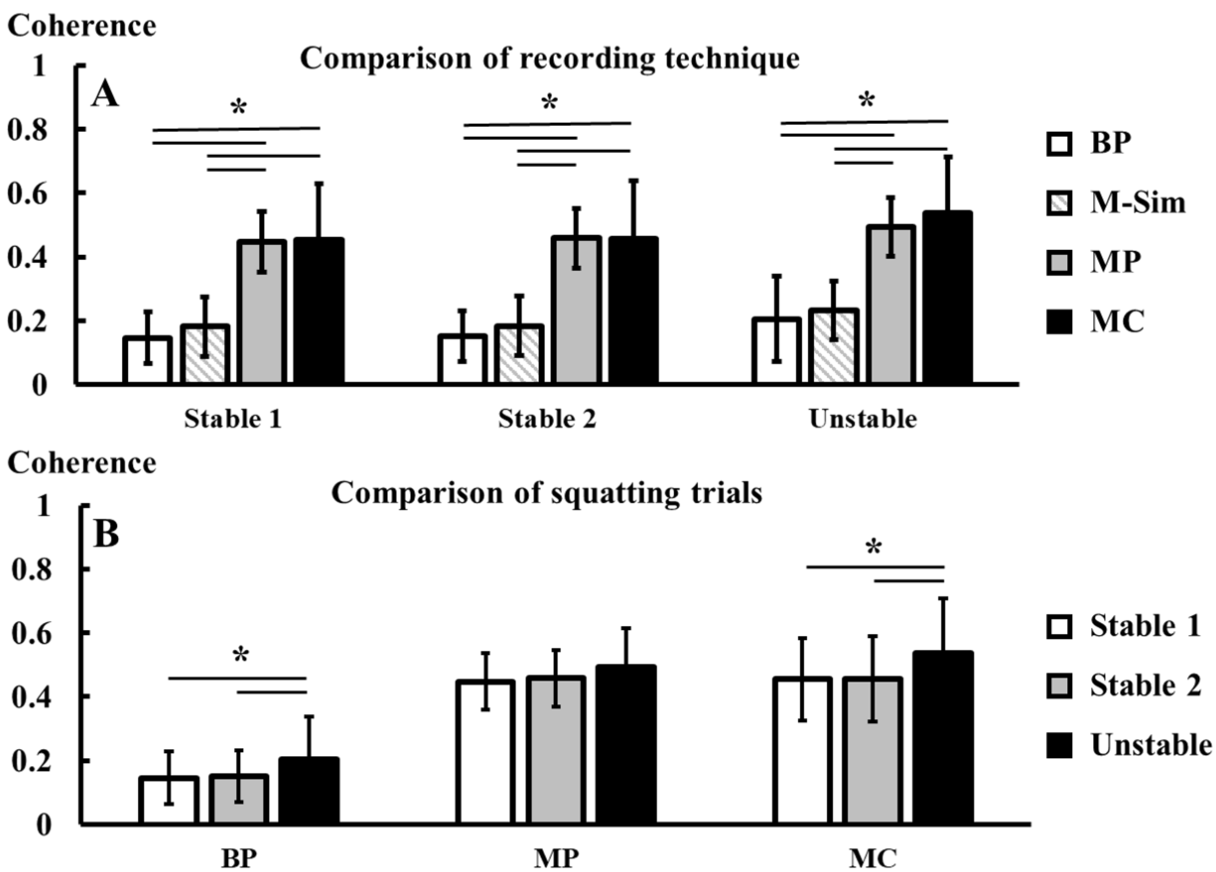

Figure 4.4. Comparison of average coherence (mean ± SD, n = 16) between recording techniques (BP – bipolar potentials, M-Sim – simulated monopolar potentials, MP – monopolar potentials, MC – monopolar currents) (A), and comparison of average coherence between squatting trials (B). Asterisks mark statistically significant differences between conditions at α = 0.05.

Regarding the second study objective, the reliability and sensitivity of the three recording systems are displayed in Table 4.2. For all recording systems, there were no average differences between the first and second stable squatting trial (Fig 4B). For both potential measurements, the lower bounds of the ICC 95% confidence intervals were above 0.75, indicating good relative reliability (Koo & Li, 2016). The monopolar current measurement showed excellent relative reliability (ICC = 0.98 (0.94,0.99)). The minimal detectable change in average VL-VM coherence during squatting was between 0.06 for the bipolar potentials and 0.07 for the two monopolar systems. Only for the monopolar current measurements, the mean difference between the unstable and stable squatting condition (trial 3 – trial 1) exceeded the minimal detectable change. Similarly, the monopolar current measurements showed the highest effect size (Cohen's *d* = 1.34), followed by the bipolar (0.91) and monopolar potentials (0.63).

Table 4.2. Reliability and sensitivity of average coherence outcomes

System	Reliability				Sensitivity		
	Relative		*Absolute*		*Unstable - Stable*		*Cohen's d*
	ICC	*95% CI*	*SEM*	*MDC*	*Mean*	*SD*	
Bipolar potential	0.93	(0.81 0.97)	0.02	0.06	0.06	0.07	0.91
Monopolar potential	0.92	(0.80 0.97)	0.03	0.07	0.05	0.07	0.63
Monopolar current	0.98	(0.94 0.99)	0.03	0.07	0.08	0.06	1.34

4.3.3 EMG overall intensity

Figure 4.5 shows the average, normalized overall EMG intensity of VL and VM during all three squatting trials. For both muscles, there was a significant interaction effect between 'trial' and 'recording technique' on the overall EMG intensity (VL: $F(2.78, 41.75) = 3.07$, $p=0.041$; VM: $F(3.27,49.02) = 4.39$, $p=0.007$). Post-hoc comparisons showed that on average, there were no significant differences in EMG intensity between the stable and unstable squatting condition for the bipolar system. For all monopolar recordings, there was a small trend for an average percentage increase of about 10% during the unstable compared to stable squatting condition. The average increase in overall EMG intensity in the unstable vs. stable condition only reached statistical significance for the current measurements of the vastus medialis (trial 1 vs. 3, p = 0.028). The large standard deviations in Figure 4.5 indicate that there was a high degree of variability between the individuals regarding which of the three squatting trials showed the highest overall EMG intensity.

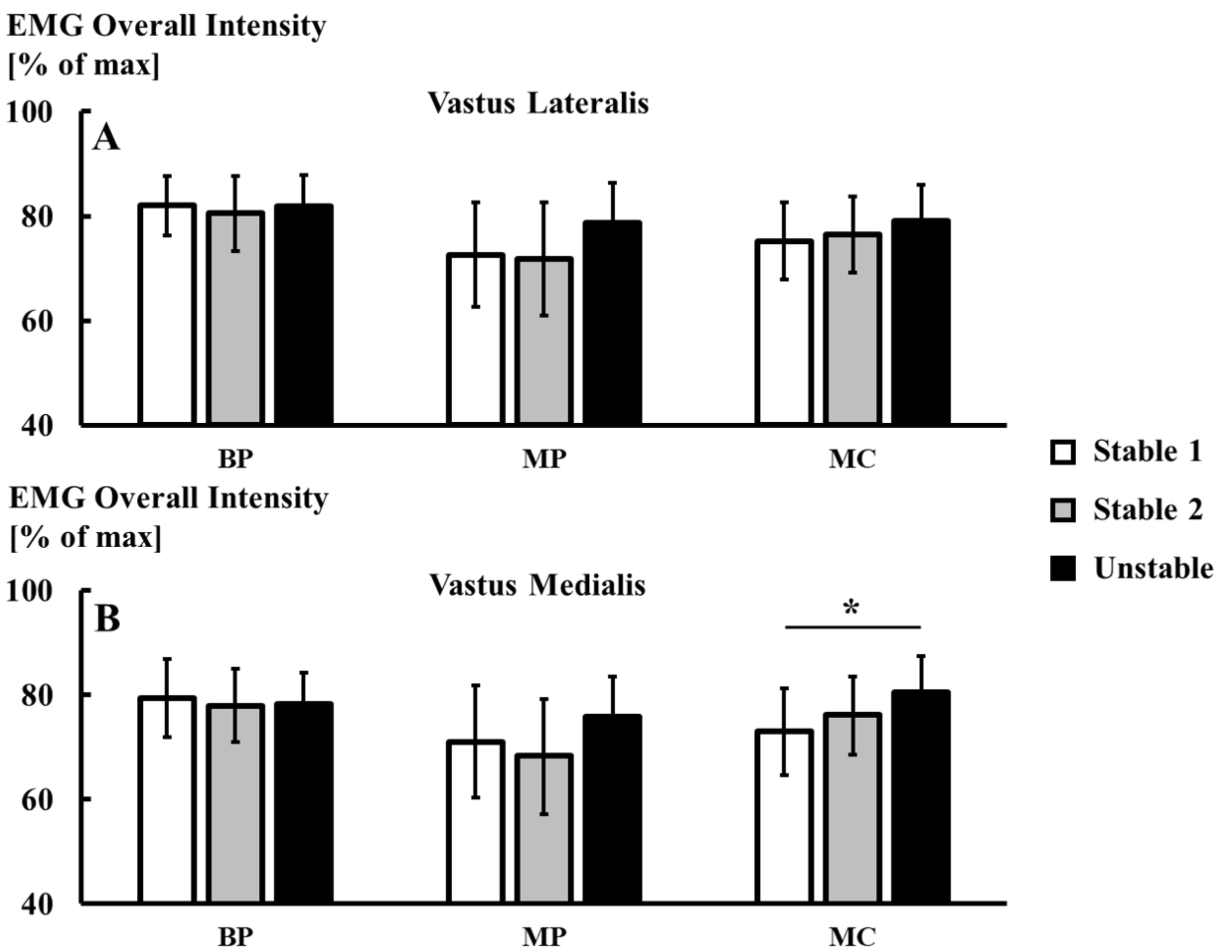

Figure 4.5. Comparison of normalized overall EMG intensity (mean ± SD, n = 16) between recording techniques (BP – bipolar potentials, MP – monopolar potentials, MC – monopolar currents) for the vastus lateralis (A) vastus medialis (B). Asterisks mark statistically significant differences between conditions at α = 0.05.

4.4 Discussion

This study aimed to investigate the effect of different EMG recording techniques on the magnitude, reliability, and sensitivity of intermuscular coherence during dynamic squatting tasks. Our first hypothesis that intermuscular coherence would be higher when computed from monopolar compared to bipolar EMG signals was confirmed by the result that the average coherence for bipolar potential measurements was significantly reduced compared to the monopolar potential and current measurements. The second hypothesis that all three systems would be sensitive to a lower VL-VM coherence during unstable vs. stable squatting was not supported by the findings that 1) the monopolar potential recordings showed low sensitivity to the change in coherence between the two squatting conditions and 2) the average coherence was in fact higher during unstable compared to stable squatting.

4.4.1 Recording techniques – Bipolar vs. Monopolar

The reduction in coherence for bipolar compared to monopolar EMG recordings may have two possible reasons: 1) the reduction or disruption of amplitude and frequency information within the bipolar signals that arose from electrode alignment and position errors and subsequent differential amplification, and 2) less cross-talk in bipolar recordings due to spatial filtering and higher spatial selectivity.

When a muscle is activated, motor unit action potentials travel along the muscle fibers, starting from the innervation zone and ending at the muscle insertion or origin (Basmajian, 1985). For bipolar EMG measurements, two adjacent electrodes are applied between innervation zone and tendon and in alignment with the muscle fiber direction to detect the same motor unit action potentials twice but spatially shifted along the muscle. Subtracting the signals from these two monopolar EMG signals yields a single-differential bipolar EMG signal, which has the advantage that common noise under both electrodes is reduced (Gallina, Merletti, & Gazzoni, 2013). Consequently, bipolar EMG measurements have been state-of-the-art in investigating muscle activation patterns during movement and have frequently been used to study intermuscular synchronization (Gibbs et al., 1995; Gibbs, Harrison, & Stephens, 1997; Kilner et al., 1999; Halliday et al., 2003; Boonstra et al., 2008; Kattla & Lowery, 2010; Reyes et al., 2017). During movements such as squatting, however, the fiber direction and location of the innervation zone of the quadriceps muscles with respect to the electrode position on the skin are functions of the knee angle and quadriceps muscle force (Rutherford & Jones, 1992; Gallina et al., 2013). Similarly, the innervation zone has been shown to move with respect to the skin as a function of knee angle (Rainoldi et al., 2000; Gallina et al., 2013). In consequence, bipolar electrodes cannot be properly aligned with the muscle fiber direction and the bipolar EMG signal will likely represent a combination of 1) the differential between propagating motor unit action potentials from the same motor units recorded twice at different locations along the muscle fiber direction and 2) the differential between motor unit action potentials that originate from different motor units (von Tscharner et al., 2013). The ratio of these differentials depends on the geometry of the muscle as well as electrode placement and will change with the pennation angle throughout a movement. It is well known that a portion of motor units within one muscle are synchronized in time, i.e. intramuscular synchronization, with an accuracy of more than 5 ms (Sears & Stagg, 1976; Bremner, Baker, & Stephens, 1991; Carlo J. De Luca, Roy, & Erim, 1993). Therefore, it is likely that signals recorded by the electrodes in a bipolar measurement setup are highly correlated and are either eliminated or at least disrupted in an unspecific and unpredictable way by the common mode rejection of the amplifier (von Tscharner, 2014). In consequence, only the signals that are uncorrelated between adjacent electrodes, i.e. not synchronized, are retained in bipolar measurements.

Evidence for this assumption can be seen in the average power spectra that were obtained using the monopolar and bipolar recording systems in this study. The monopolar recordings demonstrate a pronounced peak in the power spectrum between frequencies of 30-50 Hz. This 40 Hz peak in the EMG power spectrum of dynamic tasks has been observed previously and has been connected to rhythmic bursts of clustered motor unit activity, where multiple motor units are firing within a short time window of 10 ms (Yao, Fuglevand, & Enoka, 2000; Maurer, von Tscharner, & Nigg, 2013; Asmussen, Von Tscharner, & Nigg, 2018). If the two electrodes of a bipolar amplifier are recording motor unit action potentials from different motor units that are virtually firing at the same time, the common mode rejection would likely remove a significant amount of this information and explain why the 40 Hz peak is absent or much reduced in amplitude in the power spectra obtained from bipolar recordings.

Both intra- and intermuscular synchronization of motor units as measured by EMG-EMG coherence have been speculated to originate primarily from common, or shared inputs of the corticospinal tract to the respective motoneuron pools (Bremner et al., 1991; Farmer, Bremner, et al., 1993; Lowery, Myers, & Erim, 2007). In consequence, the degree of intra- and intermuscular synchronization is most likely correlated. If the bipolar EMG signal from one muscle only contains information about uncorrelated motor unit activity, as described above, it will be more difficult to detect intermuscular synchronization between different muscles. In contrast, monopolar EMG recordings contain the entire signal information and inherently do not need to be aligned with the muscle fiber direction. Therefore, it is speculated that the reduced or disrupted information within the bipolar EMG signal is the theoretical basis for the reduced VL-VM coherence in comparison to monopolar signals seen in this study.

The second possible origin of the difference in intermuscular coherence observed between the recording systems is a varying influence of cross-talk. Cross-talk ratios of about 10% were reported between the VL and VM during isometric knee extensions (Farina et al., 2002). Specifically, it has been shown that cross-talk was reduced in surface EMG signals from the thigh muscles when they were obtained using a double-differential vs. a single-differential recording technique (De Luca & Merletti, 1988; Farina et al., 2002). The reduction of cross-talk is most likely due to an increase in spatial selectivity of the EMG system when using double-differential amplification (Reucher et al., 1987). Albeit not systematically investigated to date, it can be speculated that monopolar surface EMG signals may thus contain more cross-talk components compared to bipolar single-differential signals. Cross-talk between EMG recordings of adjacent muscles can artificially inflate EMG-EMG coherence and should therefore be carefully addressed as a potential confounding factor in this study (Grosse, Cassidy, & Brown, 2002; Halliday et al., 2003). There are three reasons why the influence of cross-talk on the findings of this study is likely small. First, the EMG measurement set-up of this study was carefully designed to record VL and VM signals using two electronically separated circuits with separate grounds to exclude the possibility of hardware-based cross-talk (Mohr et al., 2015). Second, when measuring intermuscular EMG-EMG coherence, significant cross-talk between the two muscles of interest typically leads to a resulting coherence spectrum that shows high values across a broad range of frequencies, spanning almost the entire EMG bandwidth (Grosse et al., 2002; Halliday et al., 2003). This was not observed for any of the individuals tested in this study. Third and most importantly, we used a simple simulation to investigate the possible influence of an additional 10% of cross-talk components in monopolar compared to bipolar EMG signals. Although on average, the simulated signals show a slightly higher coherence compared to the bipolar signals (see Figure 4.4 A, BP vs. M-Sim), this difference was not statistically significant and cannot explain the large increase in coherence from the bipolar to the monopolar recording systems. In summary, while the presence of cross-talk can not be completely excluded in this study, cross-talk was not a major confounding factor in the comparison of monopolar vs. bipolar EMG systems.

4.4.2 Recording techniques – EMG Intensity

A second difference between the recording systems was observed for the level of muscle excitation according to the overall EMG intensity during the stable and unstable squatting exercise. While the bipolar EMG measurements of the vasti muscles did not show an average change in EMG intensity between the movement conditions, the monopolar recording systems showed a small, average increase in EMG intensity for both VL and VM during the unstable squat. This is in accordance with a previous study showing no or only a small percentage increase (< 10%) in thigh muscle activity when switching from squatting on an stable to squatting on an unstable surface (Anderson & Behm, 2005). The discrepancy between the monopolar and bipolar recording systems could originate from additional synchronized inputs that the vasti muscles received from the central nervous system during the unstable squat as suggested by the corresponding increase in VL-VM coherence during this exercise. Such synchronized motor unit activity would increase the overall EMG intensity (Yao et al., 2000; Asmussen et al., 2018) but may not be detected by the bipolar EMG recording system due to the elimination or reduction of common input signals as explained above.

4.4.3 Recording techniques – Potentials vs. Currents

A third difference between the recording systems was observed between the coherence spectra obtained using the monopolar current compared to the potential amplifiers. Specifically, the current amplifier detected a higher magnitude of coherence for frequencies above 80 Hz compared to the potential amplifiers. The presence of high-frequency intermuscular coherence has been reported between EMG signals for upper and lower limb muscles (Chang et al., 2012; De Marchis et al., 2015; Mohr et al., 2015; Pizzamiglio et al., 2017). Intermuscular coherence within the gamma band (30-60 Hz) and higher frequencies has been speculated to represent a coupled, descending motor command to muscles involved in movement tasks that require dynamic modulation of muscle force for error correction – such as squatting on an unstable surface in the current study (Pizzamiglio et al., 2017). For these force modulations, it may be preferable for the central nervous system to primarily activate fast motor units due to their ability to generate higher forces and faster conduction velocities (Milner-Brown, Stein, & Yemm, 1973; Wakeling & Syme, 2002; Hodson-Tole & Wakeling, 2009). In parallel, it has been suggested that faster motor units generate motor unit action potentials that contribute high-frequency components to the EMG signal (Wakeling & Rozitis, 2004), which could explain the second, smaller peak in the coherence spectra at frequencies above 100 Hz seen in this study (see Fig 3, H-I). However, direct evidence for a preferential recruitment of fast, large motor units for a mixed fiber type muscle is currently not available. Nevertheless, it is unlikely that this second high-frequency coherence peak seen for the current recordings is due to a measurement artefact but it is unclear why this peak is much reduced or absent in the potential recordings.

Previously, von Tscharner and colleagues (von Tscharner et al., 2013) had observed that the monopolar current amplifier is more sensitive in detecting EMG signal power at high frequencies, which could be a reason for the higher EMG-EMG coherence at these frequencies in the current recordings. However, Figure 4.3 does not show an obvious difference between the average power spectra of monopolar current and potential amplifiers at frequencies above 80 Hz. It may be that synchronized, fast motor units only have a negligible contribution to the average EMG power, which is dominated by frequencies below 80 Hz, but that they still contribute to the coherence spectrum, which is independent of signal amplitude. Further research is required to understand why the current amplifier may be more sensitive in resolving motor unit action potentials at higher frequencies.

4.4.4 Stable vs. unstable squat

Both the bipolar potential and monopolar current system showed an average increase in VL-VM coherence during the squat on the unstable BOSU balance trainer compared to the stable squat. Albeit not statistically significant, the monopolar potential system also showed an increase towards a higher VL-VM coherence during unstable squatting. In parallel, there was no difference in intermuscular coherence between the first and second trial of stable squatting, demonstrating the absence of a possible learning effect. For both the bipolar potential and monopolar current system, the increased VL-VM coherence during unstable squatting was equal to or exceeded the respective minimal detectable change. In combination, these findings suggest that the neuromuscular strategy to control the vasti muscles changed when adding an unstable surface to the squatting exercise.

While all three recording systems indicated an average increase in VL-VM coherence between the two movement conditions, the bipolar potential and monopolar current systems were more sensitive compared to the monopolar potential system. Therefore, if researchers are interested in studying a change in intermuscular coherence between two different tasks, the bipolar potential system or monopolar current system seem to be more suitable than the monopolar potential technique.

The squatting movement on the BOSU balance trainer was selected as a task that is comparable to squatting on a stable surface in terms of joint kinematics and net force while demanding a greater involvement of the individual quadriceps muscles in maintaining postural stability. The result of a higher coherence during unstable squatting was not expected since previous investigators have reported a reduction in intermuscular coherence when performing a task that requires more individual muscle control compared to a task that requires more synergistic muscle control (Mohr et al., 2015; Reyes et al., 2017). For example, Reyes and colleagues demonstrated a reduction in intermuscular beta-band coherence (15-30 Hz) between a finger and a thumb muscle during a task where participants pinched an unstable spring compared to a task where a stable cylinder was compressed with a matched force (Reyes et al., 2017). Furthermore, musicians who require more individual control of finger muscles showed a lower degree of motor unit synchronization within a finger muscle compared to weight lifters who have trained to use their finger muscles in synergy (Semmler & Nordstrom, 1998). It is questionable, however, whether vastus medialis and lateralis in this study were in fact controlled more individually by the central nervous system during the squat on the BOSU balance trainer compared to the stable squat. Anderson and Behm compared the general level of EMG intensity of vastus lateralis as well as of lower leg and core muscles between squatting on a stable versus unstable surface (Anderson & Behm, 2005). Vasti EMG intensity was not significantly different between the two squatting conditions, which corroborates the result of this study, whereas the EMG intensity of the core and lower leg muscles was increased by up to 50% during the unstable condition. This indicates that the role of the quadriceps in maintaining postural stability during the unstable squat is small in relation to core and lower leg muscles. As a consequence, it may not be appropriate to compare the current findings with previous studies that investigate individual muscle control paradigms. Instead, the authors speculate that during both squatting exercises, the vasti muscles act as prime movers and were thus controlled as a functional unit by the central nervous system (De Luca & Erim, 2002; Anderson & Behm, 2005). During the unstable squat, the motor units of the vasti muscles may have received additional, intermittent and synchronized inputs to achieve small adjustments in the knee flexion angle trace while squatting on the BOSU ball. Furthermore, these intermittent bursts of activity may have disturbed the rhythmic, clustered motor unit activity related to the 40 Hz peak in the VL and VM power spectra and, thus explain the reduced magnitude of this peak during the unstable squat in Figure 3. In support of this argument, Gibbs et al. (1995) showed that the motor unit synchronization between two synergistic lower leg muscles as measured by a cross-correlation analysis was higher during a balancing standing task compared to a regular standing task and compared to voluntary contractions while lying down. It was suggested that the increase in motor unit synchronization may originate from a greater involvement of the vestibular system, specifically that the muscles received synchronized inputs from increased activity in vestibulospinal neurones. The authors speculate that a similar neuromuscular mechanism could explain the finding of higher VL-VM intermuscular coherence during the balancing task in this study.

4.4.5 Reliability and sensitivity

The question remains if one of the EMG recording techniques, bipolar vs monopolar, is more suited to investigate EMG-EMG coherence as a measure of intermuscular synchronization. A higher coherence score alone does not necessarily indicate that the monopolar system is more suitable. Therefore, reliability and sensitivity analyses were performed to give further insight into this question. Comparing all three systems, it was observed that the coherence obtained from the monopolar currents showed the highest relative reliability between two stable squatting trials as well as the highest sensitivity when changing to unstable squatting with a large effect size of greater than one. The monopolar potential measurements, however, showed a low sensitivity and could not resolve the increase in coherence when changing between squatting conditions. This could be because monopolar potential recordings are more susceptible to stray potentials in the measurement environment and electrical noise that could contaminate the signals and reduce the system sensitivity (von Tscharner et al., 2013). The bipolar system showed good relative reliability and resolved a large effect between the stable and unstable squat, although with a lower sensitivity compared to the monopolar current system.

Therefore, when studying EMG-EMG intermuscular coherence to investigate the relative change in intermuscular synchronization between two or more movement conditions, both the bipolar potential and monopolar current systems seem to be suitable while the monopolar potential system should not be used. A monopolar current technique may be preferable over the traditional bipolar technique if 1) the muscles of interest are far enough apart that cross-talk between monopolar electrodes has a minor influence, and 2) the movement of interest does not involve impacts, e.g. walking or running. The latter would induce large motion artefacts in monopolar EMG measurements, which would produce a misleading EMG-EMG coherence.

When studying the magnitude of intermuscular coherence as a measure of the absolute degree of intermuscular synchronization between two muscles for a certain individual or a group of individuals, monopolar EMG recordings on the one hand may provide a more ‘global’ view on correlated motor unit activity at the whole muscle level. On the other hand, bipolar EMG recordings, particularly in combination with additional spatial filtering techniques or when applied as multi-electrode arrays, may provide better information on the behaviour and synchronization of individual motor units. Whether one or the other technique better represents the physiological origin of correlated motor unit activity, i.e. the strength of common input to the motor neuron pools of two muscles, should be the focus of future studies.

4.5 Conclusions

This study investigated the effect of three different surface EMG recording systems on the coherence between the raw EMG signals of vastus medialis and lateralis during bipedal squatting on stable and unstable surfaces. When EMG signals were obtained with the traditional bipolar potential amplifier, the magnitude of intermuscular coherence between 10-60 Hz was less than half compared to the coherence based on monopolar signals. This may be a consequence of disrupted information about motor unit activity contained in the bipolar EMG signals as a result of the elimination of common signals by the differential bipolar amplifier. A simple simulation of additional cross-talk in monopolar signals could not explain this substantial difference in coherence between the recording systems. When comparing squatting exercises on a stable and unstable surface, only the bipolar potential and monopolar current system resolved an increase in intermuscular coherence for the unstable surface, with a larger effect size for current measurements. The monopolar potential system showed low sensitivity to the change in the movement condition and should therefore not be used to determine intermuscular coherence. If cross-talk plays a minor role and in the absence of movement artefacts, both bipolar potential and monopolar current measurements are suited to study changes in intermuscular coherence as an indicator of varying levels of intermuscular synchronization between different conditions.

Chapter Five – **Quadriceps-hamstrings intermuscular coherence during single-leg squatting 3-12 years following a youth sport-related knee injury**

5.1 Introduction

Increased quadriceps-hamstrings (Q-H) muscle co-contraction during gait or other functional movements is a commonly observed neuromuscular adaptation immediately following a significant knee injury (e.g., Anterior Cruciate ligament (ACL) rupture or meniscal tear) (Rudolph, Axe, Buchanan, Scholz, & Snyder-Mackler, 2001; Hurd & Snyder-Mackler, 2007; Sturnieks, Besier, & Lloyd, 2011). This adaptation is hypothesized to be a protective adaptation of the central nervous system (CNS) to increase joint stiffness and thereby avoid excessive anterior tibial translation and/or other painful movements (Chmielewski, Rudolph, & Snyder-Mackler, 2002). Despite these immediate benefits, however, a pronounced Q-H co-contraction strategy is characteristic for individuals who show poor knee function following ACL rupture as it may hinder more effective knee stabilization strategies observed in individuals with no prior knee injury (Eastlack, Axe, & Snyder-Mackler, 1999; Rudolph et al., 2001; Chmielewski, Hurd, Rudolph, Axe, & Snyder-Mackler, 2005). Furthermore, a more pronounced Q-H co-contraction strategy that persists past the acute knee injury phase (> one year) most likely results in a permanent alteration of the magnitude and direction of forces that act across the tibio-femoral joint surfaces (Schipplein & Andriacchi, 1991; Victor, Labey, Wong, Innocenti, & Bellemans, 2010). Joint tissues that are unable to adapt to the modified mechanical environment associated with increased Q-H co-contraction may slowly degrade overtime, exposing the affected individuals to an increased risk for post-traumatic osteoarthritis (PTOA) of the knee (Andriacchi et al., 2004). Therefore, albeit protective initially, a permanent increase in Q-H co-contraction following a knee injury has been considered a maladaptive neuromuscular strategy that may impede effective knee stabilization and contribute to joint degeneration (Chmielewski et al., 2005; Tsai, McLean, Colletti, & Powers, 2012).

The few previous studies that have investigated Q-H muscle co-contraction beyond the first year following a knee injury have generally provided evidence that a pronounced co-contraction strategy does persist in previously injured individuals (Ortiz et al., 2008; Tsai et al., 2012; Hall, Stevermer, & Gillette, 2015). These authors, however, primarily focused on the question of how Q-H co-contraction may affect knee joint loading in the context of PTOA risk. Their approach can only provide limited insight into neurophysiological processes that may facilitate a stronger coupling of activity between quadriceps and hamstring muscles following knee trauma. For the design of effective neuromuscular rehabilitation aimed at improving knee stability and reducing OA risk following a knee injury, it is of importance to better understand long-term adaptations of the CNS related to controlling quadriceps and hamstrings motor unit activity (Needle, Lepley, & Grooms, 2017; An, 2018).

For the ankle joint it has been suggested, that a co-contraction strategy between antagonistic muscles, e.g. for joint stabilization, is enabled by a stronger involvement of the motor cortex that leads to a more synchronized motor unit activity between these muscles (Nielsen & Kagamihara, 1992; Hansen, Hansen, Christensen, Petersen, & Nielsen, 2002). These findings were based on an intermuscular coherence analysis between the surface electromyography (sEMG) signals of the involved muscles (Hansen et al., 2002). EMG-EMG coherence can be used to detect coupled motor unit activity between two muscles as a result of neural inputs from different sources within the CNS (Farmer, Bremner, Halliday, Rosenberg, & Stephens, 1993; Grosse, Cassidy, & Brown, 2002). Specifically during movement, intermuscular coherence in the gamma-band (30-60 Hz) has been investigated as a marker of motor unit coupling between muscles associated with common inputs from the corticospinal tract to these muscles (Clark et al., 2013; De Marchis et al., 2015; von Tscharner, Ullrich, Mohr, Comaduran Marquez, & Nigg, 2018)

Therefore, it may be hypothesized that a persistent Q-H muscle co-contraction strategy observed in individuals following a knee injury is associated with a stronger coupling of motor unit activity between the quadricep and hamstring muscles and can be detected as an increase in gamma-band intermuscular coherence between the respective sEMG signals when compared to uninjured controls.

Recent analyses of intermuscular coherence between two quadriceps muscles during a squatting task demonstrated a high degree of relative and absolute between-trial reliability and good sensitivity to detect a change in coherence between different squatting conditions (Mohr et al., 2015; Mohr, Schön, von Tscharner, & Nigg, 2018). Given that a single-leg squatting (SLS) task is a common clinical test to assess knee joint function following an injury (Escamilla, 2001), high intermuscular coherence between the quadricep and hamstring muscles during this task may be a useful marker to detect abnormal co-contraction strategies. Here, it is crucial to consider males and females separately due to sex-specific neuromuscular patterns during single-leg movement tasks (Zeller, McCrory, Ben Kibler, & Uhl, 2003) as well as sex-specific adaptations to knee injuries when accomplishing single-leg movements (Yamazaki, Muneta, Ju, & Sekiya, 2010). Furthermore, if increased Q-H co-contraction during SLS is a clinically relevant strategy to cope with compromised knee function as stated initially, then Q-H intermuscular coherence should be even higher in individuals who exhibit more severe knee symptoms and/or pain.

The primary objective of this study was to assess the relationship between a history of intra-articular youth sport-related knee injury 3-12 years ago and gamma-band Q-H intermuscular coherence during a single-leg squat taking into consideration the influence of sex and self-reported knee symptoms and pain.

The secondary objective of this study was to explore side-to-side differences in Q-H gamma-band coherence between the injured and uninjured legs in males and females.

5.2 Methods

5.2.1 Study design and participants

Participants included a subset of individuals from an ongoing longitudinal historical cohort study [The Alberta Youth Prevention of Early OA Study (PrE-OA Study)]. The Pre-OA cohort, primary outcome variables, recruitment strategies, and exclusion criteria have been described in detail previously (Whittaker et al., 2015; Toomey et al., 2017; Whittaker, Toomey, Nettel-Aguirre, et al., 2018; Whittaker, Toomey, Woodhouse, et al., 2018). Briefly, the PrE-OA study includes 200 youth / young adults (aged 15 to 26); 100 participants who have suffered a sport-related knee injury three to ten years previously and 100 participants with no history of knee injury, matched for age, sex, and sport. Sport-related knee injury was defined as a clinical diagnosis of knee ligament, meniscal or other intra-articular tibiofemoral or patellofemoral injury that required both medical consultation and disrupted regular sport participation. The current study was embedded within the larger PrE-OA study and included a convenience sample of 71 individuals (34 injured, 37 uninjured) from the PrE-OA cohort that consented to participating in an additional analysis of their leg muscle activity and lower extremity kinematics during various movements including the SLS. The biomechanical data collection for this study took place between August 2016 and October 2017 and included PrE-OA participants at varying follow-up stages of participation in the larger cohort study (e.g., year 1-3 follow-up testing). This study was carried out in accordance with the guidelines of the University of Calgary's Conjoint Health Research Ethics Board (#E-25075) and with the Declaration of Helsinki.

5.2.2 Procedures

The primary data collection site for the PrE-OA study was the University of Calgary Sports Medicine Centre. After completing a study questionnaire (e.g., demographics, knee injury/surgery details, medical history) and the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire, participants' height (cm) and weight (kg) were measured. For the purposes of this study the index leg refers to the injured leg of participants with a knee injury history and the corresponding leg of their matched-control in the larger PrE-OA cohort study.

The KOOS is a self-reported outcome measure related to knee joint health and function with five subscales: pain, other symptoms, function in daily living, function in sport and recreation, and knee-related quality of life (Roos, Roos, Lohmander, Ekdahl, & Beynnon, 1998). Each item is scored on a 5-point Likert-scale ranging from no problems to extreme problems with a cumulative, normalized score for each subscale between 100 and 0, respectively. For this analysis, the KOOS pain and symptoms subscales were evaluated as these sub-scales have shown clear associations with knee injury history in the PrE-OA and other OA cohorts, and may be indicative of symptomatic PTOA (B. E. Øiestad, Holm, Engebretsen, & Risberg, 2011; Whittaker, Toomey, Woodhouse, et al., 2018).

Biomechanical and surface EMG measurements were conducted at the University of Calgary's Human Performance Laboratory. Locations for bipolar surface EMG sensors (Ag/AgCl, 10 mm diameter, 20 mm inter-electrode distance, Norotrode Myotronics-Noromed Inc., US) were identified on the vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), and medial hamstrings (MH) of both legs according to standardized landmarks described in the widely used

SENIAM guidelines for the use of surface EMG (Hermens et al., 1999). Before sensor placement, the skin around the identified locations was shaved, lightly abraded, and cleaned with alcohol wipes to reduce skin impedance. After sensor placement, the location of the electrodes was validated according to clean, raw EMG signals during a series of test movements (i.e. squats for vasti muscles, hamstring curls for hamstrings).

Bipolar, differential EMG signals were recorded at 2400 samples per second, amplified by a factor of 1000 and bandpass-filtered between 10-500 Hz (Biovision, Wehrheim, Germany). Signals below 10 Hz were therefore not considered for drawing conclusions although there may be some EMG signal in the role off range of the frequency response. The measuring system was grounded by connecting the ground electrode of the system to the right tibial tuberosity. The analog EMG signals were digitized by a 12-bit A/D converter (National Instruments, Austin, TX). In addition to EMG recordings, 1D-accelerometers were taped to the lateral aspect of the right and left heel to later synchronize the EMG and motion capture systems during squatting. Heel accelerations were recorded simultaneously to EMG signals via the same data acquisition system. Following the EMG set-up, participants were equipped with sets of three retroreflective markers that were mounted on the thigh and shank segments to track three-dimensional lower limb kinematics during the squatting task.

Following the sensor set-up, participants performed a series of 45-degree SLSs at a rate of 17 squats/minute for 1 minute. The squatting speed was controlled for by a metronome playing at 35 beats per minute with the instruction to synchronize the highest and lowest position of the squat with a beat. The relatively small range of motion of 45 degrees was chosen to ensure that participants with knee symptomology or poor balance would still be able to complete the SLS task. To ensure a consistent squatting depth, a string was suspended between two tripods and adjusted so that it would lightly touch the anterior aspect of the participants' knee when the knee was flexed to 45 degrees. The position of the string was confirmed prior to testing by a manual goniometer. Participants were instructed to squat down until they touched the string without deforming it and to then reverse the movement. Each participant completed a warm-up of three sets of five SLS on each leg to familiarize with the instructions, the movement task, and squatting rhythm. For the actual measurement, participants performed two 60 second trials of single-leg squatting, one for each leg. Participants were instructed to initially step onto a force plate with the leg to be measured. The step on to the force plate produces a sharp peak in both the vertical ground reaction force as well as the heel acceleration signal, which was used to synchronize the motion analysis and EMG systems during the analysis. Then, participants performed the squats while keeping their upper body straight and with their hands on their hips. During the squatting movement, EMG (2400 Hz sampling), ground reaction force (2400 Hz sampling, Kistler), and 3D marker trajectories (240 Hz sampling, 8 cameras, Motion Analysis Corp, Santa Rosa, CA, USA) were recorded. If participants lost the squatting rhythm or had to set down their other leg to regain balance, the measurement was repeated after one minute of rest.

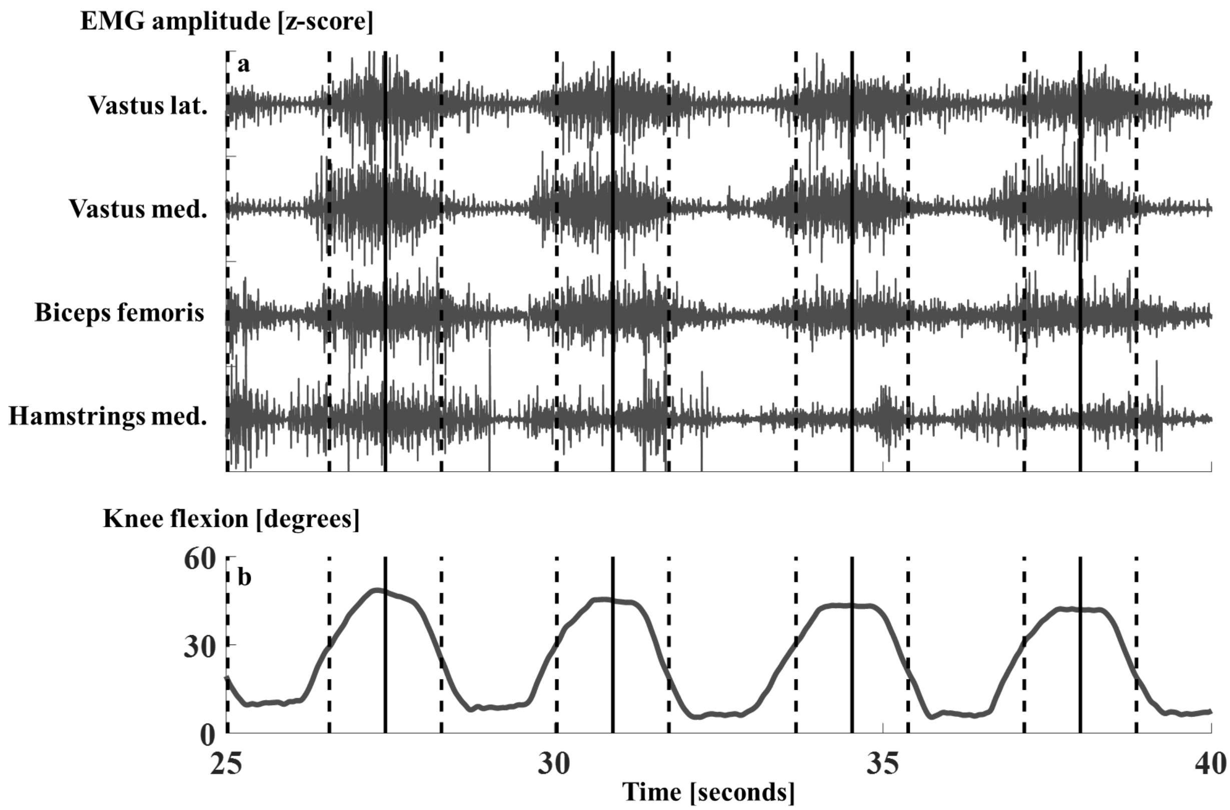

Figure 5.1. Raw EMG signals of thigh muscles during the single-leg squat (a). EMG amplitudes were normalized to their respective standard deviations for display purposes. Corresponding knee flexion angle in degrees (b). Solid vertical bars represent peak knee flexion. Dashed vertical bars represent analyzed data windows.

5.2.3 Data processing

The raw marker trajectories obtained from motion analyses were reconstructed using Cortex software (Motion Analysis Corp, Santa Rosa, CA). The remaining processing steps were performed using a custom-written software in MATLAB 2017a (Mathworks, Natick, MA, USA). First, the EMG and motion data were synchronized in time at the instant of the initial step onto the force plate according to a peak in the heel accelerometer signal (EMG system) and a sharp rise of the vertical ground reaction force (20 N threshold) provided by the force plate. Three-dimensional marker trajectories of the thigh and shank during squatting were low-pass filtered (fourth-order Butterworth, 10 Hz cut-off) and used to estimate the sagittal knee joint angle as described by Soderkvist & Wedin (Söderkvist & Wedin, 1993) (Figure 5.1b). The peak knee flexion angles during the squatting series were identified and used to extract EMG data sequences during a time window of 4096 samples (1.7 s) centered at the time of peak knee flexion. For each individual, 15 EMG data sequences were selected and further subdivided into four sequences of 1024 samples (~ 0.43 s). This procedure increased the number of available data sequences to 60 and thus, a smaller standard error of the estimated Fourier spectra and coherence analysis could be obtained. The resulting frequency increments of 2.3 Hz were still deemed acceptable to estimate spectral variables in different frequency bands.

5.2.4 Intermuscular coherence analysis

After removing the mean of the raw EMG data sequences, the FFTs were computed. The power spectra for each muscle and individual were determined by multiplying the FFT of each sequence with its complex conjugate and averaging across all data sequences (Figure 5.2a). Intermuscular coherence of the raw, unrectified EMG signals as a function of frequency λ (coherence spectrum) for each muscle pair was computed from the average cross-spectra normalized by the corresponding power spectra across s = 60 data sequences (Rosenberg et al., 1989):

$$\boldsymbol{coherence}(\boldsymbol{\lambda}) = \frac{\left|\overline{\boldsymbol{F}_{\boldsymbol{VL}}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VM}}(\boldsymbol{\lambda})^{*}}\right|^{\mathbf{2}}}{\overline{(\boldsymbol{F}_{\boldsymbol{VL}}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VL}}(\boldsymbol{\lambda})^{*})} \cdot \overline{(\boldsymbol{F}_{\boldsymbol{VM}}(\boldsymbol{\lambda}) \cdot \boldsymbol{F}_{\boldsymbol{VM}}(\boldsymbol{\lambda})^{*})}}$$

Equation 5.1

Due to the averaging procedure, the coherence between two signals decays with an increasing number of analyzed sequences unless the phase shift relationship between the signals remains constant. In consequence, even signals with no phase correlation can show a high level of coherence by chance if the number of analyzed sequences is small (s < 10). Therefore, a threshold coherence must be established, where the coherence spectrum above this threshold value represents a significant phase correlation between the two analyzed signals. The reference coherence was determined as a function of the number of analyzed sequences as introduced by Rosenberg and colleagues (Rosenberg et al., 1989):

$$\boldsymbol{threshold\ coherence = 1 - \alpha^{1/(s-1)}}$$

Equation 5.2

where α corresponds to the significance level that was set to 0.05. In this study, *s* was given by the 60 analyzed squatting sequences leading to a reference coherence of 0.0495. The reference value was subtracted from the determined coherence spectra and resulting negative values were set to zero (Figure 5.2 b,c). For each participant, intermuscular coherence was analyzed between the vastus lateralis and biceps femoris (VL-BF), and vastus medialis and medial hamstrings (VM-MH).

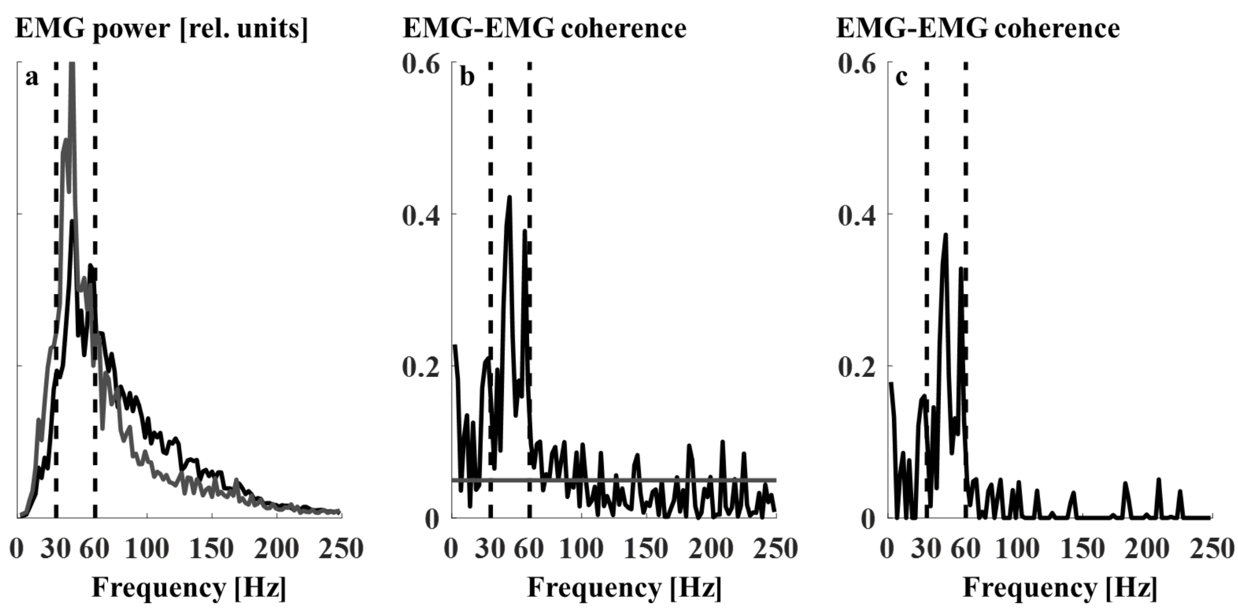

Figure 5.2. Power spectra of vastus lateralis (VL, black) and biceps femoris (BF, grey), normalized to the summed power between 0-250 Hz (a). VL-BF intermuscular coherence spectrum (black) and coherence reference value (grey) (b). Significant VL-BF

intermuscular coherence spectrum above reference value (c). Dashed vertical lines indicate the gamma-band. Spectra are displayed for one participant.

To summarize the presence and strength of significant intermuscular coherence for Q-H muscle pairs during squatting, the coherence spectra from both legs of all participants were pooled. The presence of significant coherence was then determined as the percentage of legs exhibiting intermuscular coherence above the reference value for each frequency bin (Farmer et al., 1993). To summarize the strength of intermuscular coherence across individual measurements, previous authors have recommended to first convert the raw coherence spectrum, *coherence(λ)*, into a normally distributed variable with an approximately unit standard deviation, *z-coherence(λ)*, by the following transformation (Rosenberg et al., 1989; Baker, Pinches, & Lemon, 2003):

$$\boldsymbol{z - coherence}(\boldsymbol{\lambda}) = \frac{\mathbf{tanh^{-1}}(\sqrt{\boldsymbol{coherence}(\boldsymbol{\lambda})})}{\sqrt{\mathbf{1/2}\boldsymbol{s}}}$$

Equation 5.3

where *s* corresponds to the 60 analyzed data sequences that were used to compute the raw coherence. The transformed coherence spectra, *z-coherence(λ)*, were pooled across all legs and then averaged at each frequency to compare the strength and properties of Q-H intermuscular coherence between the two muscle pairs.

To investigate associations between Q-H coherence and knee injury history, sex, and KOOS scores, VL-BF and VM-MH gamma-band z-coherence was computed as the mean of the z-transformed coherence spectra between 30-60 Hz for each muscle pair and individual. All subsequent statistical analyses were performed on these average VL-BF and VM-MH z-transformed coherence values in the gamma-range.

5.2.5 Statistical analyses

Statistical analyses were performed using STATA (v.14.2, College Station, TX, USA) at a significance level of $\alpha = 0.05$. Descriptive statistics were calculated for participant characteristics (by exposure group), KOOS pain and KOOS symptoms sub-scale scores for the index leg (by injury group), and gamma-band coherence between VL-BF and VM-MH for the index and non-index leg (by injury group and sex). A Wilcoxon rank sum test was used to evaluate significant differences in the medians of KOOS sub-scale scores between injured and uninjured participants. Regarding the first objective, multivariable linear regression (95% CI) was used to investigate the association between the predictor variables 'injury group' (exposure), 'sex', 'KOOS symptoms,' 'KOOS pain' and the two outcome variables, 'VL-BF and VM-MH gamma-band coherence'. Regression analyses began with models that included coefficients for all predictor variables and two-way interaction terms with the exposure variable (i.e. exposure x sex, exposure x KOOS symptoms, exposure x KOOS pain). After interaction terms were evaluated for statistical significance according to likelihood ratio tests, the association between 'injury group' and each coherence outcome was assessed considering confounding by sex, KOOS symptoms, and KOOS pain using a stepwise elimination approach. Covariates were removed from the model if their coefficients were not statistically significant and there was no evidence that they confounded the relationship between the exposure and outcome variables. In the presence of a significant exposure x sex interaction, Bonferroni-corrected post-hoc comparisons (Wilcoxon rank sum tests) of coherence outcomes were conducted for all combinations of the levels of 'injury group' and 'sex'. For the VL-BF coherence outcome, all assumptions for linear regression were assessed and met. For the VM-MH coherence outcome, the distribution of residuals was right-skewed and violated the assumption of normally distributed residuals, which is important for obtaining accurate F and t-statistics. Therefore, a ln-transformation of the VM-MH coherence outcomes was applied to achieve normally distributed residuals.

Regarding the second objective, a qualitative, exploratory analysis was conducted to detect possible side-to-side differences in Q-H gamma-band coherence in injured and uninjured subjects. Specifically, side-to-side differences (index – non-index leg) were determined for each individual and then displayed as boxplots by injury group and sex.

5.3 Results

5.3.1 Participants

Participant characteristics are summarized in Table 5.1. After visual analysis of the raw EMG data, six participants were excluded due to poor quality / missing data for one or two muscles (amplifier defect, n = 5; data acquisition box defect, n = 1). For these reasons, a total of 64 individuals (31 injured, 33 uninjured) were included in the analyses examining EMG power and intermuscular coherence during squatting. For assessing side-to-side differences in coherence, five additional injured individuals were excluded due to a contralateral knee injury, leaving a sample of 26 previously injured participants.

Nineteen of the previously injured participants had sustained an ACL tear and all had undergone ACL reconstruction surgery. Other injuries included isolated meniscal injuries, medial or lateral collateral ligament injuries, and one fracture. KOOS pain and KOOS symptoms median scores were significantly lower in the previously injured group compared to the control group ($p = 0.025$) indicating more symptoms and higher pain.

Table 5.1. Participant characteristics

Variable	***Uninjured n = 33***	***Injured n = 31***
% female	71	65
Age at testing in years (median, range)	25 [18 29]	24 [19 30]
Height in cm (median, range)	170 [158 194]	167 [157 196]
Weight in kg (median, range)	72.1 [54 109]	68 [49 110]
Months since injury (median, range)	n/a	98 [38 150]
Primary knee injury surgery (number)	n/a	20
ACL reconstruction (number)	n/a	19
Knee injury with meniscus involvement (number)	n/a	17
Second injury to study knee (number)	n/a	5
Contralateral knee injury (number)	n/a	5
KOOS Symptoms (median, range)	96 [71 100]	93 [64 100]
KOOS Pain (median, range)	100 [92 100]	94 [72 100]

5.3.2 Q-H intermuscular coherence during a single-leg squat

The intermuscular coherence for the VL-BF and VM-MH muscle pairs did not reach significance at all frequencies. Thus, at each frequency only a certain percentage of legs reached significant coherence between the quadriceps and hamstring muscles. The percentage of legs that showed a significant coherence for a specific frequency is summarized in Figure 5.3 as a grey line (right y-axes). The z-transformed coherence of the legs as a function of frequency yielded an average coherence spectrum shown as black line in Figure 5.3 (left y-axes).

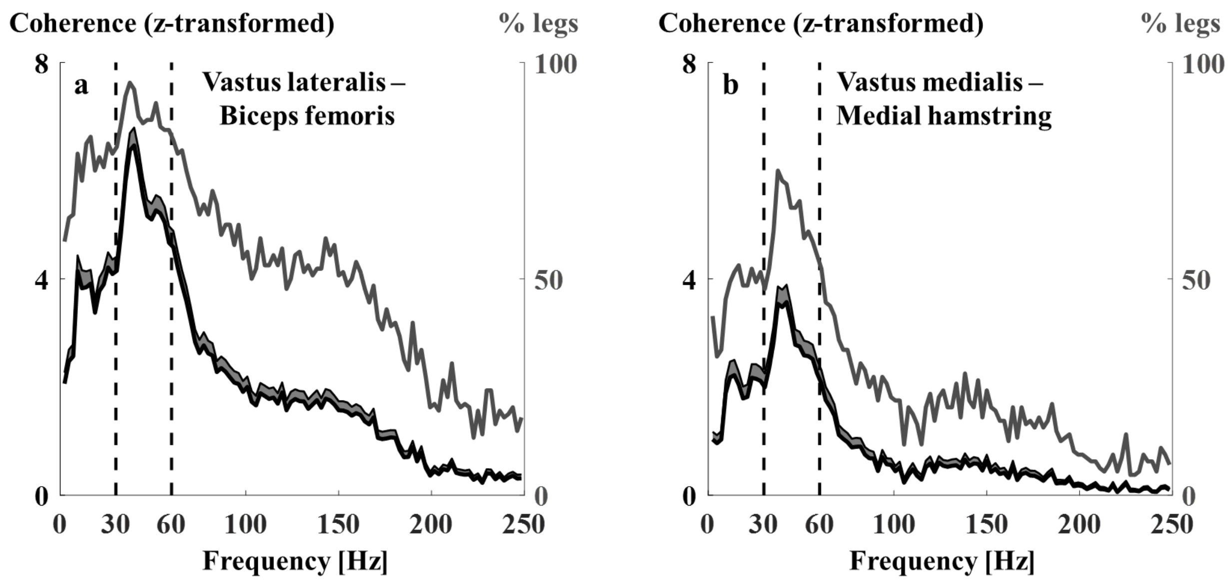

Figure 5.3. Percentage of legs with significant intermuscular coherence at each frequency bin (grey lines, right y-axes) and corresponding mean z-transformed coherence spectra across legs (black lines, left y-axes) for vastus lateralis-biceps femoris (a), and vastus medialis-medial hamstrings (b). Shaded area represents one standard error computed across legs at each frequency.

The shape of the average coherence spectra was similar for the VL-BF and VM-MH muscle pairs with the most consistent and strongest intermuscular coherence between 30-60 Hz. In the same frequency range, the gamma band, the percentage of legs that showed a significant coherence of the VL-BF muscle pairs was about 90% (64 x 2 = 128 legs). About 60% of legs showed significant VM-MH gamma-band coherence. The mean peak z-coherence between 30-60 Hz across all legs was higher for the VL-BF muscle pair (mean±SE = 6.5±0.3) compared to VM-MH (3.6±0.3).

5.3.3 Association between Q-H coherence, knee injury history, sex, and KOOS

The results of the multivariable analysis are presented in Table 5.2. Medians and ranges for VL-BF and VM-MH intermuscular coherence in the gamma-band are presented for the index leg as boxplots by injury group and sex in Figure 5.4a and 5.4b. A first general observation was that Q-H gamma-band coherence values exhibited a large between-subject variability, particularly for female individuals. There was a significant interaction effect between ‘injury group’ and ‘sex’ with respect to VL-BF gamma-band coherence (p = 0.019). While females generally showed higher VL-BF coherence compared to males (injury group median difference = 5.7, p-corrected < 0.001; control group median difference = 1.9, p-corrected = 0.19), the association between knee injury history and VL-BF coherence was different in males and females. Specifically, injured males showed a lower median VL-BF coherence compared to uninjured males while females in the injury group showed a slightly higher VL-BF coherence compared to the uninjured group (Figure 5.4a). After correction for multiple comparisons, however, the corresponding post-hoc tests were not statistically significant (median difference males = 2.6, p-corrected = 0.18; median difference females = -1.1, p-corrected = 0.41). For the VM-MH muscle pair, ln-transformed coherence values were significantly influenced by ‘sex’ (p = 0.001) but not ‘injury group’ (p = 0.89). Figure 4b shows that females showed a higher VM-MH coherence compared to males for both the injured group (median difference = 2.4) and uninjured group (median difference = 1.6). KOOS pain and symptoms scores did not show significant effects on VL-BF or VM-MH gamma-band coherence.

Table 5.2. Association between previous knee injury and quadriceps-hamstrings coherence based on multivariable linear regression

Outcome	*Constant*	*Injury Group*[a]	*Sex*[b]	*Injury x Sex*	*KOOS symptoms*	*KOOS pain*
VL-BF z-coherence	9.4 (-3.4, 22,1)	1.2 (-0.4, 2.8)	-2.1* (-4.0, -0.2)	-3.4* (-6.3, -0.6)	-	-0.0 (-0.2, 0.1)
VM-MH z-coherence[c]	0.8 (0.3, 1.3)	0.0 (-0.6, 0.7)	-1.2* (-1.8, -0.5)	-	-	-

Values represent coefficient and 95% confidence interval (CI)
* 95% CI does not encompass zero
‘-‘ variable was removed from the model after assessing for interaction with injury group and confounding
[a] Uninjured was used as the reference
[b] Female sex was used as the reference
[c] Coefficients were determined for ln(VM-MH z-coherence)

5.3.4 Side-to-side differences in Q-H coherence

Figure 5.4c and 5.4d display side-to-side differences in Q-H intermuscular coherence between the index and non-index leg by injury group and sex. For both muscle pairs, the median side-to-side differences were generally close to zero indicating no systematic differences between the index and non-index leg. The one exception were male subjects in the injury group who showed a median side-to-side difference in VL-BF coherence of -1.2, as a result of lower coherence values for the injured compared to uninjured leg in 7 out of 9 injured males with no contralateral injury (Figure 5.4c).

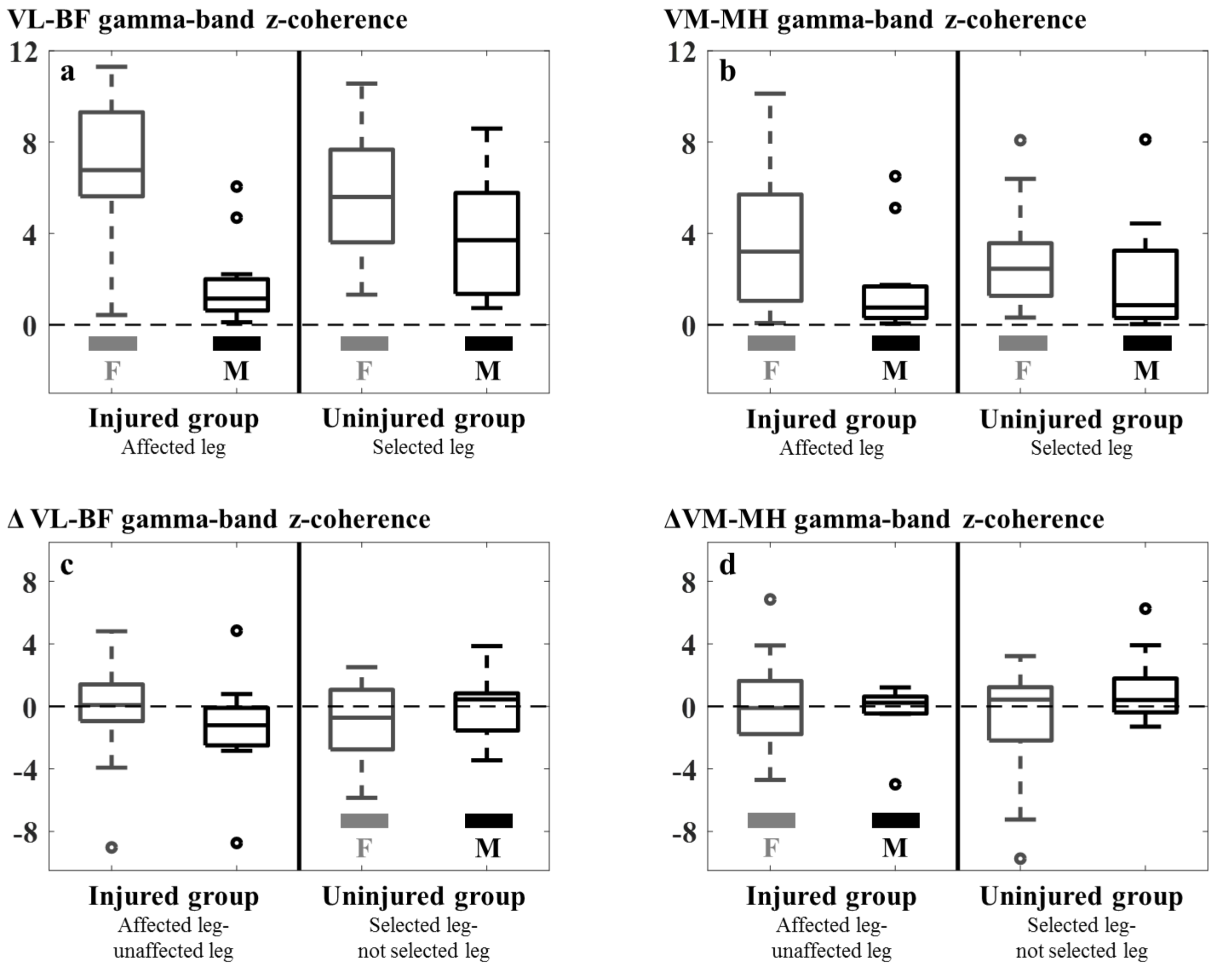

Figure 5.4. Boxplots of intermuscular coherence in the gamma band (30-60 Hz) between vastus lateralis and biceps femoris (VL-BF, a) and vastus medialis and medial hamstrings (VM-MH, b) by sex. Both graphs show the results for the affected leg of the injured group and

the selected leg of the uninjured group. Figure 4c and d show the side-to-side differences between legs for the injured group (affected-unaffected leg) and uninjured group (selected – not selected leg). Grey and black boxplots present results for females (F) and males (M), respectively. All graphs show the coherence values following z-transformation.

5.4 Discussion

This study evaluated the thigh muscle activation patterns during a SLS in participants with a previous youth sport-related knee injury 3-12 years ago in comparison to uninjured controls. Specifically, intermuscular coherence between quadriceps and hamstrings in the gamma-band frequency range was investigated as a marker for a more pronounced Q-H co-contraction strategy. Significant gamma-band intermuscular coherence between the quadricep and hamstring muscles was detected for at least two thirds of the investigated legs. However, there was no evidence to support our hypothesis that previously injured participants would generally exhibit higher Q-H gamma-band coherence compared to uninjured controls. Instead, male and female participants showed a sex-specific Q-H co-contraction strategy and possibly a sex-specific adaptation of Q-H co-contraction to a previous knee injury. These results will be discussed from a neuromuscular control perspective as well as in the context of the strengths and limitations of this study’s design and methodology.

5.4.1 Q-H intermuscular coherence during a single-leg squat

EMG-EMG coherence has been successfully used in previous studies to identify the involvement of the corticospinal tract and other neural circuits in coordinating the muscles that produce movements or accomplish stabilizing tasks (Farmer et al., 1993; Hansen et al., 2002; Clark et al., 2013; Laine & Valero-Cuevas, 2017). During the single-leg squat investigated in this study, most of the 128 investigated legs showed significant EMG-EMG intermuscular coherence in the 30-60 Hz gamma-band between vastus lateralis and biceps femoris (~ 90%) and between vastus medialis and the medial hamstrings (~ 60%). While it is known that a large portion of the neural input to the medial and lateral quadriceps muscles is shared during squatting exercises (Chang et al., 2012; Mohr et al., 2015, 2018), the consistent motor unit coupling between the antagonistic quadriceps and hamstring muscles in the gamma-band is a novel finding. Antagonistic muscle pairs, such as vastus lateralis and biceps femoris in this study, show coupled motor unit activity if they are simultaneously activated to stabilize a common joint (De Luca & Mambrito, 1987; Nielsen & Kagamihara, 1992, 1994; Geertsen et al., 2013; Pizzamiglio, De Lillo, Naeem, Abdalla, & Turner, 2017). Combined EMG and EEG recordings during ankle muscle co-contractions have suggested that descending commands of the motor cortex are responsible for coupled motor unit activity between antagonists by suppressing spinal pathways that would otherwise inhibit their coupling (Hansen et al., 2002).

During SLS, the quadriceps muscle is contracting eccentrically and concentrically to perform the downwards and upwards squatting movement, respectively. At knee flexion angles of less than 45 degrees, quadriceps forces can destabilize the knee by producing an anterior pull of the tibia with respect to the femur (Li et al., 1999). The hamstring muscles have the ability to increase knee joint stability by reducing anterior tibia translation (More et al., 1993). Furthermore, Q-H co-contraction can support knee joint loads in the frontal plane, which are typically present during single-leg squatting (Lloyd & Buchanan, 2001; Alenezi, Herrington, Jones, & Jones, 2014). Therefore, the CNS likely uses a Q-H co-contraction strategy to stabilize the knee joint during the SLS and this may be facilitated by a common, neural input from the corticospinal tract to the quadricep and hamstring muscles.

5.4.2 Association between Q-H coherence and knee injury, sex, symptoms, and pain

The functional significance of intermuscular coherence is evident as the level and frequency content of coherence is often associated with the presence or absence of neurological disorder (Farmer et al., 1993), skill-level (Geertsen et al., 2013), fatigue (Castronovoa et al., 2018), or task-specific biomechanical requirements (Mohr et al., 2015; Laine & Valero-Cuevas, 2017). This study used Q-H intermuscular coherence as a potential neurophysiological marker for a pronounced Q-H co-contraction strategy in individuals 3-12 years following a previous knee injury. Despite rehabilitation and knee surgery, individuals who have sustained a knee injury, such as an ACL rupture, may suffer from persistent quadriceps muscle inhibition, knee joint instability, and deficient hamstrings reflex arcs (Ingersoll, Grindstaff, Pietrosimone, & Hart, 2008). It was hypothesized that a strategy of the CNS to compensate for these consequences of a knee injury is to increase a shared, cortical drive to the quadricep and hamstring muscles and facilitate their antagonistic co-contraction with the goal to improve joint stability. This would likely result in increased gamma-band intermuscular coherence between these muscles. The present findings, however, suggest that a more pronounced Q-H co-contraction was not generally present in all participants with a previous knee injury. The significant 'injury group x sex' interaction effect on VL-BF gamma-band coherence suggests that a previous knee injury may affect Q-H co-contraction strategies differently in males and females. Specifically, compared to uninjured controls, previously injured males showed reduced and previously injured females showed slightly increased VL-BF gamma-band coherence. In post-hoc comparisons, these trends did not reach statistical significance and should thus be interpreted with caution. Nevertheless, the exploratory analysis of side-to-side differences supported the finding that a previous knee injury in males is associated with a reduction in VL-BF gamma-band coherence. For females, side-to-side differences did not indicate a difference between the injured and uninjured leg.

These findings disagree with previous studies reporting a persistent, pronounced Q-H co-contraction strategy in a mixed group of males and females during stair ambulation (Hall et al., 2015) and in females during drop-landing tasks (Ortiz et al., 2008; Tsai et al., 2012). Possible reasons for the disagreement may include 1) that the large-between subject variability in coherence outcomes observed for females reduced the sensitivity to observe a similar significant effect of knee injury history, and/or 2) that more strenuous tasks such as drop-landings may aggravate the need for previously injured individuals to use a stronger Q-H co-contraction strategy (Ingersoll et al., 2008). Regardless, it is questionable whether a comparison of the Q-H intermuscular coherence outcomes in this study to earlier findings is appropriate. All of the above studies used a Q-H muscle co-contraction index, which assesses the magnitude of co-contraction based on a ratio between the sEMG amplitudes of the quadricep and hamstring muscles (Rudolph et al., 2001). This ratio, however, could increase simply due to an increase in hamstring EMG intensity and a constant quadricep EMG intensity. Therefore, an increase in the Q-H muscle co-contraction index in individuals with a previous knee injury may not necessarily represent a different coordination of motor unit activity between these muscles but could just reflect a stronger neural drive to the hamstring muscle as often observed following ACL injuries (Ingersoll et al., 2008).

In addition to the observed interaction between injury group and sex, both VL-BF and VM-MH gamma-band coherence were generally reduced in males compared to females. These findings corroborate previous SLS analyses, that reported sex-specific effects of a previous ACL injury on knee and hip kinematics (Yamazaki et al., 2010) as well as systematic differences in thigh muscle activation between males and females (Zeller et al., 2003). One possible interpretation of the present findings is based on the observation that during single-leg tasks, females tend to rely more on muscles surrounding the knee to control the frontal and transverse plane alignment of the lower extremity. In contrast, males may utilize a neuromuscular strategy focused on the hip musculature, which seems to be more effective in maintaining hip and knee alignment (Zeller et al., 2003; Zazulak et al., 2005; Yamazaki et al., 2010). Therefore, it may be more common for females to activate a motor program that couples the quadricep and hamstring motor unit activity via shared neural inputs from the corticospinal tract, which could explain the observed increased Q-H intermuscular coherence in the gamma-band. For male individuals, a pronounced co-contraction between quadriceps and hamstring muscles may have not been as important due to the effective use of hip muscles such as gluteus maximus for maintaining lower extremity alignment (Zazulak et al., 2005). The further reduction of VL-BF intermuscular coherence in the previously injured leg of male participants may represent a further shift of muscular control away from the knee to the hip or ankle joint as previously demonstrated in ACL-injured individuals during walking and running (Rudolph et al., 2001; Hurd & Snyder-Mackler, 2007). Since this is the first study to compare intermuscular coherence outcomes between males and females and following a knee injury, however, the neurophysiological origin of observed differences remains speculative and requires further investigation.

Due to the lack of association between coherence outcomes and knee pain and symptoms, this study was not able to discern whether the reduced VL-BF gamma-band coherence in injured males may represent a beneficial or maladaptive neuromuscular strategy. On the one hand, a low degree of Q-H motor unit coupling may facilitate more selective motor responses of the individual quadricep and hamstring muscles and thus a better ability to react to external perturbation (Chmielewski et al., 2005; Mohr et al., 2015). On the other hand, simulation experiments have suggested that low intermuscular coherence between two co-contracting muscles is associated with out-of-phase fluctuations of the corresponding muscle forces (Santello & Fuglevand, 2004). For the knee joint it may thus be speculated, that a lower Q-H intermuscular coherence observed in injured males may result in more out-of-phase fluctuations of quadricep and hamstring muscle forces. This poor coordination of quadriceps and hamstring muscles forces may lead to relative movements of the tibia with respect to the femur (Li et al., 1999) and cause the articular cartilage to experience abnormal shear forces (Benedetti et al., 1999), a possible risk factor for knee PTOA (Chaudhari, Briant, Bevill, Koo, & Andriacchi, 2008). The effect of Q-H intermuscular coherence on the resultant forces of the knee joint is virtually unknown. Future studies aimed at exploring the clinical significance of Q-H intermuscular coherence should 1) implement simulations to provide insight into the relationship between Q-H motor unit coupling and knee mechanics, and 2) evaluate individuals with more acute knee injuries and thus more extreme knee symptoms and pain.

5.4.3 Strengths and limitations

A limitation of this study is the large variability in Q-H intermuscular coherence outcomes that remains unexplained by the investigated variables, particularly in female individuals (see Figure 4). From a methodological perspective, there are three main factors that may influence EMG-EMG coherence spectra: Data acquisition, signal processing, and cross-talk. Cross-talk between neighbouring electrodes manifests in significant bands of coherent activity that span across the entire frequency range of the EMG spectrum (Grosse et al., 2002). However, for each measured leg, there were frequencies where low or non-significant coherences were detected (see Figure 3), thus indicating, that there was no substantial cross-talk between the investigated Q-H muscle pairs. In contrast, due to the small distance of about 2-3 cm between the two sEMG electrodes on the hamstring muscles, it is possible that the biceps femoris EMG signal included a cross-talk component from the medial hamstrings and vice versa. This may have reduced our ability to obtain fully independent medial and lateral Q-H coherence spectra. However, a recent study simulated the influence of 10% of cross-talk between the vastii muscles on their intermuscular coherence and observed a negligible effect (Mohr et al., 2018). Therefore, cross-talk between the hamstring muscles may have been present but likely had a minimal effect on this study's conclusion.

Other methodological considerations related to data acquisition and signal processing techniques and their effects on intermuscular coherence outcomes have been the topic of many recent investigations (Keenan et al., 2012; Negro & Farina, 2012; Boonstra & Breakspear, 2012; Mohr, Schön, von Tscharner, et al., 2018). For example, the electrode location with respect to the muscle's innervation zone and its inclination with respect the muscle fiber pennation angle may affect the magnitude of the intermuscular coherence spectrum (Keenan et al., 2012; Mohr et al., 2018). Thigh muscles such as vastus medialis can show region-specific activation patterns (Gallina, Blouin, Ivanova, & Garland, 2017) and thigh muscle architecture such as the pennation angle may be different between males and females (Gallina et al., 2018). These are potential methodological factors that could explain the large variability in coherence outcomes but were not investigated in this study and has lowered our sensitivity to support or reject the hypothesized association between Q-H intermuscular coherence and knee injury history. Due to the novelty of this research question, future studies should be aimed at replicating the present results while further minimizing methodological influencing factors of sEMG on intermuscular coherence, potentially by using more sophisticated high-density EMG grid technology.

From a study design perspective and based on a recent review (Shanbehzadeh, Bandpei, & Ehsani, 2017), the present study is among the largest investigations of knee injury effects on quadriceps and hamstrings muscle activity. In addition to previous studies, this study also included a bilateral analysis of knee muscle activity to determine both group and side-to-side differences in muscle activity related to a previous knee injury and thus provides a full picture of knee injury effects on Q-H intermuscular coherence. The sample size of n > 60 enabled a sex-specific comparison of knee muscle activity between injured and uninjured legs, which is a strength of this study given the observed differences in neuromuscular control between males and females. However, the uneven distribution of males and females in this study (~ 70% female) resulted in a low number of available male subjects and reduced our statistical power. Therefore, the observed reduction in VL-BF coherence in previously injured males was not statistically significant and has to be confirmed by future studies. Such studies should aim to further increase the sample size of similar analyses to improve statistical power and enable the analysis of additional confounding and effect modifying variables such as time since injury and type of sports (Whittaker, Toomey, Nettel-Aguirre, et al., 2018). The skill level and type of skill/sport (e.g. weightlifters vs. musicians) have been shown to influence intermuscular coherence estimates and may provide further explanations of this study's findings, e.g. the large variability in gamma-band coherence in females or the reduced coherence seen in males (John G. Semmler, Sale, Meyer, & Nordstrom, 2004; Geertsen et al., 2013). Finally, the findings of this study may not be generalizable to older, more sedentary post-injury populations. Due to the high percentage of individuals who return to sport and frequent physical participant after a knee injury in this young population (Toomey et al., 2017), 'normal' neuromuscular control may be recovered more quickly.

5.5 Conclusions

This study investigated the EMG-EMG intermuscular coherence between medial and lateral Q-H muscle pairs during a SLS to investigate whether an increase in Q-H co-contraction persists as a joint stabilization strategy in individuals who sustained an intra-articular knee injury 3-12 years ago. A Q-H co-contraction strategy represented by higher intermuscular coherence in the 30-60 Hz frequency range was more pronounced in female compared to male individuals. This finding suggests that compared to males, females may utilize a different neuromuscular control strategy during the SLS, that relies more heavily on coupled activity between muscles surrounding the knee. This study did not provide evidence for a pronounced Q-H muscle co-contraction strategy as assessed by an intermuscular coherence analysis more than three years following a knee injury. In contrast, male participants showed slightly reduced intermuscular coherence between vastus lateralis and biceps femoris in the injured leg. The clinical relevance of this observation with respect to knee joint stability and post-traumatic knee osteoarthritis should be the focus of subsequent studies.

Chapter Six – **Classification of gait muscle activation patterns according to knee injury history using a support vector machine approach**

6.1 Introduction

Following a significant knee injury, the neuromuscular control system shows multifaceted adaptations in order to maintain movement performance despite compromised knee joint stability, reduced muscle strength, and joint pain related to the injury (Hart, Pietrosimone, Hertel, & Ingersoll, 2010b; Pietrosimone et al., 2012). During gait in the first year after a rupture of the anterior cruciate ligament (ACL), characteristic adaptations in lower extremity muscle activation have been observed frequently including reduced quadriceps muscle activation ('quadriceps avoidance') and/or increased hamstring muscle activation, and/or increased quadriceps-hamstring co-contraction during early stance. These alterations may be aimed at avoiding excessive anterior translation of the tibia in individuals who are missing the passive stability and sensory feedback provided by the ACL (Berchuck et al., 1990; Li et al., 1999; Kvist & Gillquist, 2001). At the same time, these neuromuscular adaptations permanently alter the forces that are acting across the articulating joint surfaces of the knee (Victor, Labey, Wong, Innocenti, & Bellemans, 2010). Thus, abnormalities in lower extremity muscle activation have been suggested as possible risk factors for the slow development of post-traumatic osteoarthritis (PTOA) of the knee over the course of 10-15 years following previous joint trauma (Palmieri-Smith & Thomas, 2009; Roos, Herzog, Block, & Bennell, 2011; Pietrosimone et al., 2012). However, long-term adaptations in muscle activation patterns past the first few years after knee injury are not well characterized and vary widely between studies (Limbird, Shiavi, Frazer, & Borra, 1988; Roberts, Rash, Honaker, Wachowiak, & Shaw, 1999; Lindström, Felländer-Tsai, Wredmark, &

Henriksson, 2010; Hall, Stevermer, & Gillette, 2015), which hinders an analysis of their relationship with knee osteoarthritis.

For example, Roberts and colleagues observed abnormal timing of muscle activity for the quadriceps and hamstrings muscles but not for lower leg muscles during the stance phase of gait in ACL-deficient individuals at 47 months post-injury (Roberts et al., 1999). In contrast, Lindström and colleagues showed differences in on-offset timing of the tibialis anterior and gastrocnemius but no differences for the thigh muscles between ACL-deficient individuals and a control group at 35 months post-injury (Lindström et al., 2010). Furthermore, conflicting evidence exists for the persistence of a ‘quadriceps avoidance’ gait more than one year post-knee injury (Limbird et al., 1988; Roberts et al., 1999). One underlying reason for the disagreement may be a low sensitivity to detect differences in the timing of quadriceps and hamstrings muscle activity as reported by Lindström and colleagues. Furthermore, pre-selected muscle activity variables (e.g. on-offset timing or average amplitudes) at pre-selected time intervals (e.g. 5% gait cycle increments or first/second half of stance) in previous studies may not capture the existing abnormalities in neuromuscular control in a previously injured leg (Chau, 2001).

Therefore, the aim of this study was to develop a sensitive analytic approach that enables a holistic investigation of abnormal lower extremity muscle activation patterns during gait persistent past the acute knee injury and rehabilitation period (>3 years post-injury). We suggest that the methodological approach to classify ‘normal’ and ‘abnormal’ muscular control during gait should attempt to represent the multi-muscle activation pattern of the lower extremities as a whole, taking into account the time- and frequency-dependent changes during the gait cycle as well as the interactions between muscles (Nigg & Herzog, 2007). Such patterns can be used as an input to machine learning classification algorithms such as a support vector machine, which are highly sensitive to detecting systematic differences between multi-dimensional data sets (Begg, Palaniswami, & Owen, 2005; Lai, Levinger, Begg, Gilleard, & Palaniswami, 2009). Furthermore, once such classifiers have been trained with sufficient data from individuals with and without previous knee injuries, they could be used to decide whether or not a previously injured individual still shows abnormal neuromuscular control features during gait (Begg & Kamruzzaman, 2005). Ultimately, pattern recognition and machine learning could enable an objective data-driven approach to explore the relationship between abnormal muscular control and post-traumatic knee osteoarthritis and guide rehabilitation.

Von Tscharner & Valderrabano (2010) combined a principal component analysis of lower leg EMG time-frequency patterns with a support vector machine (SVM) analysis and could successfully classify whether patterns belonged to legs with or without ankle osteoarthritis (von Tscharner & Valderrabano, 2010). The classification rate of this approach, however, only reached about 70%. This may have been because males and females were combined in their analysis. It is known that males and females show sex-specific muscle activation features during gait (von Tscharner & Goepfert, 2003; Di Nardo, Mengarelli, Maranesi, Burattini, & Fioretti, 2015) and may respond differently to musculoskeletal pathology (Yamazaki, Muneta, Ju, & Sekiya, 2010; Ko, Simonsick, Husson, & Ferrucci, 2011), which may have hindered a higher classification rate according to ankle osteoarthritis. Therefore, classifiers that are specific to either male or female subjects may be more accurate in predicting lower extremity pathologies based on muscle activation features.

The objective of this study was to test the application of a support vector machine analysis to classify multi-muscle EMG patterns of the lower extremities during gait between limbs with a previous knee injury more than three years ago and uninjured limbs. The following hypotheses were tested:

Hypothesis 1: Individuals who sustained a previous intra-articular knee injury 3-12 years ago show systematic differences in average multi-muscle patterns during gait between the affected and not affected leg.

Hypothesis 2: The affected legs of individuals who sustained a previous intra-articular knee injury 3-12 years ago show systematic differences in average multi-muscle patterns during gait compared to multi-muscle patterns legs of individuals with no history of knee injury.

Hypothesis 3: The separation and classification of average multi-muscle patterns according to knee injury history is more accurate when conducting sex-specific analyses rather than a combined analysis of males and females.

6.2 Methods

6.2.1 Study design and participants

Participants of this study were a subset of an ongoing longitudinal 3-year historical cohort study [The Alberta Youth Prevention of Early OA Study (Pre-OA Study)] (Whittaker et al., 2015; Toomey et al., 2017; Whittaker, Toomey, Nettel-Aguirre, et al., 2018; Whittaker, Toomey, Woodhouse, et al., 2018). The Pre-OA study includes 200 youth / young adults (aged 15 to 26); 100 participants who have sustained a sport-related intra-articular knee injury three to ten years ago and 100 participants with no history of knee injury, matched for age, sex, and sport. Recruitment strategies and inclusion/exclusion criteria have been described in detail in previous publications (Whittaker et al., 2015; Toomey et al., 2017; Whittaker, Toomey, Nettel-Aguirre, et al., 2018; Whittaker, Toomey, Woodhouse, et al., 2018). For this sub-study, a convenience sample of 71 individuals (34 injured, 37 uninjured) of the Pre-OA study volunteered and gave written informed consent to participate in an analysis of their leg muscle activity during gait. This study was carried out in accordance with the guidelines of the University of Calgary's Conjoint Health Research Ethics Board (#E-25075) and with the Declaration of Helsinki.

6.2.2 Gait measurements

Biomechanical and surface EMG measurements were conducted at the University of Calgary's Human Performance Laboratory. Locations for bipolar surface EMG sensors (Ag/AgCl, 10 mm diameter, 20 mm inter-electrode distance, Norotrode Myotronics-Noromed Inc., US) were identified on the vastus lateralis (VL), biceps femoris (BF), medial hamstrings (MH), gastrocnemius lateralis (GL), and gastrocnemius medialis (GM) of both legs according to standardized landmarks described in the SENIAM guidelines (Hermens et al., 1999). Before sensor placement, the skin around the identified locations was shaved, lightly abraded, and cleaned with alcohol wipes to reduce skin impedance. After sensor placement, the EMG signal quality was visually inspected and validated during a series of test movements, i.e. squats for vastus lateralis, hamstring curls for hamstrings, and calf rises for the gastrocnemii. The acceleration of a 1D-accelerometers that was taped to the lateral aspect of the right and left heel and was used to detect the time of heel strike.

Bipolar, differential EMG signals and the accelerometer signal were recorded simultaneously at 2400 samples per second. The EMG signals were amplified by a factor of 1000 and bandpass-filtered between 10-500 Hz (Biovision, Wehrheim, Germany). The measuring system was grounded by connecting the ground electrode of the system to the right tibial tuberosity. The analog signals were digitized by a 12-bit A/D converter (National Instruments, Austin, TX).

After the sensor set-up, participants walked on a treadmill (Quinton Q55, Mortara Instrument Inc., Milwaukee, WI, USA) for a duration of two minutes at a speed of 4.5 km/h. The first minute of walking was considered a warm-up and familiarization period whereas the second minute was used for EMG data acquisition.

6.2.3 EMG signal processing

All data processing steps were performed using a custom-written software in MATLAB 2017a (Mathworks, Natick, MA, USA). Fifty heel strikes (about 1 minute of walking), thus 49 gait cycles were considered per subject for the analysis. The heel strikes were determined as the onset of high frequency oscillations in the heel acceleration signal (vertical dashed lines, Figure 6.1a) (Meyer et al., 2017).

Step 1: The EMG wavelet transform

Raw EMG signals were transformed into time-frequency space using a wavelet analysis (von Tscharner, 2000; Barandun, von Tscharner, Meuli-Simmen, Bowen, & Valderrabano, 2009). Specifically, the raw EMG signals were convolved with 30 non-linearly scaled wavelets (scale = 1.6 in von Tscharner, 2000) spanning center frequencies between 1 and 500 Hz to extract the EMG power in 30 frequency bands as a function of time. The square root of the power yields the EMG intensity, which was used as a measure of muscle excitation. A typical wavelet intensity pattern of two steps is shown in Figure 6.1b and was used for further processing steps.

Step 2: Creation of low dimensional EMG intensity patterns.

A time window of EMG intensities was selected starting 30% of gait cycle duration before the current heel strike and ending 70% of gait cycle duration after the heel strike. Within this window the EMG intensities were resampled to 250 time points which yielded a time resolution, which is shorter than the time resolution of the wavelets (von Tscharner, 2000). This procedure ensured that the main activation bursts during gait were not disrupted by the edges of the constructed patterns.

Based on a study of running, the EMG frequency content can be subdivided into three frequency bands (von Tscharner, Ullrich, Mohr, Marquez, & Nigg, 2018). The wavelet center frequencies were 32, 40.9, 50.9 Hz for the low frequency band, 62, 74.1, 87.4 Hz for the mid frequency band, and 101.6, 117, 133.4, 150.8, 169.3, 188.8, 209.4, 231, 253.6, 277.3 Hz for the high frequency band. Frequencies below 25 Hz were not considered as they may be contaminated by movement artifacts (Conforto, D'Alessio, & Pignatelli, 1999). EMG intensities at higher frequencies (> 300 Hz) showed negligible contributions (Figure 6.1b) and were thus excluded from the analysis. The overall EMG intensity was obtained by summing the intensities across all 3 frequency bands and 250 time points and averaging across gait cycles. The patterns were normalized by dividing the EMG intensities by the overall EMG intensity. The normalization was necessary to make the EMG intensities comparable in magnitude between muscles and legs. Normalized EMG intensities were then averaged across the 49 gait cycles, yielding one EMG intensity pattern for each of the five muscles, representing 3 frequency bands and 250 time points. These five patterns were stacked on top of one another and can be represented as a multi-muscle pattern (MMP) shown in Figure 6.1c. Thus, there are 122 MMPs, one for each leg of the 61 participants that were used for the pattern recognition analysis.

Each MMP was reshaped into a one-dimensional vector and appended row-wise into a matrix *M*. Each vector was normalized to a length of 1 so that the patterns of each leg and subject contributed equally to the analysis. *M* consisted of 122 rows and 3750 columns with odd rows representing right leg EMG patterns and even rows representing left leg EMG patterns. Next, *M* was used as an input to a principal component analysis to further reduce the dimensionality.

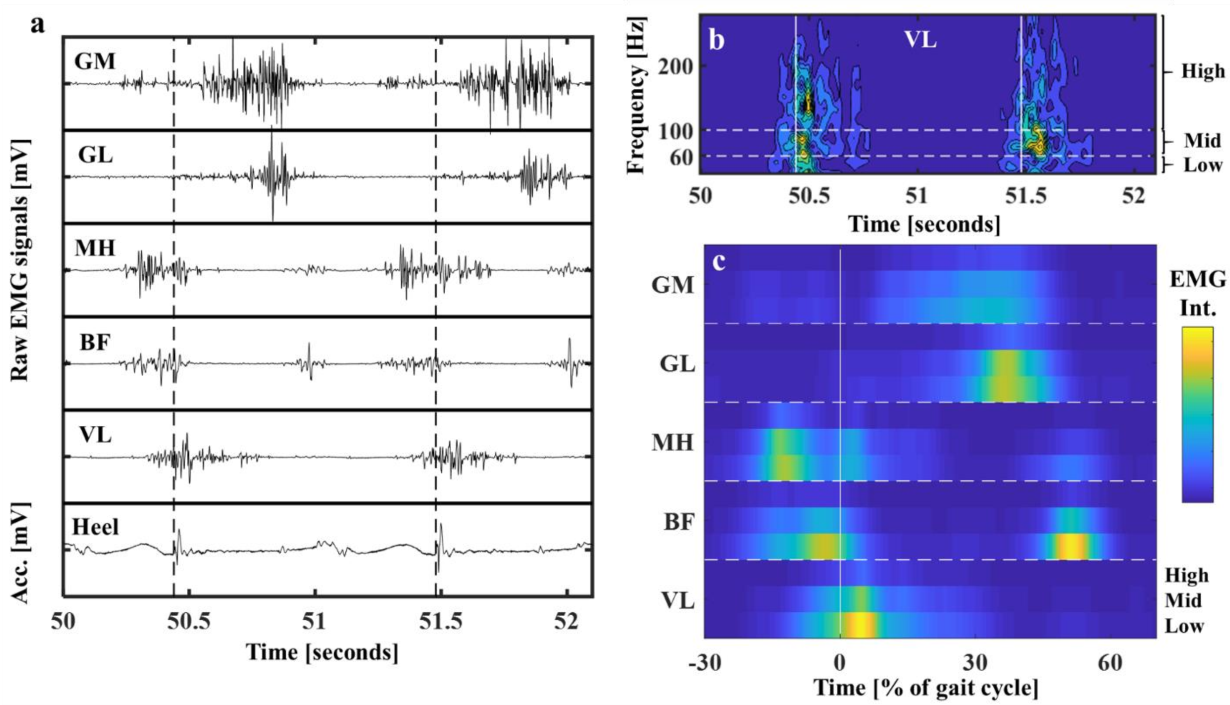

Figure 6.1. Raw EMG signals and heel acceleration for two gait cycles. Dashed, vertical lines mark the heel strikes. All y-axes have the axis limits of -0.5 to 0.5 mV (a). Wavelet-transformed EMG intensity of vastus lateralis (VL) corresponding to the shown raw signal. Solid, vertical lines mark the heel strikes. Dashed, horizontal lines mark the separation of the EMG intensity into a low, mid, and high-frequency band (b). Final, averaged, and interpolated multi-muscle pattern. Solid, vertical line represents the heel strike. Dashed, horizontal lines separate the five muscles (c). Warm and cold colors represent higher and lower EMG intensity. All graphs are displayed for one leg of a representative participant.

Step 3: Principal component analysis (PCA)

A PCA was applied to the data matrix *M* (von Tscharner, Ullrich, Mohr, Marquez, & Nigg, 2018). In summary, the mean of *M* across observations (rows) was subtracted and the eigenvectors (PC-vectors) and eigenvalues of the covariance matrix of *M* were computed. The number of PC-vectors that explained 70% of the variance in *M* and the residual mean vector, referenced as PC-vector#0, formed the base of the lower dimensional vector space. The residual mean was added because the discrimination may include the parts of the signal that are not explained by the PCs but are captured in the remaining part, which is represented by the residual mean. Because the PC-vectors represent points of the MMPs that change in a correlated way, the PC-vectors were called features (F0 = PC-vector#0, F1 = PC-vector#1, etc.). The number of features is much smaller than the points in the MMPs while containing most of their variance, which represents a successful reduction of dimensionality via the PCA. The PC-weights indicate the fraction with which each feature contributes to the MMP of a given observation (leg of a subject) and were obtained by projecting the corresponding row vector in M onto the PC-vectors. The weights are arranged in a new matrix W with 122 rows that represent the observations in M. The columns of W contain the weights, one for each of the 11 features. Thus, the first column corresponds to weights on F0, the second column to weights on F1, etc. The whitened weights of a feature (w_f) for the 122 observations were computed by dividing the weights by their standard deviation.

The matrix W with 122 whitened weights (rows) and 11 features (columns) was used for SVM comparisons between different conditions. Each previously injured participant has one injured leg (INJ_X) and one contralateral leg (INJ_C). Each uninjured participant has one non-dominant leg (CON_D) and one non-dominant leg (CON_N). The comparisons investigated in this study are shown in Table 2.

Step 4: SVM analyses

In Hypothesis 1, the assumption is that MMPs are specific to each person whereas there is a systematic side-to-side difference in MMPs that is not subject-specific as a result of the previous knee injury. To isolate side-to-side differences and reduce the influence of between-subject variation, the mean of the whitened weights across the right and left leg was subtracted for each subject so that each pair of weights was centered around zero. The centered weights were used as the input to the SVM when investigating within-subject comparisons (INJ_X vs. INJ_C and CON_N vs. CON_D, Table 2). If side-to-side differences in MMPs are randomly distributed (Null Hypothesis 1), there will be no successful SVM classification of the two conditions.

In Hypothesis 2, the assumption is that a previous knee injury results in systematic differences in MMPs between legs of injured und uninjured individuals despite between subject-variation. In this case, the whitened weights were centered by subtracting their overall mean across all subjects included in the comparison and then used as the input to the SVM (INJ_X vs. CON_D, Table 2).

Linear-kernel support vector machines (SVMs) were used to test the hypothesis that the centered weights are systematically different between legs. The SVM is a supervised learning algorithm, which determines a separating hyperplane that optimizes the margin between the two groups of the input data based on known group labels (e.g. injured vs. not injured) (Vapnik, 2000). The SVM was trained by using the *fitcsvm* function of the MATLAB 2017a Statistics and Machine Learning Toolbox with the default setting but without scaling of the input data as the whitening process was done manually. If observations of group 1 fall on one side of the optimized hyperplane and observations of group 2 on the other side, the SVM separation rate (SR) is 100%.

To assess the statistical significance and generalization of the trained SVM models, a leave-one-out (LOO) cross-validation method was used (von Tscharner & Valderrabano, 2010). During the LOO procedure, each individual of the analyzed comparison was once excluded from the data set to train the SVM. Each LOO iteration included the re-computation of the PC-vectors and corresponding weights (*Step 3* above) to ensure that the SVM training data does not include any information about the left-out observation. The MMPs of the left-out subject were then projected onto the re-computed LOO PC-vectors to determine the weights and use them as an input to the MATLB *predict* function to predict the group assignment. The number of correctly predicted test observations divided by the number of participants yields the generalized classification rate (CR).

Each assignment of a left-out observation can either be correct or wrong. Thus, a binomial test (MATLAB function *binoinv* with a probability of success $P = 0.5$) was used to assess whether the CR is statistically higher than chance at a confidence level of 99%.

Step 5: Feature selection

When using machine learning algorithms, a high risk of overfitting is typically present when the training data set contains a high number of features relative to the number of observations. To lower the risk of overfitting, we limited the number of our features using a feature selection method. SVM analyses have demonstrated that besides 3-5 important gait features that enable a successful classification, further gait features are often redundant and do not provide additional discriminatory information (Begg et al., 2005; Begg & Kamruzzaman, 2005). It was therefore decided that three features are sufficient for the classification. This bears the further advantages that 1) the SVM model can still be visually inspected in a 3D-plot, 2) each combination of three input features still explained a minimum of 10% of the original variance (see Results section). 3) it reduces the complexity of the analysis.

The selection of the three features was done using a trial and error process. There are 165 possible ways to select three features from 11 available features. Therefore, the product of separation rate and classification rate, which will be called the ‘feature selection factor’ (FS) was determined for all 165 feature combinations. Non-significant classification rates were set to zero, thus yielding FS = 0. The maximum FS was used to select the final input feature set. This feature selection approach is computationally costlier compared to the 'Hill-Climbing' approach described by Begg and colleagues (2005) who build up their feature set by iteratively adding features with discriminatory information in a feed-forward fashion. However, we noticed that the inclusion of one feature in the feature set may influence the discriminatory ability of another feature. Therefore, albeit computationally more demanding, this approach avoided erroneously excluding a feature with good discriminatory ability.

Step 6: Discriminatory multi-muscle patterns

Each SVM comparison resulted in three features that best solved the classification problem. To interpret the discriminatory multi-muscle patterns (DMP) encoded in these features, the following analytical steps were taken. For a given comparison and the selected best set of features f = 1,2,3, the linear combinations of features F_f and corresponding input weights w_f were determined for each observation k considered in the comparison according to:

$$\boldsymbol{Z}(\boldsymbol{k}) = \sum_{\boldsymbol{f}=\boldsymbol{1}}^{\boldsymbol{3}} \boldsymbol{w}(\boldsymbol{k})_{\boldsymbol{f}}' \cdot \boldsymbol{F}(\boldsymbol{k})_{\boldsymbol{f}}$$

Equation 6.1

Z(k) are reconstructed patterns with the structure of the original MMPs but contain only the muscle activation features that enabled the classification between two groups. Then, the DMP was determined as the average difference in *Z* between the individuals belonging to the first group, e.g. injured leg, and the individuals belonging to the second group, e.g. contralateral leg.

DMPs can be displayed similarly to the MMP in Figure 6.1c and indicate the location and direction of differences in muscle activation that were identified between the two groups.

6.2.4 Data sharing

Data underlying the analysis described above has been uploaded to the Mendeley public data repository (Mohr, von Tscharner, Emery, Nigg, 2018, doi:10.17632/f2fv7gb577.1, *reserved but not active*). These data include 1) raw EMG and heel strike data, 2) the matrix *M* containing the wavelet-transformed, interpolated, and amplitude-normalized multi-muscle patterns, the principal component vectors PC#0-10 (=F0-F10), the matrix *W* containing the weights *before* whitening, and anonymized labels for the grouping of patterns according to injury history, leg dominance, contralateral knee injury, and sex.

6.3 Results

Participant characteristics are summarized in Table 1. After visual analysis of the raw EMG data, ten participants had to be excluded due to defects of the recording equipment (n=6) or insufficient signal-to-noise ratio for one or two muscles (n=4). Due to these reasons 61 individuals (28 injured, 33 uninjured) were included in the EMG pattern analysis. For the within-subject comparisons in the injury group, five additional individuals had to be excluded because they had also previously injured their contralateral knee. The final sample size for this comparison was therefore n = 23 (INJ_X vs. INJ_C in Table 2). Fifteen individuals had sustained a full tear of the anterior cruciate ligament and all of them underwent surgical treatment. Other injuries included isolated meniscal injuries, medial or lateral collateral ligament injuries, and one fracture.

Table 6.1. Participant characteristics

Variable	*Not injured*		*Injured*	
n (all [female])	33	[23]	28	[16]
Age at Testing (median [range])	25	[18 29]	24.5	[19 30]
Height in cm (median [range])	169	[158 192]	167	[157 196]
Weight in kg (median [range])	71.8	[54.2 108.5]	68.2	[49.3 109.5]
Months since injury (median [range])	n/a		100	[38 150]
Primary knee injury surgery	n/a		16	
ACL reconstruction	n/a		15	
Knee injury with meniscus involvement	n/a		15	
Contralateral knee injury	n/a		5	
Stride duration [s] (median, range)	1.06	[0.96 1.18]	1.04	[0.95 1.19]

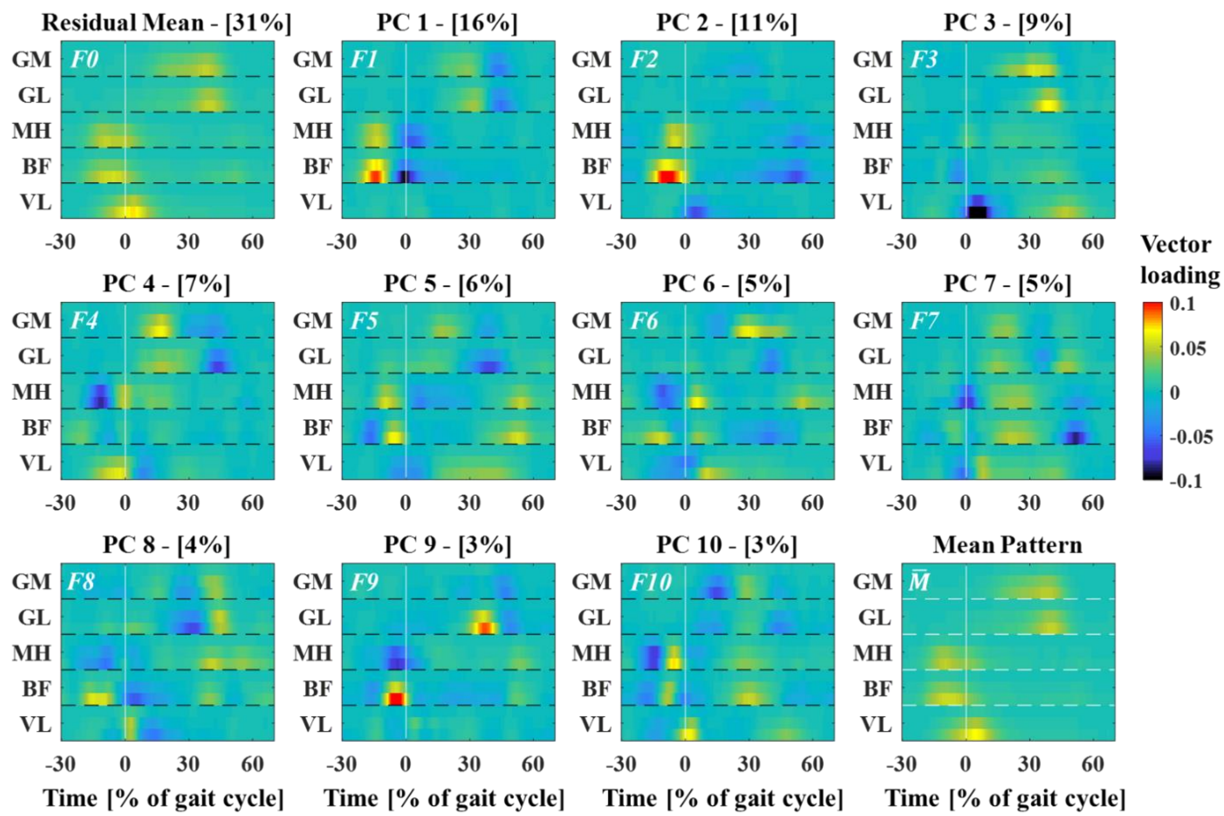

Figure 6.2. Output of the PCA. The residual mean and the first ten principal component vectors are displayed in order of their relative explained variance, shown in brackets. Their contribution to the original MMP input patterns is indicated by their color-coded PC-vector loading. These 11 patterns were called features F0-F10 and used for machine learning analyses. The bottom right image shows the average MMP $\bar{M}$ obtained from the mean of all input MMPs (2 legs for 61 subjects = 122 MMPs).

6.3.1 Principal components

Ten PC-vectors explained 70% of the variance contained in the original 122 input MMPs. The residual mean and the PC-vectors were called features 0-10 and were ordered according to their relative, explained variance. The residual mean (F0), PC-vectors (F1-F10), and the mean across all 122 input MMPs ($\overline{\mathrm{M}}$) are displayed as heat maps in Figure 6.2.

The mean MMP shows the characteristic activity bursts of the lower extremity muscles during gait, e.g. the main vastus activation burst shortly after heel strike, the main hamstrings activation burst right before and during heel strike, and the main gastrocnemius activation burst during the second half of the stance phase. The averaging procedures to calculate $\overline{M}$ removed most of the high-frequency components and EMG signal fine structure that were still visible in the wavelet patterns of individual steps in Figure 6.1b. The residual mean vector largely resembles the mean pattern and is of importance for the full reconstruction of original MMPs from the reduced set of features. An example for the interpretation of features in Figure 6.2 is as follows. If the weight on feature#1 is positive then the person exhibits higher hamstrings activation before heel strike (yellow area in Figure 6.2, PC1) and lower hamstrings activation during heel strike (blue area in Figure 6.2, PC1). This deviation in hamstrings activity before and during heel strike combined with a trade-off in the timing of gastrocnemius activity explained 16% of the total variance. Features 2 and 3 start to describe EMG intensities of the vastus lateralis as well as features that are related to muscle activity events outside of the main activation bursts, e.g. hamstrings and vastus lateralis activity during the late stance phase. Higher numbered features explain a smaller amount of variance, their interpretation becomes more complex, and they are less relevant. However, any set of three muscle activation features selected from the 11 features explained a minimum of 10% of the total variance. The whitened weights corresponding to the 11 features were used as the input for the SVM classification analysis.

Table 6.2. SVM algorithm and feature selection output for all comparisons

Comparison (G1, G2)	*Participant Group*	*Sample size (G1, G2)*	*Critical CR** [%]*	*Selected feature set*	*Explained variance [%]*	*SR [%]*	*CR** [%]*	*FS*
*INJ_X, INJ_C**	All Subjects	23, 23	74	4,8,10	15	78	83	65
	Females	13, 13	85	2,3,9	34	100	100	100
INJ_X, CON_D†	All Subjects	28, 33	66	no solution	-	-	-	-
	Females	16, 23	69	no solution	-	-	-	-
*CON_N, CON_D**	All Subjects	33, 33	70	2,9,10	22	79	70	55
	Females	23, 23	74	0,2,3	56	78	78	61

SR – separation rate, CR – classification rate, FS – feature selection factor
* within-subject design; † between-subject design.
** classification rates were significant if they exceeded the 'Critical classification rate' as determined by a binomial test with a 99% confidence level.

6.3.2 SVM and feature selection

Table 2 summarizes the separation rate (SR), classification rate (CR), and feature selection factor (FS) of the selected best feature sets and their explained variance for all comparisons of this study. Classification rates above the critical classification threshold were obtained when comparing the injured and uninjured legs of the injury group (INJ_X vs. INJ_C) as well as the dominant and non-dominant legs of controls (CON_N vs. CON_D) but not when comparing individuals with and without a previous knee injury (INJ_X vs. CON_D). Therefore, feature sets that classify between a previously injured leg and the dominant leg of a control subject could not be obtained. The feature sets that led to the highest product of separation and classification rate (feature selection factor, FS) were different for all four successful classifications. The variance explained by selected features was generally higher when considering only female individuals

(34% for INJ_X vs. INJ_C, 56% for CON_N vs. CON_D) compared to all individuals (15% for INJ_X vs. INJ_C, 22% for CON_N vs. CON_D). The classification rates and feature selection factors were higher for the comparison of injured vs. uninjured legs compared to non-dominant vs. dominant legs (Table 2). For 100% of female individuals with a knee injury history, the injured and uninjured legs could be separated and classified successfully by the SVM algorithm.

6.3.3 Discriminatory multi-muscle patterns

Figure 6.3 shows the discriminatory multi-muscle patterns (DMPs) that enabled to separate and classify the original MMPs between injured and uninjured legs of individuals with a previous knee injury (Figure 6.3, a-b) and non-dominant and dominant legs of healthy controls (Figure 6.3, c-d). The discrimination was most clear when comparing the previously injured and contralateral legs in female individuals. Compared to the contralateral leg, muscle activation of the previously injured leg was mostly characterized by a lower vastus lateralis activation during the first 10% of stance combined with a stronger biceps femoris activation before heel strike and stronger medial hamstrings activation during heel strike (Figure 6.3b). When considering both male and female injured individuals, differences in muscle activation were less clear but included a lower medial hamstring and higher biceps femoris activity before heel strike and a difference in timing of medial gastrocnemius activity for the injured compared to contralateral leg (Figure 6.3a). DMPs were not pronounced for the comparison of the non-dominant and dominant legs of healthy controls. For the whole group as well as for female subjects separately, the non-dominant leg showed slightly higher vastus lateralis activation post-heel strike and lower hamstring activity pre-heel strike compared to the dominant leg (Figure 6.3, c-d).

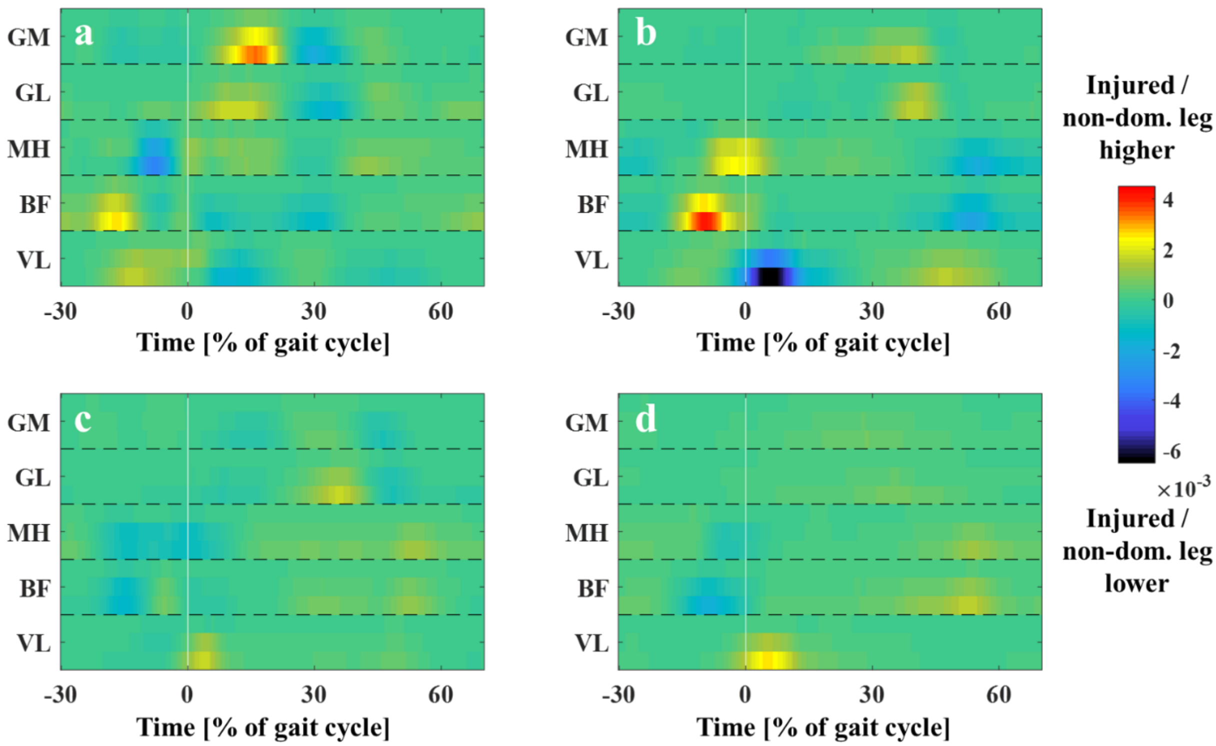

Figure 6.3. Discriminatory multi-muscle patterns reconstructed from the combination of three features that enable the best SVM separation and classification of multi-muscle EMG patterns between a) INJ_X vs. INJ_C (n = 23), b) INJ_X vs. INJ_C, female only (n = 13), c) CON_N vs. CON_D (n = 33), d) CON_N vs. CON_D, female only (n = 23). The color scale is constant in all images and was selected based on the highest and lowest values across all four comparisons.

6.4 Discussion

In this study, a pattern analysis of lower extremity muscle activation patterns during gait was applied and combined with a support vector machine analysis to distinguish patterns from individuals with and without a previous knee injury 3-12 years ago. The aim of this analysis was to test whether abnormal muscle activation features still exist in individuals more than three years following intra-articular knee injury and whether these features can be used to predict if a newly measured pattern belongs to an injured or uninjured leg. The findings support our first hypothesis that muscle activation features can be used to predict whether an activation pattern recorded from a previously injured subject belongs to the affected or unaffected leg. In contrast, muscle activation features could not be used to successfully classify whether an activation pattern belonged to a previously injured individual versus a control subject with no knee injury history and thus falsifying our second hypothesis. Finally, and in support of our third hypothesis, the classification according to knee injury history was more accurate when conducting a sex-specific analysis of females compared to an overall analysis of males and females.

6.4.1 Injured leg vs. contralateral leg

When comparing muscle activation features during gait between the previously injured leg and contralateral leg of individuals with a knee injury 3-12 years ago, the pattern recognition approach presented in this study classified over 80% of the injured and contralateral legs correctly. This indicates that the previously injured leg still shows systematic differences in muscle activity surrounding the knee joint more than three years post-injury corroborating earlier results (Limbird et al., 1988; Roberts et al., 1999; Lindström et al., 2010; Hall et al., 2015). The discriminatory pattern consisted of many subtle differences in activation distributed over all measured muscles at different time intervals, since they only explained a small portion of the variance contained in the original MMPs. In comparison, the separate analysis of females resulted in a higher classification rate of 100%, more explained variance by the selected features, and a different, more obvious discriminatory multi-muscle pattern. In support of previous reports (Yamazaki et al., 2010), this indicates that the neuromuscular adaptations observed for female individuals were sex-specific and were not shared by all male individuals. In turn, the training of a linear classifier for the assignment of lower extremity muscle activation patterns to the injured vs. uninjured leg was less successful when both sexes were combined in the analysis. Consequently, the discriminatory muscle activation features presented in Figure 6.3a are likely a mix of features that are characteristic for male and female gait adaptations and should thus not be generalized. Instead, these findings suggest that future analyses should always be designed to examine sex-specific gait patterns following a knee injury.

The specific neuromuscular differences that were observed for previously injured female individuals were characterized by reduced quadriceps (VL) activity during early stance and an increase in hamstring activity before and during heel strike, thus not at the same time in the gait cycle. In contrast, a simultaneous increase in quadriceps and hamstring activation, i.e. co-contraction, was not observed. Thus, we can confirm that in female individuals with a previous knee injury, 'quadriceps avoidance' and 'increased hamstrings activity' but not 'increased quadriceps-hamstrings co-contraction' persist more than 3-12 years following knee injury (Limbird et al., 1988; Beard et al., 1996; Bulgheroni, Bulgheroni, Andrini, Guffanti, & Castelli, 1997; Bulgheroni, Bulgheroni, Andrini, Guffanti, & Giughello, 1997; Ferber, Osternig, Woollacott, Wasielewski, & Lee, 2002; Knoll et al., 2004; Hurd & Snyder-Mackler, 2007b; Hall et al., 2015). Since this study used gait cycle-averaged, amplitude-normalized MMPs as the input to the pattern recognition and machine learning analysis, the identified differences in muscle activity have to be interpreted from a probabilistic point of view (von Tscharner & Valderrabano, 2010). For example, increased biceps femoris EMG intensity pre-heel strike on the injured side could represent 1) a more consistent biceps femoris EMG intensity peak at this time point across gait cycles, and/or 2) higher biceps femoris EMG intensity relative to the average intensity during walking. The first scenario may result from a less variable neuromuscular control program for the biceps femoris whereas the second scenario may result from an increased number of recruited motor units or a more synchronized activation of multiple motor units of the biceps femoris (De Luca, 1997; Asmussen et al., 2018). This analysis, however, could not discern the neurophysiological origin of differences in EMG intensity, which provides targets for future studies. Regardless, changes in quadriceps and hamstring muscle activity most likely alter tibio-femoral kinematics and articular contact forces, which may facilitate the degradation of knee joint tissues and increase the risk for future PTOA (Andriacchi, Briant, Bevill, & Koo, 2006; Wellsandt et al., 2016). Previous analyses of previously injured individuals in this cohort have reported reduced self-reported knee joint health and function as well as a higher incidence of MRI-defined knee OA, which is consistent with a trajectory towards clinical knee OA (Whittaker et al., 2015; Whittaker, Toomey, Nettel-Aguirre, et al., 2018; Whittaker, Toomey, Woodhouse, et al., 2018). The methodological approach presented here may be a useful tool to assist the monitoring of progress in neuromuscular rehabilitation following a knee injury or to assess the risk of developing PTOA. Specifically, a classification model trained by a sufficiently large data set ($n > 100$) could be used to decide if the previously injured leg still shows abnormal muscle activation patterns during gait in comparison to the uninjured leg.

When comparing the MMPs between the non-dominant and dominant legs of individuals with no knee injury history, the feature based-SVM approach also resulted in successful classifications regarding leg dominance. This was not necessarily surprising since limb dominance has been shown to systematically influence muscle activity of the lower extremities during gait (Õunpuu & Winter, 1989). Even though scientific debate remains related to specific biomechanical functions of the dominant and non-dominant legs during gait (Sadeghi, Allard, Prince, & Labelle, 2000), the SVM discrimination between the two in the current study helps to put the knee injury analysis into perspective. For comparisons involving all subjects or female subjects separately, the classification and separation rates were lower when classifying according to leg dominance compared to knee injury history. Similarly, the discriminatory patterns in Figure 6.3 related to leg dominance demonstrated less pronounced average differences in muscle activation features compared to patterns related to knee injury history. Therefore, systematic side-to-side differences in muscle activation between the injured and uninjured leg of individuals with a knee injury 3-12 years ago, specifically females, are not observed to the same degree in individuals with no knee injury history, thus emphasizing the relevance of these neuromuscular differences in injured subjects.

6.4.2 Injured leg vs. healthy control

In contrast to the successful within-subject classifications of injured and uninjured legs, no significant classification rates were obtained when comparing the muscle activation patterns between the previously injured leg with the dominant leg of healthy controls. This was true for analyses including all subjects and only female subjects and is in contrast to other previous machine learning analyses, which resulted in successful classifications of kinematic or electromyographic gait patterns between individuals with and without ankle osteoarthritis (von Tscharner & Valderrabano, 2010), idiopathic hip osteoarthritis or rheumatoid arthritis (Nair, French, Laroche, & Thomas, 2010), and patellofemoral pain (Lai et al., 2009). This suggests that the natural between-subject variation in the investigated muscle activation features among the previously injured and uninjured individuals was larger than any potential systematic variation due to knee injury history, thus prohibiting the training of a successful classifier. The discrepancy to former studies likely results from the fact that these authors investigated musculoskeletal conditions such as end-stage osteoarthritis, which lead to much more dramatic effects on neuromuscular control during movement that are larger than natural between-subject variation and can thus be detected by machine learning analyses. A second methodological reason for the successful classifiers in previous studies may be that non-linear machine learning techniques such as neural networks (Nair et al., 2010) or SVMs with non-linear kernel functions (von Tscharner & Valderrabano, 2010) were applied. Such techniques can improve the classification rates between two classes but the exact features responsible for the classification cannot easily be reconstructed (von Tscharner & Valderrabano, 2010; Stirling, von Tscharner, Kugler, & Nigg, 2011a). While such methods could likely improve the classification rates in this study, it was our intention to keep the machine learning procedure as simple as possible so that identified differences can still be displayed and potentially be used by clinicians to guide the rehabilitation process. The findings of this study suggest that either systematic differences due to a previous knee injury 3-12 years ago were too small to be resolved by the applied technique or long-lasting systematic effects of a previous knee injury on the neuromuscular system are resolved in this young population more than three years post-injury.

6.4.3 Methodological considerations

In this study, the decision was made to only include the first 10 PCs to explain 70% of the original variance. Furthermore, the fine-structure of the EMG signal particularly in the high-frequency band was largely reduced due to the averaging across gait cycles. Finally, we set the significance level for the assessment of classification rates to 1% to lower the risk of type 2 error when testing 165 feature sets. These three steps represent a conservative approach and were aimed at 1) avoiding misleading conclusions due to overfitting of the data and 2) ensuring that identified feature sets still explained a minimum of 10% of the original variance. Despite the conservative approach, high classification rates of 80-100% were achieved, which emphasizes the sensitivity of the applied techniques. With the present sample size, this study already belongs to the largest EMG investigations during gait following knee injuries (Shanbehzadeh, Bandpei, & Ehsani, 2017). Nevertheless, larger sample sizes would enable a direct comparison of sex-specific discriminatory multi-muscle patterns and inform the most important aspects of neuromuscular rehabilitation in males and females following a knee injury.

Other studies that focus on a more homogeneous injury history than this study, e.g. isolated ACL rupture with hamstring tendon graft reconstruction may observe more systematic injury dependent differences. Furthermore, the findings of this study may not be generalizable to older, more sedentary populations. A previous analysis of this PrE-OA cohort has shown that despite lower physical activity levels compared to controls, the previously injured sample is highly physically active with over 87% participation in sports at the time of testing (Toomey et al., 2017). Participation in physical activity may have helped this population to resolve abnormal neuromuscular control pattern more quickly. Nevertheless, it may be the more physically active populations for which abnormal neuromuscular control following knee injuries represents a more relevant problem in the context of risk for re-injury or post-traumatic osteoarthritis.

6.5 Conclusions

Lower extremity muscle activation patterns during gait from individuals with and without a previous knee injury 3-12 years ago were used to train linear SVM classifiers. Systematic side-to-side differences in neuromuscular control still existed more than three years post-injury and thus enabled a successful recognition of affected and unaffected legs. Differences in patterns between injured and uninjured legs in female individuals were characterized by increased hamstring activity before heel strike and reduced vastus lateralis activity post-heel strike. Male individuals did not show the same neuromuscular adaptations to a knee injury and thus future studies should always conduct sex-specific analysis of knee injury effects on gait patterns. The presented machine learning approach may be used to guide post-injury rehabilitation and inform the presence or absence of abnormal neuromuscular patterns in the injured compared to the contralateral leg. In contrast, the trained classifiers were not able to successfully discriminate between muscle activation patterns that belonged to a previously injured leg vs. the dominant leg of healthy controls. As compared to controls, neuromuscular gait adaptations to a previous knee injury are either resolved or not systematic at more than three years post-knee injury.

Chapter Seven – **Summary and Conclusions**

This thesis aimed to use surface electromyography (sEMG) techniques to determine if individuals who sustained a previous knee injury more than three years ago still show altered lower extremity muscle activation patterns during functional movement tasks. The motivation was to explore the potential role, that persistent, abnormal neuromuscular control of the knee joint following joint trauma may play for the development of post-traumatic knee osteoarthritis (PTOA) of the knee.

7.1 Summary of experimental studies

When comparing sEMG-based variables to describe neuromuscular control strategies between two experimental conditions, i.e. previous knee injury vs. no history of knee injury, the total variance in outcome variables may be partitioned into 1) variance due to injury history (injury vs. no injury, type of injury, etc.), 2) variance due to other sources of between-subject variability (sex, age, type of sport, etc.), and 3) variance due to random and/or systematic measurement error. If the second and third sources of variance become too large, the primary effect of interest will not be observed. The influence of between-subject variability is difficult to avoid in human studies but may be reduced by the appropriate study design (e.g. matching) or data analysis (e.g. accounting for covariates in a linear regression). The third source of variance arises, among other factors, from an error associated with the measurement technique. For the purpose of this thesis, sEMG was the primary measurement technique. To substantiate conclusions related to knee injury history, the first part of this thesis aimed to investigate the influence of methodological factors on sEMG-based outcome variables that can be used to describe neuromuscular control strategies of the lower extremities, i.e. muscle co-contraction indices and intermuscular coherence.

The specific aims of the first part were (1a) to determine the reliability of a commonly used sEMG-based measurement, named the muscle co-contraction index, during gait in individuals with and without a knee injury history, and (1b) to elucidate the effect of different sEMG acquisition techniques on the magnitude, reliability, and sensitivity of a measure of intermuscular synchronization between knee extensor muscles during squatting exercises in healthy individuals.

The third and fourth studies incorporated lessons learned from the first methodological part and investigate if altered lower extremity muscle activation patterns are present in individuals with a previous knee injury compared to an uninjured control group. Both studies were conducted as part of the 'Alberta Youth Prevention of Early OA Study (PrE-OA Study)', an ongoing, longitudinal cohort study with the goal to determine long-term health outcomes following knee injury in youth. The Pre-OA Study includes a variety of structural, clinical, and functional outcome variables (Whittaker, Woodhouse, Nettel-Aguirre, & Emery, 2015; Toomey et al., 2017; Whittaker, Toomey, Nettel-Aguirre, et al., 2018; Whittaker, Toomey, Woodhouse, et al., 2018). Studies three and four included a participant subset of 71 young adults who were recruited from the cohort of the PrE-OA Study with more than 200 participants. About half of this cohort had sustained a sport-related, intra-articular knee injury 3-12 years before and the other did not have a history of sustaining a knee injury.

The specific aims of the second part were (2a) to investigate the association between a knee injury history, sex, and knee pain and symptoms on the degree of quadriceps-hamstrings co-contraction as quantified by an intermuscular coherence analysis between the respective sEMG signals during a single-leg squatting task, and (2b) to apply a pattern recognition analysis to compare lower extremity multi-muscle activation patterns during gait between individuals with and without a knee injury history.

The following subsections will review the main conclusions of each specific aim, evaluate the limitations associated with each conclusion, and synthesize the findings to comment on the role of abnormal neuromuscular control of the knee following joint trauma for the development of knee osteoarthritis.

7.2 Reliability of a muscle co-contraction index

Muscle co-contraction indices are frequently used in the clinical biomechanics literature to investigate whether individuals with a knee injury history exhibit abnormal levels of muscle co-contraction, specifically between medial and lateral quadricep and hamstring muscle pairs, compared to individuals with no previous knee injury (Rudolph, Axe, Buchanan, Scholz, & Snyder-Mackler, 2001; Hurd & Snyder-Mackler, 2007; Ortiz et al., 2008; Sturnieks, Besier, & Lloyd, 2011; Tsai, McLean, Colletti, & Powers, 2012; Hall, Stevermer, & Gillette, 2015). These indices are typically based on the sEMG signal amplitudes from the examined muscles, which under certain circumstances are related to their produced muscle force (Lippold, 1952; Hof & van den Berg, 1977; Potvin & Brown, 2004). Due to the many physiological and non-physiological aspects that can influence sEMG amplitudes (De Luca, 1997), however, amplitude-based variables such as the muscle co-contraction index may exhibit poor test-retest reliability when obtained on different days (Ball & Scurr, 2010; Murley, Menz, Landorf, & Bird, 2010). The first study quantified the absolute and relative within- and between-day measurement error of muscle co-contraction indices between knee flexors and extensors during gait in 10 young adults with a previous knee injury and 10 young adults with no knee injury history. Co-contraction indices were derived from sEMG amplitudes of the examined muscles that were normalized to the corresponding peak amplitudes during maximum voluntary isometric contractions (MVIC) in a dynamometer. This represents a state-of-the-art sEMG gait protocol in clinical biomechanics research (Burden, 2010). The main finding was that such co-contraction indices exhibit poor reliability and substantial measurement errors of up to 300% when measured on two different days. In contrast, acceptable reliability and much smaller measurement errors (~50%) were observed between two subsequent measurements on the same day that were both normalized to the same MVIC normalization value. Consequently, a main source of measurement error was identified in the MVIC normalization procedure, which does not allow the researcher to obtain a reliable reference value for the normalization of sEMG amplitudes during gait and as a result, introduces substantial variation in the derived co-contraction indices. A systematic effect of knee injury history on measurement errors in co-contraction indices was not observed. It was concluded that sEMG amplitude-based co-contraction indices should only be used when investigating within-subject designs, that do not require the reapplication of sEMG electrodes and thus allow a relative comparison of sEMG amplitudes without MVIC normalization. In turn, the use of sEMG amplitude-based co-contraction indices was not recommended for studies that involve repeated measurements on different days or comparisons of independent groups.

7.3 sEMG recording techniques and intermuscular coherence

Due to the poor reliability of sEMG amplitude-based co-contraction indices, this analysis was not further considered for investigating abnormal neuromuscular control of the knee between individuals with and without a previous knee injury. Intermuscular EMG-EMG coherence may be used as an alternative measure of co-contraction between quadricep and hamstring muscles with the potential to give insight into neural adaptations to a knee injury. Like the sEMG amplitude, however, the frequency information encoded in the sEMG signal may also be influenced by methodological factors related to EMG data acquisition and analysis, e.g. sEMG electrode placement or sweat accumulation on the skin (Abdoli-Eramaki, Damecour, Christenson, & Stevenson, 2012; Keenan, Massey, Walters, & Collins, 2012). To lower the influence of these methodological factors, previous studies have suggested that monopolar instead of bipolar sEMG recordings obtained with an EMG current instead of traditional potential amplifier may be more suitable for the study of intermuscular EMG-EMG coherence (von Tscharner, Maurer, Ruf, & Nigg, 2013; Mohr, Nann, von Tscharner, Eskofier, & Nigg, 2015). Therefore, the second study of this thesis compared three sEMG data acquisition systems, i.e. (1) bipolar with potential amplifier, (2) monopolar with potential amplifier, and (3) monopolar with current amplifier, with respect to the measurement of EMG-EMG intermuscular coherence between the vastus lateralis and medialis muscles during a stable and an unstable squatting exercise in healthy, young adults. The main finding was that monopolar configurations resulted in higher intermuscular coherence compared to the bipolar configuration irrespective of the amplifier used. The difference in coherence could not be explained by the presence of more cross-talk in monopolar signals since the addition of simulated cross-talk components had a negligible effect on coherence outcomes. Instead, it was speculated that reduced intermuscular coherence in bipolar signals resulted from random errors in the alignment of the bipolar electrodes with the muscle fiber direction, which may alter the information content in the bipolar sEMG signals and thus, the ability to detect intermuscular coherence (Rainoldi et al., 2000; Gallina, Merletti, & Gazzoni, 2013; von Tscharner, 2014). Further, more spatial filtering in the bipolar system due to differential amplification likely reduced the electrode detection volume and thus the number of MUs that contributed to the intermuscular coherence estimate (De Luca & Merletti, 1988). Therefore, it was concluded that monopolar recordings provide a more ‘global’ view on correlated MU activity at the whole muscle level. On the other hand, bipolar EMG recordings, particularly in combination with additional spatial filtering techniques may provide better information on the behavior and synchronization of smaller groups of MUs. When comparing between the stable and unstable squatting condition, only bipolar potentials and monopolar currents showed high sensitivity to detect an increased intermuscular coherence during unstable squatting. Therefore, both bipolar potential and monopolar current measurements were considered suitable to study a change in intermuscular coherence between two movement conditions. The monopolar potential system did not show good sensitivity, potentially due to its susceptibility to recording other potentials from the measurement environment (von Tscharner et al., 2013), and was not considered a useful system for intermuscular coherence measurements.

The limitation of this study was the absence of measurements to quantify the activity of individual MUs, e.g. via intramuscular EMG or high-density sEMG in combination with decomposition algorithms (Laine, Martinez-Valdes, Falla, Mayer, & Farina, 2015; Dideriksen et al., 2018). Such measurements could have helped to indicate if the higher intermuscular coherence based on monopolar currents or the lower intermuscular coherence based on bipolar potentials provides a more valid estimate of the 'true' underlying degree of shared, neural input from the CNS to the muscles of interest. However, technologies that can capture the activity of many individual and concurrently active MUs within a muscle are in their infancy and generally restricted to recordings during isometric contractions (De Luca, Chang, Roy, Kline, & Nawab, 2014; Farina, Negro, Muceli, & Enoka, 2016). Future research should be aimed at developing methodologies that can quantify the activity of individual MUs and their association with brain activity during movement. Recent evidence suggests that a history of knee injury leads to a wide range of adaptations in neural pathways at the spinal and cortical level and that these adaptations are partially responsible for long-lasting abnormalities in muscle activation and knee joint loading patterns possibly associated with knee PTOA or risk of re-injury (Needle, Lepley, & Grooms, 2017; An, 2018). Therefore, a better understanding of how the CNS organizes the neural input to the MUs of multiple muscles during complex movement tasks in healthy and injured populations will be crucial for the development of targeted rehabilitation aimed at resolving CNS maladaptation post-injury.

Despite the possible advantages of measuring monopolar EMG currents in certain situations as described above, the traditional, bipolar potential EMG amplifier was used for the remainder of this thesis. The reasons this system was used were: 1) The available technology to measure monopolar EMG currents in our laboratory is limited to measuring a maximum of two muscles at a time, and 2) The problem of movement artefacts in monopolar recordings during gait measurements has yet to be solved.

7.4 Quadriceps-hamstrings co-contraction following a knee injury

A pronounced quadriceps-hamstrings (Q-H) co-contraction strategy has been observed in individuals with the goal to increase joint stability following a recent knee joint injury (Rudolph et al., 2001; Hurd & Snyder-Mackler, 2007). Due to the change in knee joint contact mechanics caused by increased Q-H co-contraction (Li et al., 1999; Victor, Labey, Wong, Innocenti, & Bellemans, 2010), a persistent co-contraction strategy after the acute knee injury phase was speculated to be a risk factor for the slow development of knee PTOA (Chmielewski, Hurd, Rudolph, Axe, & Snyder-Mackler, 2005; Tsai et al., 2012). Long-lasting effects of a previous knee injury on Q-H co-contraction after the acute injury and rehabilitation phase (i.e., greater than three years post-injury), however, are not well characterized. The second study showed that the intermuscular coherence between the bipolar sEMG signals of the vastii muscles is a sensitive measure to quantify changes in the coordination of MU activity between these muscles during squatting. Moreover, and in contrast to co-contraction indices based on the sEMG amplitude, intermuscular coherence spectra can give insight into the neural mechanisms underlying a muscle co-contraction strategy (Pizzamiglio, De Lillo, Naeem, Abdalla, & Turner, 2017). Specifically, higher EMG-EMG intermuscular coherence between two antagonistic muscles indicates a stronger coupling of MU activity between them (Hansen, Hansen, Christensen, Petersen, & Nielsen, 2002; Geertsen et al., 2013).

The third study applied an intermuscular EMG-EMG coherence analysis to determine Q-H co-contraction during single-leg squatting in individuals with and without a previous knee injury 3 to 12 years prior to the test date. Further, the influence of sex and the severity of knee pain and symptoms on the association between knee injury history and Q-H co-contraction was characterized. The main finding was that Q-H intermuscular coherence in the gamma range (30-60 Hz) was significantly higher in females compared to males and that the association between knee injury history and Q-H coherence was modified by sex. Specifically, a previous knee injury in males was associated with reduced gamma-band coherence between vastus lateralis and biceps femoris while females did not show this effect. Neither males, nor females showed an influence of knee symptoms or knee pain on Q-H coherence outcomes. Sex-specific neuromuscular strategies to perform the single-leg squat as well as sex-specific movement adaptations to a previous knee injury during single-leg squatting have been observed before (Zeller, McCrory, Ben Kibler, & Uhl, 2003; Yamazaki, Muneta, Ju, & Sekiya, 2010). This study provides corroborating evidence that females use a different strategy for the coordination of quadricep and hamstring muscle activity during the single-leg squat compared to males. Previous authors noted that females tend to utilize more thigh muscle activity while males tend to use more hip muscle activity for controlling lower extremity alignment during single-leg tasks (Zeller et al., 2003; Zazulak et al., 2005). Therefore, it was speculated that a more knee muscle-focused single-leg squatting strategy in females required a stronger common, neural drive from the CNS to the quadricep and hamstring muscles, which resulted in higher Q-H gamma-range intermuscular coherence (Hansen et al., 2002; Clark, Kautz, Bauer, Chen, & Christou, 2013; Geertsen et al., 2013).

In disagreement with previous studies that observed a persistent increase in Q-H co-contraction more than one year following a knee injury (Ortiz et al., 2008; Tsai et al., 2012; Hall et al., 2015), this study did not provide evidence for increased Q-H coherence post-knee injury. The disagreement may be due to the different approaches and concepts to determine co-contraction. Previous studies used sEMG-amplitude based co-contraction indices discussed in 7.2, which typically represent the ratio of average quadriceps and hamstrings EMG intensity during a certain time period (Rudolph et al., 2001). This ratio can change because of an isolated increase/reduction of muscle activity in either the quadricep or the hamstring muscles but does not necessarily represent a more or less pronounced strategy of the CNS to couple the MU activity of these muscle groups. In contrast, the degree of gamma-band intermuscular coherence during movement has been used as a marker for a coupling of MU activity between two muscles as a result of a shared, neural input from supraspinal sources (Clark et al., 2013; De Marchis, Severini, Castronovo, Schmid, & Conforto, 2015; Pizzamiglio et al., 2017). Based on the latter approach, Q-H co-contraction did not seem to be increased 3-12 years following a knee injury. Intermuscular coherence outcomes, however, were characterized by a large between-subject variability, particularly for females. Large variability in coherence outcomes may have originated, partially, from random errors in the alignment of the bipolar sEMG electrodes as discussed in section 7.3 of this thesis. This would lower the sensitivity to detect differences in coherence outcomes and possibly bias leg and group comparisons towards failing to reject the null hypothesis.

In contrast to previous studies, males with a knee injury history showed reduced Q-H co-contraction for their injured side compared to their uninjured side and compared to uninjured controls. This observation hints at a maladaptation of the CNS to a knee injury and associated symptoms that is still present 3-12 years later (Needle et al., 2017; An, 2018). Due to the absence of an association between Q-H coherence and knee pain and symptoms as well as an unknown relationship between Q-H coherence and knee joint mechanics and/or stability, the clinical significance of this finding requires further investigation. Further, due to the small sample size of the male group, i.e. 11 previously injured males, this study did not have enough statistical power to substantiate the observed reduction in Q-H intermuscular coherence. Future studies should overcome the limitation of a small sample size and investigate intermuscular coherence outcomes between groups that exhibit larger differences in knee function, e.g. in individuals closer to the knee injury event. Moreover, the use of more direct physiological measurements of MU control as discussed in section 7.3 of this dissertation may help to further increase the sensitivity to detect injury-related adaptations in the functional coordination of the quadricep and hamstring muscles.

7.5 Lower extremity muscle activation patterns during gait following a knee injury

The few studies that have evaluated sEMG-based lower extremity muscle activation during gait more than three years post-knee injury have provided conflicting evidence regarding the long-term associations between a knee injury history and neuromuscular control of the knee during movement (Limbird, Shiavi, Frazer, & Borra, 1988; Roberts, Rash, Honaker, Wachowiak, & Shaw, 1999; Lindström, Felländer-Tsai, Wredmark, & Henriksson, 2010; Hall et al., 2015). Likewise, the finding of the third study of this thesis that increased Q-H co-contraction during single-leg squatting is likely not present in individuals 3-12 years after a knee injury conflicts with the result of Hall and colleagues (2015) who demonstrated higher Q-H co-contraction during stair ambulation at an average of 5 years post-injury. One reason may be the analysis of different pre-selected sEMG variables, e.g. sEMG-amplitude based variables (Limbird et al., 1988; Hall et al., 2015), sEMG on-/offset variables (Roberts et al., 1999; Lindström et al., 2010), or sEMG-frequency based variables as described in section 7.4, which may capture different aspects of neuromuscular control.

Therefore, the final study of this thesis aimed to examine whole patterns of lower extremity muscle activation during gait that include time, amplitude, and frequency information of the sEMG signals from multiple lower extremity muscles. These multi-muscle patterns (MMPs) were constructed by using a wavelet transform and a principal component analysis (PCA) and then compared between legs with and without a previous knee injury using a support vector machine (SVM) approach. The main findings were that 1) MMPs contained systematic differences in muscle activation features between the affected and unaffected leg in previously injured individuals that could be correctly classified by the SVM, 2) adaptations in muscle activation following the knee injury may be sex-specific, and 3) systematic differences in MMPs could not be identified between the previously injured legs and the dominant legs of control subjects according to to non-significant SVM classification rates. It was confirmed that 3-12 years following a previous knee injury, adaptations in lower extremity muscle activation are present when comparing the affected and unaffected leg 3-12 years post-knee injury. In contrast, adaptations were not apparent when comparing injured to uninjured individuals, most likely due to the high between-subject variability in MMPs. In females with a previous knee injury, the affected leg showed a reduction in quadriceps activity post-heel strike and a higher hamstrings activity pre-heel strike compared to the unaffected leg. In agreement with the third study of this thesis, the fourth study did not provide evidence to suggest a strategy of persistent, increased Q-H co-contraction in individuals with a history of knee injury. These adaptations were specific to females and should not be generalized to males. Nevertheless, adaptations in females were consistent with the neuromuscular strategies of ‘quadriceps avoidance’ and ‘hamstrings facilitation’, which have been shown to reduce excessive motion of the tibia (Li et al., 1999; Shelburne, Torry, & Pandy, 2005; Victor et al., 2010) but may alter tibio-femoral kinematics and kinetics in a way that accelerates the degeneration of joint tissues after a knee injury, i.e. PTOA (Chaudhari, Briant, Bevill, Koo, & Andriacchi, 2008). Since this study did not assess joint mechanics, future investigations should examine to what degree the persistent alterations in quadriceps and hamstrings activity affect the forces that are acting on the knee joint tissues and whether these kinetic changes increase the risk of knee PTOA. The developed analysis approach for MMPs of the lower extremities during gait could assist the rehabilitation of individuals following joint injuries by providing a tool to decide whether abnormal muscle activation patterns are still present in the affected limb.

7.6 The role of muscle activation for post-traumatic knee osteoarthritis

The motivation of this thesis was to investigate persistent, abnormal neuromuscular control of the lower extremities following a knee injury as a possible risk factor for the development of knee PTOA. Ample evidence exists for the presence of abnormal muscle activation patterns during the acute knee injury phase and in individuals with a clinical diagnosis of knee osteoarthritis (for reviews on these topics, see Ingersoll et al., 2008; Mills, Hunt, Leigh, & Ferber, 2013). Further, in people with knee OA, abnormal muscle activation patterns, such as increased Q-H co-contraction, have been shown to be associated with signs of disease progression, i.e. the slow degradation of articular cartilage (D. Rutherford, Hubley-Kozey, &

Stanish, 2013; Hodges et al., 2016). In contrast, there is a paucity of research studies investigating effects of previous knee injuries on muscle activation patterns that are present past the acute injury and rehabilitation phase but before the clinical diagnosis of knee OA, i.e. 3-12 years post-injury when individuals are at increased risk of PTOA development (Whittaker et al., 2015). Not a single study could be identified to demonstrate an association between abnormal neuromuscular control of the knee joint greater than three years post-injury with the presence or severity of early signs for the development of knee OA. The aim of this thesis was to address this gap in the existing literature and use sEMG technology to explore abnormal muscle activation strategies of the lower extremity 3-12 years post-knee injury and possible associations with early knee OA development. In chapter 1, three possible reasons were suggested for the lack of evidence to link abnormal neuromuscular control following a knee injury to an increased risk of knee PTOA. These reasons included a methodological explanation, a study design explanation, and a conceptual explanation and will be revisited based on the findings of this thesis.

7.6.1 Possible methodological explanation

From a methodological perspective, it was argued that sEMG-based outcome variables that are typically used to investigate knee injury effects on lower extremity muscle activation may exhibit poor reliability. Poor reliability would blur possible associations between injury-related abnormal control of lower extremity muscles during movement and an increased risk for knee PTOA. The first study of this thesis confirmed that a commonly used knee muscle co-contraction index during gait, which is based on normalized sEMG amplitudes indeed shows poor reliability and substantial measurement error if obtained on two different days (Mohr, Lorenzen, Palacios-Derflingher, Emery, & Nigg, 2018). This lack of repeatability across days is problematic because a large measurement error may bias a group comparison of muscle co-contraction indices between a previously injured and uninjured group towards failing to reject the null hypothesis. In contrast, both the amplitude-based muscle co-contraction index and an alternative frequency-based measure, i.e. intermuscular coherence, demonstrate good to excellent reliability between two sessions on the same day and are sensitive to changes in neuromuscular control in response to different movement conditions (Chmielewski et al., 2005; Elias, Hammill, & Mizner, 2015; Mohr, Lorenzen, et al., 2018; Mohr, Schön, von Tscharner, & Nigg, 2018). As a result, these variables are helpful for investigating the acute effects of different movement tasks or therapeutic interventions on neuromuscular control of the lower extremities and thus, inform rehabilitation strategies following knee injury.

The between-day reliability of intermuscular coherence outcomes is currently unknown. It has been reported, however, that EMG-EMG coherence magnitude between two sEMG recordings from the proximal and distal anterior tibialis muscle (intramuscular coherence) only exhibits poor to moderate reliability (Asseldonk, Campfens, Verwer, Putten, & Stegeman, 2014). Similarly, the magnitude of EEG-EMG coherence between electrical recordings from the motor cortex and upper extremity muscles is characterized by low reproducibility between sessions on different days (Pohja, Salenius, & Hari, 2005). The second study of this thesis showed effects of two methodological factors (sEMG electrode configuration and amplifier technology) on the magnitude of intermuscular coherence outcomes (Mohr, Schön, et al., 2018) while additional influencing factors have been reported by others (Keenan et al., 2012; Boonstra & Breakspear, 2012; Steeg, Daffertshofer, Stegeman, & Boonstra, 2014). Consequently, intermuscular EMG-EMG coherence outcomes are susceptible to many physiological and non-physiological sources of variability, which may lead to poor between-day reliability and low sensitivity to detect differences between independent groups. This limitation may partially explain the large between-subject variability observed in Q-H coherence outcomes that was observed in the third study of this thesis and must be considered when interpreting the comparison of Q-H coherence between individuals with and without a previous knee injury.

In conclusion, sEMG-based analyses of muscle activity enable non-invasive measurements that provide insight into how the CNS coordinates the activity of multiple muscles and have the potential to detect maladaptive neural strategies. As such, I still believe that sEMG is an essential technology for exploring the function of the neuromuscular system during movement. Current sEMG amplitude and frequency measures to characterize neuromuscular control during movement, however, often demonstrate poor reliability. This increases the risk of type II error when comparing lower extremity muscle activation between groups with and without a knee injury history and makes it difficult to explore the association between markers of abnormal neuromuscular control post-injury and knee PTOA development. Therefore, future studies must address the issue of poor reliability of sEMG procedures when estimating variables to quantify intermuscular coordination during movement. To achieve this, sEMG hardware should include multi-electrode arrays or high-density grids that can capture the activity of many motor units distributed across the surface of the muscles of interest (Farina et al., 2016). The further development of such technologies may allow to better account for many of the physiological factors, e.g. motor unit clustering (Asmussen, Von Tscharner, & Nigg, 2018), and non-physiological factors, e.g. muscle fiber direction, location of the innervation zone, and cross-talk (Farina, Merletti, Indino, Nazzaro, & Pozzo, 2002; Gallina et al., 2013), that would typically influence the single-differential sEMG signal and thus improve sEMG reliability. Further, the prediction of muscle forces from sEMG may become possible when activity data from many individual motor units obtained via with high-density sEMG grids are combined with musculoskeletal models (Sartori, Yavuz, & Farina, 2017). Here, the challenge will be to innovate technology that can capture the activity of large groups of individual motor units during movements that are relevant for everyday activities of humans. In addition, the sEMG software should consider the natural variability in intermuscular coordination from one movement cycle to the next. EMG time-frequency patterns may exhibit highly individual features from one step to the next during locomotion, e.g. during running (von Tscharner et al., 2018). Therefore, average measures of sEMG amplitude or frequency variables across a small number of repeated measurements as typically used in clinical biomechanics research may not adequately represent the underlying neuromuscular strategy of the CNS in different individuals.

7.6.2 Possible study design explanation

There is a lack of studies to investigate abnormal neuromuscular patterns in individuals in the time after the acute knee injury and rehabilitation phase but before the clinical diagnosis of knee PTOA, i.e. 3-12 years post-injury. Despite initial biological healing processes within the joint and surgical treatment, the previously injured joint remains in a vulnerable state during this period 3-12 years post-injury, e.g. due to the permanently altered mechanical properties of the articular cartilage and subchondral bone (Riordan, Little, & Hunter, 2014; Carbone & Rodeo, 2017). Abnormal neuromuscular control of the lower extremity muscles and the resulting abnormal tibio-femoral kinematics during this time period may further increase the rate of joint degeneration and thus, present a target for PTOA prevention (Chaudhari et al., 2008; Anderson et al., 2011; Guilak, 2011; Stiebel, Miller, & Block, 2014). Disagreement exists between the few existing studies regarding whether or which abnormal lower extremity muscle activation features persist during movement more than three years post-injury (Roberts et al., 1999; Knoll, Kiss, & Kocsis, 2004; Lindström et al., 2010; Hall et al., 2015). Further, there is a lack of previous studies that attempted to explore the association between markers of persistent abnormal neuromuscular control following knee injury and early symptoms of knee PTOA such as worse knee pain, and symptoms. This makes it difficult to design large, prospective studies aimed at modifying abnormal neuromuscular control strategies through neuromuscular rehabilitation and investigating whether such modifications can reduce the risk or rate of progression of knee PTOA.

The three neuromuscular adaptations to a previous knee injury that have been discussed most extensively are 1) ‘quadriceps avoidance’, 2) ‘hamstrings facilitation’, and 3) ‘increased Q-H co-contraction’ (Ingersoll et al., 2008; Shanbehzadeh, Bandpei, & Ehsani, 2017). The third and fourth study of this thesis provided new insight into the presence of such neuromuscular strategies in individuals with a previous knee injury more than three years ago.

Quadriceps-hamstrings co-contraction

In both studies, there was no evidence for the persistence of an increased Q-H co-contraction strategy in individuals with a history of knee injury compared to uninjured controls. Previous authors have argued that an excessive Q-H co-contraction strategy during movement resembles a neuromuscular pattern observed during early stages of motor skill acquisition, i.e. although this strategy stiffens the joint and may be effective at avoiding excessive joint motion, it is not effective in stabilizing the joint in response to sudden, external perturbation (Chmielewski et al., 2005). The findings of this thesis suggest that young adults with a previous sport-related intra-articular knee injury 3-12 years ago may have recovered neuromuscular strategies for the dynamic stabilization of the knee during walking and squatting and do not have to rely on increased Q-H co-contraction. Other analyses of the PrE-OA cohort have shown that the previously injured individuals are highly physically active and show only marginal reductions of sport participation compared to a matched, control group (Toomey et al., 2017). The frequent return to sports and high level of physical activity in these young adults may have helped to avoid the development of chronic Q-H co-contraction patterns at more than three years post-injury. More demanding movement tasks such as single-leg landings may exacerbate the need for increased Q-H co-contraction in individuals with a knee injury history as was observed by other authors (Ortiz et al., 2008; Tsai et al., 2012). The walking and single-leg squatting tasks investigated for this thesis, however, are common during everyday human activities and were thus considered to be more relevant for questions related to the mechanisms of slow, joint degeneration following a knee injury. Besides the influence of Q-H co-contraction on dynamic knee stability, a stronger simultaneous contraction of the quadricep and hamstring muscles most likely results in increased knee joint contact forces, which has been hypothesized to be a risk factor for future knee PTOA and faster progression of idiopathic knee OA (Chmielewski et al., 2005; Hurd & Snyder-Mackler, 2007; Hodges et al., 2016). Based on the findings of this thesis, however, a faster progression to PTOA due to a permanently increased Q-H co-contraction strategy following a knee injury and the associated increase in tibio-femoral contact force appears unlikely. This is in agreement with musculoskeletal modeling studies with the result, that tibio-femoral contact forces may in fact be reduced in individuals with a knee injury more than two years ago compared to uninjured controls (Saxby et al., 2016). In conclusion, although possibly associated with disease progression in clinical knee OA patients, increased Q-H co-contraction is not common in young adults with a previous knee injury 3-12 years ago who are at risk of future knee OA.

In contrast to the hypothesis of increased Q-H co-contraction following a knee injury, the third study of this thesis indicated that previously injured males demonstrate a reduction in Q-H intermuscular gamma-band coherence, possibly indicative of reduced coupling of MU activity between the quadricep and hamstring muscles. The relationship between EMG-EMG intermuscular coherence of thigh muscles and knee joint forces are not well understood. On one hand, reduced Q-H intermuscular coherence may facilitate a more independent control of MU activity for the quadricep and hamstring muscle groups and enable effective stabilization strategies in response to perturbations as previously suggested for the medial and lateral vastii muscles (Mohr et al., 2015) or finger muscles (Reyes, Laine, Kutch, & Valero-Cuevas, 2017). On the other hand, it is possible that reduced Q-H intermuscular coherence leads to less coordinated MU activity between quadricep and hamstring muscles during squatting (Santello & Fuglevand, 2004) and thus small anterior-posterior shear forces between the tibia and femur (Serpell et al., 2015). In a feline ACL transection model, Hasler and colleagues (1997) showed that the muscle activity of the vastus lateralis (quadricep muscle) and semitendinosus (hamstring muscle) become visibly uncoordinated in the animals 7 days after an ACL transection. As a possible result of uncoordinated quadricep muscle forces, the measured patellofemoral contact force traces during one gait cycle appear "jerky" in comparison to much smoother traces in control animals. Although the knee injury in the animal model was more acute than in the current study, this example shows that poor coordination of the MU activity from muscles surrounding the knee could possibly affect forces acting across the articulating knee joint surfaces. Such changes in how the knee joint tissues are loaded may contribute to the slow degradation of the joint tissues over time and pose a threat for long-term joint heath (Chaudhari et al., 2008; Guilak, 2011). The absence of a relationship between Q-H intermuscular coherence and self-reported knee pain and symptoms, however, questions the clinical significance of the finding of reduced Q-H coherence in previously injured males. Due to the many unknowns in how intermuscular coherence affects resultant joint moments and forces of an adjacent joint, this novel area of study in post-knee injury populations requires further investigation before intermuscular coherence outcomes may be confirmed or rejected as useful markers for abnormal neuromuscular control post knee-injury (Farina et al., 2016; Needle et al., 2017). Nevertheless, findings from other research areas, e.g. the fact that Q-H intermuscular coherence during walking was associated with improved motor function in individuals with spinal cord injuries (Norton & Gorassini, 2006), strengthens the argument for the functional significance of this variable.

Altered quadriceps and hamstrings activation patterns

In contrast to increased Q-H co-contraction, the fourth study of this thesis did provide evidence that a history of knee injury 3-12 years ago is associated with other alterations of quadriceps and hamstrings activation during walking and single-leg squatting. Further, both the third and fourth studies indicated that the association between a knee injury history and lower extremity muscle activation patterns may be different between males and females. The latter finding has the important implications that biomechanical and neuromuscular adaptations to a knee injury observed for one sex should not be generalized to the other and that future neuromuscular studies should generally conduct sex-specific analyses of neuromuscular control of movement.

In the fourth study of this thesis, a pattern recognition and machine learning analysis demonstrated that the previously injured legs of females show reduced quadriceps activity post-heel strike and increased hamstrings activity prior to heel strike compared to their contralateral, uninjured legs. These adaptations are consistent with a ‘quadriceps avoidance’ and ‘hamstrings facilitation’ strategy typically observed in individuals with an acute knee injury who try to limit excessive motion of the tibia with respect to the femur (Berchuck, Andriacchi, Bach, & Reider, 1990; Kvist & Gillquist, 2001; Rudolph et al., 2001). Further, reduced quadriceps muscle activity post-heel strike may also explain why previous studies have observed reduced internal knee extension moments during walking and running in individuals with a previous meniscectomy 2-8 years ago (Willy, Bigelow, Kolesar, Willson, & Thomas, 2017). Reduced quadriceps activity may originate from a persistent inhibition of the quadriceps muscle, which is commonly observed in ACL-injured individuals for years after the injury (Hart, Pietrosimone, Hertel, & Ingersoll, 2010). Reduced isometric quadricep muscle strength has also been observed in the previously injured participants of the larger PrE-OA cohort compared to a matched control group (Whittaker, Toomey, Nettel-Aguirre, et al., 2018). Since walking only requires about 20-30% of the maximum quadricep muscle force (Besier, Fredericson, Gold, Beaupré, & Delp, 2009), however, it is questionable whether muscle inhibition is responsible for the observed reduction in quadriceps activity.

Reduced knee joint loading, e.g. due to reduced quadriceps activation, in individuals with a previous ACL reconstruction has been suggested as a risk factor for the development of knee PTOA due to the lack of mechanical stimuli that would typically maintain a healthy homeostasis of the joint structures (Herzog, Longino, & Clark, 2003; Palmieri-Smith & Thomas, 2009; Wellsandt et al., 2016). Similarly, a change in tibio-femoral kinematics during walking, e.g. due to altered quadriceps and hamstrings activation, may result in the mechanical loading of areas of the knee joint articular cartilage that are not accustomed to this mechanical environment and degenerate as part of the PTOA pathogenesis (Chaudhari et al., 2008). Higher hamstrings activity before heel strike that was observed for the affected leg of previously injured females may position the tibia more posteriorly compared to the femur leading to shifted load bearing areas on the articular cartilage (Li et al., 1999). Reduced quadriceps activity after heel strike may result in reduced knee joint contact forces during the stance phase of gait (Winby, Lloyd, Besier, & Kirk, 2009). Therefore, the differences in lower extremity muscle activation during gait that were observed for females with a previous knee injury 3-12 years ago may be linked to knee joint kinematics and mechanical loading that expose these individuals to a higher risk for knee PTOA.

In this context, participants who demonstrate a particularly strong reduction in quadriceps activity and/or increase in hamstring activity should also demonstrate early symptoms of knee PTOA. This relationship was difficult to address, because early markers for the development of PTOA with high prognostic capability are scarce (Anderson et al., 2011; Harkey et al., 2015; Carbone & Rodeo, 2017). Arguably, the most common modality for an early diagnosis of PTOA is magnetic resonance imaging (MRI). Results from the larger PrE-OA cohort, of which a subset was used in this dissertation, showed that about 30% of individuals with a previous knee injury 3-10 years ago exhibit knee OA as defined by the number and severity of defects in various knee joint structures on MRI images, e.g. cartilage, menisci, subchondral bone defects. (Hunter et al., 2011; Whittaker, Toomey, Woodhouse, et al., 2018). In comparison, only 4% of individuals with no previous knee injury showed MRI-defined knee OA. This indicates that a third of the previously injured group may be on a trajectory towards future radiographic knee OA. For this thesis, it was not possible to explore the statistical association between abnormal muscle activation patterns and the risk of MRI-defined knee OA. This limitation occurred because the selected participant subset for the muscle activation analyses described in chapters five and six showed a very low prevalence of MRI-defined knee OA. Only one previously injured individual demonstrated MRI-defined knee OA out of 19 previously injured individuals who completed MRI imaging. This low probability of knee OA prevalence may have been the result of the convenience sampling method that was used to recruit the participant subset from the larger PrE-OA cohort. With only one participant showing MRI-defined knee OA, any statistical analysis to explore possible associations with this PTOA marker would have failed. From a different perspective, this result indicates that none of the differences in lower extremity muscle activation identified in chapters five and six were associated with structural signs of early knee PTOA in the evaluated participants. Regardless, the presence of MRI-defined knee OA is unrelated to self-reported markers of joint symptoms, pain, and function 3-10 years post-knee injury, which generally questions the value of MRI-based markers as early indicators for clinical PTOA (Whittaker, Toomey, Woodhouse, et al., 2018).

A limitation of the studies presented in chapters five and six is that the tested participants had sustained different types of knee injuries. Over half of the participants had sustained full ACL ruptures and underwent surgical reconstruction. Knee injuries of the other participants included incomplete tears of the cruciate and/or collateral ligaments, isolated meniscal lesions, patellar subluxations/dislocations, and intra-articular fracture. Although not systematically studied to date, the latter injuries may result in different neuromuscular adaptations during movement compared to ACL ruptures and reconstructions. Further, 3-10 years following the injury event, less severe knee injuries such as incomplete tears of the cruciate or collateral ligaments without surgical intervention resulted in a lower prevalence of MRI-defined knee OA (13%) compared to ACL reconstructions (39%) or isolated meniscal lesions (30%) (Whittaker, Toomey, Woodhouse, et al., 2018). Therefore, considering the type of knee injury for the analyses presented in chapters five and six may have increased the sensitivity to detect injury-related differences in lower extremity muscle activation patterns and the associated PTOA risk. After separating the participants into a female and male group, however, the sample size of these subgroups did not allow to systematically compare between injury types.

In conclusion, chapters five and six provided evidence that 1) ‘quadriceps avoidance’ and ‘hamstrings facilitation’ neuromuscular strategies during gait are present in females more than three years following a previous knee injury, and 2) an increased Q-H co-contraction strategy is not present in males or females more than three years following a knee injury. Further, both chapters have provided evidence for sex-specific neuromuscular strategies as well as sex-specific associations between a previous knee injury and altered lower extremity muscle activation patterns. The synthesis with previous findings suggests that reduced quadriceps activity and increased hamstrings activity observed for the affected leg of previously injured females may be linked to suggested mechanical risk factors for the development of knee PTOA. A quantitative relationship between these neuromuscular adaptations and early symptoms of knee PTOA such as MRI-defined knee OA, knee pain and knee symptoms, however, could not be identified in this dissertation.

7.6.3 Possible conceptual explanation

From a conceptual perspective, it may be that past approaches as well as the approach of this dissertation to identify risk factors for knee PTOA do not adequately represent the nature of the disease and thus limit their success.

A growing body of evidence suggests that there will not be one single marker for PTOA development that is valid for each individual with a previous knee injury. Rather, the combination of multiple structural, biological, and mechanical variables may predict the risk of PTOA development for each unique patient (Griffin & Guilak, 2005; Chu & Andriacchi, 2015; Carbone & Rodeo, 2017; Whittaker et al., 2018). For example, after a severe injury such as intra-articular fracture, the trauma may result in sufficient articular cartilage and subchondral bone damage that the affected individual will develop knee PTOA regardless of a successful rehabilitation of neuromuscular control or a healthy, inflammatory joint environment (Anderson et al., 2011). In contrast, for individuals who sustain lower-energy injuries, such as an isolated ACL rupture, immediate structural damage of the joint tissues may be relatively small so that mechanical and biological markers become more relevant risk factors for knee PTOA. For an individual with a low-energy injury who has become obese as a result of reduced physical activity following the knee injury, low-grade systemic inflammation and its negative effects on cartilage health may be the dominant risk factor (Guilak, 2011; Toomey et al., 2017). Alternatively, for individuals with low-energy injuries who maintain a healthy bodyweight and relatively healthy range of body fat but demonstrate persistent, abnormal muscle activation patterns post-injury, the resulting modified loading environment of the injured joint may be the dominant PTOA risk factor (Chaudhari et al., 2008). As is obvious from the examples, the contribution of mechanical, structural, and biological factors to the risk of knee PTOA may be different for each patient. However, as long as only one risk factor is present, e.g. abnormal joint loading as a result of reduced quadriceps muscle activity, while the other two systems – in this case joint structure and biology – operate in normal ranges, knee PTOA may not develop (Chu & Andriacchi, 2015). In fact, 85-100% of individuals with an isolated ACL rupture and 50-80% of individuals with combined ACL and meniscal injuries may not show knee PTOA more than 10 years post-injury (Øiestad, Engebretsen, Storheim, & Risberg, 2009). The analysis in the fourth study of this thesis showed classification rates of up to 100% when discriminating between muscle activation patterns of a previously injured and uninjured leg during gait. This suggests that systematic differences in neuromuscular control of movement persist more than three years post-knee injury. Considering the arguments above, however, these side-to-side differences in lower extremity muscle activation may not lead to PTOA in most individuals while they are important PTOA risk factors for some individuals who also exhibit additional structural and biological risk factors. In this case, no study will be successful in demonstrating a consistent relationship between abnormal lower extremity muscle activation following a knee injury and the development of knee PTOA.

7.7 Neuromuscular and biomechanical research for PTOA prevention

Although this dissertation demonstrated that lower extremity muscle activation patterns may be altered in individuals who are at an increased risk of PTOA development, there was no evidence that such alterations are associated with a higher risk or faster progression to PTOA. Therefore, this dissertation could not provide support for a neuromuscular pathway of knee PTOA in young adults with a previous sport-related knee injury 3-12 years ago. The results of this thesis raise the question: "How should research related to lower extremity biomechanics and muscle activation be utilized for reducing the burden of knee PTOA?".

In contrast to this dissertation, animal models have provided good evidence for PTOA risk factors related to abnormal activation of the muscles surrounding the knee joint following a knee injury (O'Connor, Visco, Brandt, Albrecht, & O'connor, 1993; Herzog et al., 2003; Egloff et al., 2014). The reasons that animal models have been more successful in identifying risk factors may include that 1) structural signs of knee OA develop much faster in the studied animals 2) abnormal muscle activation patterns can be experimentally induced and are thus similar between tested animals, and 3) other risk factors for PTOA such as meniscal pathology or obesity can be minimized (Mahmoudian et al., 2018). The findings of this dissertation suggest that in the human model, current sEMG-based markers of muscle activation strategies following a previous intra-articular knee injury exhibit too much between-subject variability to investigate PTOA risk factors from a mechanistic perspective. The large variability in these neuromuscular markers may be partitioned into 1) the influence of different subject characteristics on neuromuscular strategies such as type of previous knee injury, age, and sex (Wexler, Hurwitz, Bush-Joseph, Andriacchi, & Bach, 1998; Hoerzer, Tscharner, Jacob, & Nigg, 2015), 2) the inherent variability of the neuromuscular system when accomplishing the same task (Bernstein, 1967), and 3) measurement error associated with the selected neuromuscular outcome variables. On one hand, variability related to different subject characteristics may be accounted for by multivariable statistical models and large sample sizes (Slemenda & Brandt, 1997; Whittaker, Toomey, Nettel-Aguirre, et al., 2018). On the other hand, accounting for the natural within-subject variability and reducing the measurement error of neuromuscular measurements in humans likely requires a considerably greater experimental effort than logistically possible in the studies presented in chapters five and six. Specifically, more intricate sEMG technology and longer protocols in combination with the application of musculoskeletal models are needed to more accurately quantify MU activation patterns of lower extremity muscles and their relationship with mechanical knee joint loading at the tissue level. Such models exist (Adouni, Shirazi-Adl, & Shirazi, 2012; Sartori et al., 2017) but require lengthy experimental procedures and manual post-processing efforts. To apply these methods in studies with large enough sample sizes for multivariable analyses is not very feasible and requires an extraordinary amount of resources. This disconnect represents a current limitation of multidisciplinary PTOA research at the intersection of biomechanics and epidemiology and may be one of the reasons why this doctoral research project was not successful in identifying neuromuscular risk factors for knee PTOA.

Given the neuromuscular pathways of knee PTOA identified from animal models, I believe that the study of lower extremity muscle activation and joint mechanics remains an important pillar of research related to PTOA prevention. To bridge the abovementioned disconnect, I suggest that future biomechanical protocols in PTOA research projects should be implemented based on the following framework.

Animal models remain the most suitable scientific approach to study neuromuscular pathways of PTOA from a mechanistic perspective. I believe that experimental studies to confirm such pathways in humans should be designed to minimize the influence of between-subject characteristics (age, sex, type of knee injury and surgery, etc.) as much as possible. In this case, small samples can be studied with the most advanced technology for assessing neuromuscular strategies and their effect on *in vivo* joint mechanics and tissue morphology. Once evidence for a certain PTOA pathway in humans exist, research efforts should be aimed at developing surrogate markers for the neuromuscular and biomechanical constructs that are underlying the identified pathway. For example, if reduced quadriceps force during the gait stance phase has been confirmed as a risk factor for PTOA development in humans, a recently developed wearable technology to estimate tendon forces during movement may represent a suitable surrogate marker for this risk factor (Martin et al., 2018). Surrogate markers must exhibit good test-retest reliability and must not rely on lengthy experimental protocols. Only once these previous steps have been achieved, the developed markers should be implemented in large-scale, longitudinal epidemiological studies investigating the combined PTOA risk as a function of biomechanical, structural, and biological factors in more heterogeneous groups. This way, patient groups may be identified that could benefit from neuromuscular rehabilitation or gait retraining and thus, help to reduce the public health burden of PTOA.

7.8 Conclusions

This thesis had the goal to explore abnormal muscle activation patterns during movement following a knee injury 3-12 years prior as assessed by sEMG technology and their possible involvement in the development of knee PTOA. Limitations in existing sEMG-based markers for neuromuscular control hindered the immediate analysis of injury effects and instead, warranted two methodological investigations related to sEMG-based assessment of muscle activation. The main findings and conclusions of these studies were:

1) sEMG amplitude-based indices of muscle co-contraction during gait that are normalized to maximum voluntary isometric contractions show poor between-day reliability and are not recommended for the analysis of knee injury effects on co-contraction between muscles surrounding the knee joint.

2) intermuscular EMG-EMG coherence between thigh muscles during squatting is an alternative to amplitude-based measures of muscle coordination but depends strongly on the sEMG electrode configuration and alignment.

Based on these methodological findings, effects of knee injuries on lower extremity muscle activation patterns were investigated during gait and during a single-leg squat. The main findings and conclusions of these studies were:

3) Muscle co-contraction between medial and lateral quadriceps-hamstrings muscle pairs as quantified by gamma-band EMG-EMG intermuscular coherence during a single-leg squat was stronger in females compared to males indicating sex-specific co-contraction strategies. There was no evidence for the presence of an increased quadriceps-hamstrings co-contraction strategy in individuals with a previous knee injury 3-12 years ago. This

finding questions the role of increased quadriceps-hamstrings co-contraction as a risk factor for the development of post-traumatic knee osteoarthritis.

4) Multi-muscle activation patterns of the lower extremities during gait show persistent differences between the affected and non-affected leg of individuals with a previous knee injury 3-12 years ago. These knee-injury related adaptations may be different for males and females. For female individuals, the injury resulted in a reduction of quadriceps muscle activity post-heel strike and an increase in hamstring activity pre-heel strike, which could be linked to mechanical risk factors for PTOA development.

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Closing remark

The mountains are calling, and I must go.

John Muir, 1873

Appendix A

Journal of Electromyography and Kinesiology (Chapter Three)

Maurice Mohr

From:	Permissions Helpdesk [redacted]
Sent:	September-21-18 1:47 PM
To:	Maurice Mohr
Subject:	RE: Use of Elsevier journal article in own thesis

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Laura

Laura Stingelin
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Appendix B

Co-author permissions

UNIVERSITY OF CALGARY

FACULTY OF KINESIOLOGY

Benno Nigg, Dr.sc.nat., Dr.h.c.mult.
Professor Emeritus Biomechanics
Human Performance Laboratory
University of Calgary
[redacted]

Letter of permission for Maurice Mohr

Calgary, November 21, 2018

To whom it may concern,

This is to confirm that I allow Maurice Mohr to include the manuscripts listed below in his PhD thesis entitled: "Lower Extremity Muscle Activation Following a Previous Knee Injury: Implications for Post-Traumatic Knee Osteoarthritis.

List of co-authored manuscripts:

1) **Mohr, M.**, Lorenzen, K., Palacios-Derflingher, L., Emery, C. A., & **Nigg, B. M.** (2018). Reliability of the knee muscle co-contraction index during gait in young adults with and without knee injury history. *Journal of Electromyography and Kinesiology*, 38, 17–27.

2) **Mohr, M.**, Schön, T., von Tscharner, V., & **Nigg, B. M.** (2018). Intermuscular Coherence Between Surface EMG Signals Is Higher for Monopolar Compared to Bipolar Electrode Configurations. *Frontiers in Physiology*, 9.

3) **Mohr, M.**, von Tscharner, V., Whittaker, J. L., Emery, C. A., & **Nigg, B. M.** (2018). Quadriceps-hamstrings intermuscular coherence during single-leg squatting 3-12 years following a youth-sport related knee injury. *Submitted to Human Movement Science, October 2018.*

4) **Mohr, M.**, von Tscharner, V., Emery, C. A., & **Nigg, B. M.** (2018). Classification of gait muscle activation patterns according to knee injury history using a support vector machine approach. *Submitted to Human Movement Science, September 2018.*

Sincerely,

Benno M. Nigg,

[redacted]

UNIVERSITY OF CALGARY
FACULTY OF KINESIOLOGY
Sport Injury Prevention Research Centre

Calgary, November 21, 2018

Letter of permission for Maurice Mohr

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Sincerely,

[redacted]

Dr. Carolyn Emery
Professor, Faculty of Kinesiology
Chair Sport Injury Prevention Research Centre
Professor, Pediatrics and Community Health Sciences
Chair Pediatric Rehabilitation
Cumming School of Medicine
University of Calgary
[redacted]

UNIVERSITY OF ALBERTA
FACULTY OF REHABILITATION MEDICINE
Department of Physical Therapy

Letter of permission for Maurice Mohr

Edmonton, November 22, 2018

To whom it may concern,

This is to confirm that I allow Maurice Mohr to include the manuscripts listed below in his PhD thesis entitled: "Lower Extremity Muscle Activation Following a Previous Knee Injury: Implications for Post-Traumatic Knee Osteoarthritis."

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Sincerely,

Jackie L. Whittaker

[redacted]

Jackie L. Whittaker BScPT, PhD
Assistant Professor, Department of Physical Therapy, Faculty of Rehabilitation Medicine,
Research Director, Glen Sather Sports Medicine Clinic
University of Alberta. Edmonton, Canada

[redacted]

[redacted]

Letter of permission for Maurice Mohr

Calgary, November 21, 2018

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Sincerely,

[redacted]

Kristin Lorenzen

UNIVERSITY OF CALGARY
FACULTY OF KINESIOLOGY
Sport Injury Prevention Research Centre

Dr. Luz Palacios-Derflingher
[redacted]

Letter of permission for Maurice Mohr

Calgary, November 21, 2018

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Sincerely,

[redacted]

Luz Palacios-Derflingher, PhD
Senior Research Associate/Biostatistician -Sport Injury Prevention Research Centre
Adjunct Professor, Faculty of Kinesiology
Adjunct Assistant Professor, Dept of Community Health Sciences, Cumming School of Medicine
University of Calgary

[redacted]

Tanja Schön
[redacted]

Letter of permission for Maurice Mohr

Calgary, November 21, 2018

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Sincerely,

Tanja Schön,

[redacted]

UNIVERSITY OF CALGARY

FACULTY OF KINESIOLOGY

Dr. Vinzenz von Tscharner
Adjunct Professor
Human Performance Laboratory
University of Calgary
[redacted]

Letter of permission for Maurice Mohr

Calgary, November 21, 2018

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Sincerely,

[redacted]

Vinzenz von Tscharner