Abstract

Individuals who have anterior cruciate ligament reconstruction (ACLr) surgery are frequently unable to return to pre-injury functionality and are more predisposed to developing knee osteoarthritis (OA) than uninjured individuals. The internal mechanism driving structural and functional decline is not fully understood. Musculoskeletal models are an excellent approach to complement previous research.

Firstly, dynamic knee stability was defined as the combination of knee and leg stiffness, co-contraction and joint contact loading. One observational study examined movement strategies in uninjured and individuals with ACLr combining movement analysis and musculoskeletal modelling (SIMM/OpenSim) using the University of Wisconsin-Madison lower leg model. Two smaller validation studies explored treadmill step width changes and compared experimental (EMG) with modelled muscle activation results. Four steady-state walking speeds were compared between ACLr and healthy individuals. For the interaction between speed and group, there were lower internal flexion moments (165%, p= 0.003) at peak knee flexion, and higher Co-Contraction Index (CCI) Four values (9%, p= 0.039) for slow and normal walking in individuals with ACLr. CCI Four is influenced by the soleus muscle suggesting an ACL agonist role aiding tibial stability.

Secondly, step width was 129% wider on a treadmill than over ground (p= 0.002), with 64% higher external knee adduction moments (p= 0.010) and higher peak (21%, p= 0.028) and total mean (25%, p= 0.028) medial knee loading.

Finally, the modelled and collected muscle data were in good agreement for both groups.

Comparing dynamic knee stability during gait in uninjured and injured populations provided important quantitative insight into internal knee biomechanics. Knee injury and subsequent reconstruction led to altered joint movement control and loading patterns. It is recommended for healthcare settings to focus on maintaining a large knee flexion range of motion in individuals with ACLr to support a shock-absorbing gait style.
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<tr>
<td>2D</td>
<td>Two-Dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three-Dimensional</td>
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<tr>
<td>ACL</td>
<td>Anterior Cruciate Ligament</td>
</tr>
<tr>
<td>ACLd</td>
<td>Anterior Cruciate Ligament deficient/ deficiency</td>
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<tr>
<td>ACLr(s)</td>
<td>Anterior Cruciate Ligament reconstruction(s)</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>A-P</td>
<td>Anterior-Posterior</td>
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<tr>
<td>ARUK</td>
<td>Arthritis Research UK Centre</td>
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<tr>
<td>ASIS</td>
<td>Anterior Superior Iliac Crest</td>
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<tr>
<td>B</td>
<td>Viscous Stiffness</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BPTB</td>
<td>Bone-Patellar Tendon-Bone</td>
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<tr>
<td>CCI(s)</td>
<td>Co-Contraction Index (Indices)</td>
</tr>
<tr>
<td>CMC</td>
<td>Computed Muscle Control</td>
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<tr>
<td>CoM</td>
<td>Centre of Mass</td>
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<tr>
<td>COMAK</td>
<td>Concurrent Optimization of Muscle Forces and Kinematics Algorithm</td>
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<tr>
<td>DOF(s)</td>
<td>Degree(s)-of-Freedom</td>
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<tr>
<td>ECG</td>
<td>Electrocardiography</td>
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<tr>
<td>eKAM(s)</td>
<td>External Knee Adduction Moment(s)</td>
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<tr>
<td>EMD</td>
<td>Electromechanical Delay</td>
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<td>EMG</td>
<td>Electromyography</td>
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<td>GRAIL</td>
<td>Gait Real-Time Analysis Interactive Laboratory</td>
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<td>GRF(s)</td>
<td>Ground Reaction Force(s)</td>
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<td>HMvPM</td>
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<tr>
<td>HS</td>
<td>Heel Strike</td>
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<td>I</td>
<td>Inertial Stiffness</td>
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<tr>
<td>IC</td>
<td>Initial Contact</td>
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<td>IK</td>
<td>Inverse Kinematics</td>
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<td>Description</td>
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<tr>
<td>K</td>
<td>Elastic Stiffness</td>
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<tr>
<td>KJCE</td>
<td>Knee Joint Center Excursion</td>
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<tr>
<td>KOOS</td>
<td>Knee Injury and Osteoarthritis Outcome Score</td>
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<tr>
<td>LCL</td>
<td>Lateral Collateral Ligament</td>
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<td>MAE</td>
<td>Mean Absolute Error</td>
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<td>MCL</td>
<td>Medial Collateral Ligament</td>
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<td>MFD</td>
<td>Muscle Force Distribution</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MSE</td>
<td>Maximum Stance Extension</td>
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<tr>
<td>MSF</td>
<td>Maximum Stance Flexion</td>
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<tr>
<td>MVC(s)</td>
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<td>MWF</td>
<td>Maximum Swing Flexion</td>
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<td>OA</td>
<td>Osteoarthritis</td>
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<td>PCL</td>
<td>Posterior Cruciate Ligament</td>
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<td>Posterior Superior Iliac Crest</td>
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<td>QT</td>
<td>Quadriceps Tendon</td>
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<tr>
<td>RCCK</td>
<td>Research Centre for Clinical Kinesiology</td>
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<tr>
<td>RMS</td>
<td>Root Mean Square</td>
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<tr>
<td>S.D.</td>
<td>Standard Deviation</td>
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<tr>
<td>SGT</td>
<td>Semitendinosis and Gracilis Tendon</td>
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<tr>
<td>SIMM</td>
<td>Software for Interactive Musculoskeletal Modeling</td>
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<td>SPSS</td>
<td>Statistical Product and Service Solutions</td>
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<tr>
<td>ST</td>
<td>Semitendinosis Graft</td>
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<td>Tibial Collateral Ligament</td>
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<td>Toe Off</td>
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Acknowledgements

Firstly, I would like to thank all the uninjured and patient volunteers who participated in this work, without you this research would simply not have been possible.

Next a massive thank you to my supervisors Prof Robert van Deursen and Prof Ilse Jonkers for all their input and support; their guidance has been invaluable over these last few years. A thank you too to Dr Rob Letchford and his colleagues at the Aneurin Bevan University Health Board for facilitating access to patient volunteers and the many hours that Rob provided during the research.

Thank you to my colleagues in the Department of Movement Sciences and the Human Movement Biomechanics Research Group at KU Leuven. Particular thanks go to Sam Van Rossom for all his guidance with the modelling, and Maarten Afschrift, Susana Meireles, Amber Bruijnes, and Tessa Hoekstra for their help and the welcome they provided me in Leuven. And of course, an additional thank you to Prof Darryl Thelen, Dr Colin Smith and others at the University of Wisconsin-Madison for providing me with access to the University of Wisconsin-Madison model.

Thinking of the support that was provided in the RCCK laboratory, a thank you goes to additional academics Dr Jennifer Davies, Dr Mohammad Al-Amri and Dr Catherine Purcell. Of course, none of the research would have happened without excellent behind-the-scenes RCCK laboratory support, from IT, the simulation team and the porters in Ty Dewi Sant. Thank you to Karl Rowe, Richard Vallender, Tomos Davies, Aaron Morgan, John Moore, Joanne Owen, Craig Arthur and Wayne Cole. An additional thank you to Selma Papegaaij from Motek Medical who helped with the development of the D-Flow applications.

For research support, a massive thank you to Penelope Farthing for all her support, Natasha Williams, Cheryl Cleary and others at the Versus Arthritis (formally Arthritis UK) Biomechanics and Bioengineering Centre at Cardiff University. And of course, thank you to all members of the School of Healthcare Sciences Research Team for their support through the years behind the scenes on the administrative side of this PhD.

Another thank you to visiting academics Claudia Höchner and Prof Jennie Scarvell for their contributions. Thank you to Fatma Alothman, Alaa Kattan and Riccardo Pozzoli for their many hours in the RCCK laboratory collecting healthy participant data; I remember those data collection weeks when we lived in the laboratory fondly.

Thank you to my wonderful support network; academic friends Michael Smith, Nicola Gale, Ken Yan Wong and Dalal Alothman. You all made the challenging times in this PhD a little easier. And a massive thank you to my parents Margaret and Harry Jones, sister Julie Jones, and the Jones and the extended Hughes family for their inspiration and
motivation. My wonderful friends who kept me sane, you know who you are! And of course, my amazing and extremely supportive husband Chris Hughes, who deserves the biggest thanks of all.

Finally, a message to Lily. You came into this world when this PhD was near its end, but you changed me and the way I work profoundly. I am beyond grateful to have you in my life.
1. Introduction and Research Aim

1.1 Introduction

Injury of the anterior cruciate ligament (ACL) of the knee affects between 0.8 and 11.5 in 1,000 people in a population (Griffin et al. 2006; Frobell et al. 2007; Gianotti et al. 2009), and an ACL injury lowers the functional capabilities of an individual. ACL injury is painful, timely and costly to recover (Herzog et al. 2017). Additionally, individuals who sustain ACL injuries are more predisposed to developing OA in their knees than those who have not sustained a knee injury (Blagojevic et al. 2010; Silverwood et al. 2015). Some with ACL injuries recover naturally, but for others, knee surgery can be perceived as the only option to improve functionality and reduce rehabilitation time due to symptomatic outcomes from the injury (Francis et al. 2001; Button et al. 2008). This is addressed most commonly through ACLr surgery (Beard et al. 2022). The costs for one individual who receives ACLr are approximately £4,200 to the NHS and an individual is extremely dependent on their ability to recover to restore their previous lifestyle (Davies et al. 2020).

However, the levels of knee functionality can sometimes not increase to the pre-injury level, and issues with knee stability during functional movement for these often young individuals remain (Button et al. 2014). There can be a long-term impact on the knee joint kinematics, kinetics, muscle contraction patterns and joint contact loading meaning OA can develop, and ACLr does not reduce the onset of OA (Roos 2005; Racine and Aaron 2014). The mechanism of decline is not fully understood due to its internal nature, though it is known that during this deterioration osteoarthritic indicators can start to affect the individual further (Marks 2017). Well-established musculoskeletal models are excellent for identifying internal and dynamic information at the knee joint such as knee joint contact forces and muscle activation information (Lenhart et al. 2015a).

There is limited evidence comparing uninjured and injured cohorts with a strong focus on musculoskeletal modelling particularly with knee kinematics and kinetics, muscle activation patterns, internal knee joint loading and other dynamic knee stability measures (Schrijvers et al. 2019). This information would help to understand the movement adaptations of a patient group. This would aid the identification of mal-adaptations and rehabilitation strategies can be modified to produce healthier movement patterns (Hicks et al. 2015). This in turn can slow down or reduce the pain, further injury risk and OA development for these individuals who have had effects on their daily functionality (Jenkins et al. 2022).
Gait was a particular movement of interest, due to its common daily usage (Andriacchi et al. 2004; Wikstrom et al. 2006), and its historic representation of the functionality of an individual and usage within knee research (Hopper et al. 2003; Button et al. 2008; Zouita Ben Moussa et al. 2009; Button et al. 2014; Samaan et al. 2016). Different gait speed was also analysed to provide more knowledge on how the dynamic joint stability changes.

The abilities of the populations were deduced through advanced software musculoskeletal models that are well established in the field (Lenhart et al. 2015a), as it is possible to input a combination of the kinematic and kinetic data into these models to obtain the internal contact forces on the knee joint at any point in time during movement. If the data from a set of ‘healthy’ volunteers was input into these models, it would be possible to establish a dataset for a ‘healthy’ loading pattern that could then be used for direct comparison with subjects who have had ACLr surgery. Then associated electromyography (EMG) data can be analysed through established measures such as co-contraction indices alongside other crucial calculations such as joint and leg stiffness to create a fuller comparison of overall dynamic knee joint stability (Schrijvers et al. 2019).

Therefore, this research is interested in the functionality and movement adaptation strategies of non-coper patients, who have had ACLr surgery, in comparison to what would normally be expected in a ‘healthy’ population. This can be addressed using the concept of dynamic knee joint stability, which is the ability of the knee to react within its natural limitations to a gait challenge (Schrijvers et al. 2019), and can be defined quantifiably by a variety of measures, with this research using co-contraction, knee stiffness and leg stiffness. A fourth measure, knee joint contact loading has been added to give a fuller understanding of dynamic knee joint stability at the knee joint surface and is one of the advances in this research.

This research builds on a longitudinal study (Letchford et al. 2014) which studied the same patient cohort as used in this study but investigated the subjects pre and post-surgery. Generally, these patients were not recovering well as demonstrated in that study. Hence this research required to establish if, approximately 6 years post-surgery, there was still a lack of return to pre-injury functionality through the innovative usage of dynamic knee joint stability measurements which could indicate longer-term mal-adaptation strategies.

To satisfy the questions raised in this research, a literature review was performed to analyse the existing knowledge, and then three studies were carried out in succession, with a large focus on musculoskeletal modelling to answer the research problem. To
minimise a sequencing effect from the researcher improving physical data collection methods over time, an initial feasibility study occurred. This served to understand data collection methods and musculoskeletal modelling knowledge, the latter garnered at K.U. Leuven, Leuven, Belgium.

The first research study established the initial factors that define dynamic knee stability. From there, information on healthy individuals and individuals with ACLr and the effects of changing a steady-state walking speed were collected. The results were examined to analyse the effect on the resultant internal joint contact loading of the knee and to identify the link to limb and joint stiffness and co-contraction indices. The analysis was based on a cross-sectional observational study of 18 participants in general good health (12 males, 6 females, average age 32 years, with half having no history of musculoskeletal injuries, and the other half sourced from a 6-year post-ACLr group).

From this initial investigation, and due to the nature of the data collection occurring on a split-belt treadmill setup, modelled internal knee forces using SIMM suggested significant lateral offloading using treadmill-based kinematics and kinetics. The second study was therefore to compare the difference in resultant biomechanics and tibiofemoral knee joint contact loading between a split-belt treadmill-based and an over ground-based data set, with a particular focus on step width differences. This study was based on a cross-sectional observational study (3 males, 3 females, average age 39 years) with no history of musculoskeletal injuries or knee issues and in general good health.

Finally, as validation of using the musculoskeletal model on injured and uninjured groups, the modelled muscle activations and collected EMG readings for healthy individuals and an ACLr patient group were compared. The purpose of this study was to establish the variation between the model and the collected EMG readings to establish the accuracy of the model for individuals with ACLr. The variables examined included timing, also known as phase error, and magnitude, also known as peak error, and root mean square (RMS) for both healthy and patient groups.

1.2 Research Aim
The overarching aim is to identify the impact of the injured and reconstructed ACL on dynamic joint stability measures, more specifically knee and leg stiffness, co-contraction and tibiofemoral knee joint loading obtained through the use of musculoskeletal modelling and other common biomechanical measures.

From this overarching research aim, three research questions were identified as listed below:
(1) How are the movement strategies and muscle activation patterns changed for a patient population following ACLr compared to a healthy ‘normal’ population and what changes occurred to dynamic knee joint stability including knee joint contact loading during dynamic functional movements?

(2) Are there step width changes between over ground and treadmill-based gait and how do these affect the resultant kinematics and kinetics, in particular, varus knee angles and internal tibiofemoral joint loading comparing the medial and lateral compartments?

(3) Knowing that the musculoskeletal model cannot be adapted readily for an injured population, what are the differences between the collected EMG and modelled muscle activations for healthy and patient subjects?
2. Literature Review and Basis for Research Protocol

2.1 Literature Review

This literature review comprises 4 main components to identify existing knowledge. Firstly, ACLr background such as knee anatomy, the mechanism of ACL injury, and ACLr. Secondly, physiological and biomechanical implications such as the association of ACLr to OA. Thirdly the dynamic knee and how it functions and stabilises during motion. Finally, functional testing of the knee joint and musculoskeletal modelling. This literature review identifies gaps in the knowledge base to inform the three research questions and each of their associated individual studies.

2.1.1 ACLr Background

2.1.1.1 Anatomy of the Knee

The knee is comprised of four bones; the proximal tibia, the distal femur and the anterior patella, creating two interacting joint surfaces, the tibiofemoral joint and the patellofemoral joint (Gray and Lewis 1918). The proximal fibula bone does not form a direct joint at the knee joint (Gray and Lewis 1918). The tibiofemoral joint can be further separated into two portions; the medial and lateral compartments (Gray and Lewis 1918), and these two halves of the joint are mostly discussed in terms of joint loading patterns (Lenhart et al. 2015a; Lenton et al. 2018). Furthermore, there are two fibrocartilaginous menisci, a patella ligament, two collateral ligaments, two cruciate ligaments, a transverse ligament that connects the medial and lateral menisci, coronary ligaments and an articular capsule (Gray and Lewis 1918). The two collateral ligaments are the medial collateral ligament (MCL), also known as the tibial collateral ligament (TCL), and the lateral collateral ligament (LCL), also known as the fibular collateral ligament (Gray and Lewis 1918). The two cruciate ligaments are the posterior cruciate ligament (PCL) and the ACL (Gray and Lewis 1918). The oblique popliteal ligament is often quoted in medical text (Gray and Lewis 1918) though it is insinuated by some that it is more of a tendon than a ligament (Benninger and Delamarter 2012).

2.1.1.2 The ACL

The ligaments in the knee ensure some stability of the joint, though, during movement, stability of the joint is more complicated and is heavily influenced by the muscles and their coordinated activity (Sinkjaer and Arendt-Nielsen 1991). Knee stability is the ability of the knee to keep stable through the use of primary and secondary stabilisers (Kakarlapudi and Bickerstaff 2001) and is discussed in sections 2.1.1, 2.1.2 and 2.1.3. While the knee has been thought of as a simple hinge in some studies and especially when it comes to modelling the knee joint (Schipplein and Andriacchi 1991; Andriacchi
et al. 2006), the knee is more complex in its movement (Gray and Lewis 1918). The ACL is responsible for restricting tibial translation anteriorly and tibial rotation (Grood et al. 1984; Bhardwaj et al. 2018) and stability of the knee joint along with the other ligaments (Esfandiarpour et al. 2013).

Of all of the ligaments in the knee, injury of the ACL is one of the most common (Kupczik et al. 2013; Hetta and Niazi 2014), and rupture of the ACL is one of the most common knee injuries in general (Erickson et al. 2016; Bhardwaj et al. 2018). It has been deduced that ACL injury or deficiency causes internal rotation in the knee joint or anterior translation of the tibia (Bhardwaj et al. 2018), though it has been argued the translation is greater for the lateral than the medial compartment, essentially meaning only internal rotation occurs (Scarvell et al. 2005).

Therefore after ACL injury, these altered positions that were not present beforehand cause loading on areas of the knee that are not prepared for this task, potentially allowing for cartilage breakdown (Andriacchi et al. 2006). Even after ACLr, knee function can continue to be sub-optimal (Samaan et al. 2016; Ithurburn et al. 2019). ACL deficiency (ACLd) is a term used to define the lack of a fully functioning ACL and recurring instability (Goldstein and Bosco 2001), and it is well established that there is an increased likelihood of OA in ACL-deficient knees (Roos et al. 1995; Andriacchi et al. 2006; Fox et al. 2018). It is therefore pertinent to investigate functional instability following ACLr to establish if there is still an ACLd.

2.1.1.3 The Risk Factors and Mechanism of ACL Injury

Injury of the ACL is a painful and traumatic process and is common due to it being in the middle of the femur and the tibia, the two longest lever arms in the body (Fleming et al. 2005). ACL injury normally occurs through non-contact mechanisms (Boden et al. 2000), accounting for between 70% and 84% of all ACL tears (Jamil et al. 2017). ACL injury commonly occurs in two distinct categories (Boden et al. 2000; Olsen et al. 2004; Kimura et al. 2010). In the first, the knee is close to full extension with a plant-and-cut deceleration movement combining forceful valgus motion and either internal or external rotation (Boden et al. 2000; Olsen et al. 2004; Kimura et al. 2010). In the second, during a single-legged landing movement with the knee close to full extension with a forceful valgus motion combined with external rotation (Boden et al. 2000; Olsen et al. 2004; Kimura et al. 2010). ACL injury affects many sporting and active individuals and takes a considerably long time to recover, to the point where some may not fully recover (Kaur et al. 2021). Individuals who sustain an ACL injury are termed ACL deficient (Paterno 2017) and those who maintain stability in dynamic situations are termed ‘copers’, while those with knee instability are ‘non-copers’ (Hurd and Snyder-Mackler 2007). Non-
Copers must seek other options to return to previous levels of ability and activity. For many this requires surgery, which is often considered the only solution to restore function, particularly to reduce rotation and anterior translation of the tibia in the tibiofemoral joint during flexion of the knee (Dhillon et al. 2011). However, this opinion is often questioned, as ACLr surgery is not without its risks and mixed outcomes (Georgoulis et al. 2010; Sharifi et al. 2018).

2.1.1.4 Obesity, Gender and Age and their Role in Knee Injury

It is important to understand any other influencing factors for sustaining ACL damage to identify the full mechanism of a knee injury, the most pertinent of which are obesity, gender and age.

Whilst there is much discussion between the trio of obesity, knee injury and subsequent knee OA as discussed in section 2.1.2, there is much less literature discussing the established link between obesity and ACL knee injury (Evans et al. 2012; Bojicic et al. 2017). However, there is some evidence of the effect an ACL injury has on obesity (de Oliveira et al. 2020).

Gender has been suggested as a risk factor for knee injuries, such as ACL rupture, and the potential onset of OA (Peterson and Krabak 2014). Over 65% of all ACLrs occur from sport (Gianotti et al. 2009), with more men sustaining an ACL injury (Gianotti et al. 2009). However, for sporting events, women are five to seven times more likely to sustain an ACL injury compared to men (Templeton 2021), due to kinematic and muscle activation differences (Editorial 2016; Templeton 2021). An example includes larger frontal moments for healthy females compared to males (Obrębska et al. 2020), with these differences not improving after ACLr (Asaeda et al. 2017). Also hormonal, environmental and anatomical factors, the latter of which feed into the kinematic differences, have their influence (Editorial 2016). There is an argument for movement classes to begin from an early age to address external influencing factors (Editorial 2016), though improper conditioning leading to ACL injuries in female athletes is less significant in recent years (Oliphant and Drawbert 1996). This disparity in the numbers between female and male ACL injuries has been suggested to be because males are more likely to participate in sporting activities (Gianotti et al. 2009; Templeton 2021).

With these differences between genders in mind, some have suggested separating females and males when analysing changes in biomechanics linked to an ACL injury (Asaeda et al. 2017) and muscle activation patterns (Mohr et al. 2019), though it is worth noting that not all gender differences are risk factors for ACL injury (Lin et al. 2012).
Whilst older age is linked with knee OA as discussed in section 2.1.2.1, younger age appears to be linked to initial knee injury (Holm et al. 2021). This may be due to the participation in sporting activities decreasing with age (Chen et al. 2023), suggesting ACL injuries are not common in older individuals (Hurd and Snyder-Mackler 2007). Interestingly, ACLr percentage decreases as the age group increases, with males in each age group having more ACLrs, until in their 60s and above, when females had slightly more ACLrs than males (Collins et al. 2011).

Obesity, gender and age are all risk factors for sustaining a knee injury and are worth considering to ensure appropriate spread across a population when selecting participants for this research. These are unfortunately often also risk factors for the development of knee OA as discussed in section 2.1.2.1, showing the sustained influence of these factors.

2.1.1.5 Other Injuries from ACL Rupture

Other common structures in the knee can be damaged when an ACL rupture occurs; the meniscus, other ligaments in the joint, chondral bone and the articular cartilage (Jones et al. 2003; Fleming et al. 2005), and can be sustained post-trauma too, ultimately leading to knee OA (Jones et al. 2003), the development of which is explored in further detail in section 2.1.2.2. Meniscal damage with an ACL injury is relatively common (Øiestad et al. 2010; Risberg et al. 2016; Costa-Paz et al. 2019), between 16 to 82% in acutely ACL-deficient knees, and up to 96% with a chronic ACL deficiency (Jones et al. 2003). Combined ACL injury and meniscus tears produce a significantly higher prevalence of OA 10 to 15 years after ACLr (Øiestad et al. 2010), so much so that meniscus injury is an independent predictor of the development of OA in the 20 years following injury (Costa-Paz et al. 2019).

This means that while it is desirable in research to reduce the number of variables being studied at any one time, this is not practical when trying to analyse an ACLr in isolation without other related injuries (Jones et al. 2003). ACLr is a known preventative to meniscal tears, however, if ACLr is delayed beyond 12 months from the injury, there is the potential for, most commonly, medial meniscal tears (Church and Keating 2005). Therefore it is crucial to restrict the time between injury and surgery to minimise damage and to provide a patient with the best chance for some amount of recovery (Church and Keating 2005). And, if the amount of damaged meniscal tissue removed from the knee during surgery increases, so does the risk for OA (Roos 2005). However, there is debate as to whether the presence of meniscal tears with ACL causes damage to the articular cartilage or subchondral bone underneath, which is a sign of OA development (Fleming et al. 2005). This suggests that whilst meniscal tears may
encourage the progression of OA, they may not define the end result (Fleming et al. 2005). However, this thought is contested by others suggesting meniscal injuries could be an independent predictor of OA (Costa-Paz et al. 2019). Either way, the presence of meniscal damage in the majority of cases further plays into the concern about the development of OA after an initial knee injury.

2.1.1.6 ACLr including Surgical Options
Understanding the type of ACLr performed on the patient population used in this research, which has the same cohort used in a pre-existing study (Letchford 2015), can aid in further understanding the success of the surgery.
There are two types of graft and three common surgery types when conducting an ACLr (Kapoor et al. 2004; Macaulay et al. 2012). The graft types are either allograft, where the graft is from another donor, or autograft, where the graft is from the same individual (Kapoor et al. 2004; Macaulay et al. 2012). The surgery can be conducted as either bone-patellar tendon-bone (BPTB), quadriceps tendon (QT), or hamstring semitendinosis and gracilis tendon (SGT) (Kapoor et al. 2004; Macaulay et al. 2012). There is also a simplified SGT graft where only one tendon is involved, otherwise known as a semitendinosis (ST) graft (Gobbi et al. 2005). Furthermore, there are commonly two different types of bundle; single or double bundled, with the latter being more popular due to it being more anatomically like the original ACL position and more kinematically correct (Yagi et al. 2002; Macaulay et al. 2012).
In the United Kingdom, approximately 58% of surgeons use bone-patellar tendon-bone autografts while 33% of surgeons use semitendinosis/gracilis autografts to repair a ruptured ACL, with there being great variation of type chosen for similar scenarios depending on the surgeon’s preference (Kapoor et al. 2004). The injured individuals in this research all received a semitendinosis and gracilis tendon (SGT) autograft (Letchford 2015), no doubt due to it being the most cost-effective method for the average ACL-deficient patient (Genuario et al. 2011).
When comparing grafts, BPTB grafts are meant to allow faster healing (Macaulay et al. 2012), whilst SGT grafts are meant to cause less anterior knee pain (Macaulay et al. 2012; Janani et al. 2020). ST and SGT grafts have similar function results, though there are internal rotation weaknesses in patients treated with the ST graft compared to the SGT graft (Gobbi et al. 2005). However, an ST graft is considered better than an SGT graft, as there is a suggestion that the more tendons involved in a graft, the weaker the knee joint becomes when performing activities at a larger flexion or rotation angle (Gobbi et al. 2005).
Considering alternative approaches to ACLr, one proposed alternative is for the ACL to be repaired instead of reconstructed (Chahla et al. 2019). Initial findings show similarity to an ACLr and it preserves more natural anatomy and natural biomechanical properties of the ligament (Chahla et al. 2019). Although this area of ACL preservation was originally discussed many years ago (Genelin et al. 1993), it has not been fully investigated until relatively recently (DiFelice et al. 2015; van der List and DiFelice 2017). Therefore, non-copers are still commonly treated with an ACLr (Kanto et al. 2023). ACLr does not restore full function, leading to incorrect articular loading patterns and the potential for OA, muscle imbalance and the potential for re-injury (Georgoulis et al. 2010). An alternative approach for an ACLr group is to then conduct post-ACLr exercise therapy to reduce pain, increase function and ultimately prevent the onset of OA in young adults, suggesting optimistic alternative options to protect from OA (Patterson et al. 2019).

2.1.1.7 Rehabilitation
Rehabilitation after ACL surgery is crucial to restore physical function (Edwards et al. 2018). Considering that many individuals sustain injuries through sport, understanding how many patients return to sport can define how effective their recovery has been, though not returning to sport does not mean a total lack of success (Hamrin Senorski et al. 2017). Less than 45% of ACLr patients return to their previous level of sporting activity, even though over 85% wanted to return to their same level of sport before surgery (Walker et al. 2021). Even though sporting patients who sustained an injury firmly believe that rehabilitation is essential for recovery from surgery so that they can return to sports, rehabilitation for ACLr patients is a difficult process (Walker et al. 2021). The adherence to rehabilitation is irregular, with fear of re-injury being the most common reason for lack of commitment (Walker et al. 2021). Other factors include poor self-motivation and external locus of control (Colaco et al. 2009), and hence a good therapeutic relationship and longer supervised rehabilitation aids a faster and more likely return to sport (Walker et al. 2021). Those who did complete their rehabilitation were nearly 8 times more likely to return to sport, assuming complete rehabilitation was at least 6 months, and incomplete rehabilitation was 3 months or less (Edwards et al. 2018). Crucially, those who return to sport, regardless of whether they have completed their rehabilitation programme or not, have fewer ongoing knee problems (Thomeè et al. 2015; Hamrin Senorski et al. 2017; Walker et al. 2021). This suggests an accidental continuation of rehabilitation driven by the individual, though many risk re-injury due to returning to sport with improper initial physical criteria (Edwards et al. 2018).
The patients in this research were periodically monitored under a rehabilitation programme for one year post-surgery including education through various closed and open chain activities for strength and coordination (Letchford 2015).

2.1.1.8 Recovery Outcomes from ACLr
As discussed in section 2.1.1.3, while some believe ACLr restores most function and is deemed a successful intervention, others think ACLr is not the ‘cure’ some hope for, as a return to previous levels of function does not always occur (Sharifi et al. 2018). Furthermore, ACLr does not necessarily prevent long-term deterioration of the joint and OA (Jones et al. 2003; Sharifi et al. 2018). This is because mechanistic OA begins at the time of the injury (Fleming et al. 2005). Even in the long term, such as one on average 22 years post-ACLr, patients display normal or near normal radiography scores in 73% of cases, though this does not evaluate the joint in true dynamic function (Costa-Paz et al. 2019). Realistically it also does not identify how the function of other surrounding joints and contralateral limb joints is affected and if they are adapting instead.
Loss of range of motion through arthrofibrosis after ACLr can affect between 4-35% of ACLr patients (DeHaven et al. 2003), while there is increased co-activation for these individuals compared to healthy individuals, causing changes in biomechanics and once again, heightened likelihood of OA (Blackburn et al. 2019).
Revised ACLr is also becoming increasingly common and is required when the original ACLr failed due to post-operative issues, like loss of motion or infection, persistent instability or pathological abnormalities (Dodds et al. 2014). Unfortunately, ACLr revisions are not as successful as initial ACLrs and individuals are less likely to return to sporting activities and hence, patients tend to be less satisfied with revision surgery (Dodds et al. 2014).
Now that the literature review has reflected on ACL injury and recovery, the physiological and biomechanical implications of ACL injury and ACLr are investigated.

2.1.2 Physiological and Biomechanical Implications
2.1.2.1 OA and the Link to ACL Injury
Previous knee injury is the most common reason for the onset of OA in older (Blagojevic et al. 2010) and younger individuals (Roos 2005), and can also accelerate the progression of knee OA (Driban et al. 2014). Knee injuries in young adults increase the chance of developing knee OA sixfold (Snoeker et al. 2020), with such a rapid deterioration of the joint after a knee injury that development of knee OA can occur within 14 years (Roos 2005) and in some cases, as little as one year (Driban et al. 2014).
As ACL injury is the most common injury of the knee as discussed in section 2.1.1.2, the link between ACL injury and OA is considered to be strong (Roos 2016).

Furthermore, it is pertinent to consider the particular link between ACLr and OA. ACLr does not potentially protect from (Risberg et al. 2016; van der List and DiFelice 2017; Sharifi et al. 2018; Vaishya et al. 2019), and in some cases may cause, OA onset (Vaishya et al. 2019). This can be when injury to the chondral bone or meniscus is present alongside the ACLr (Vaishya et al. 2019). Some individuals with ACLr demonstrate some biomechanical differences too to what would be expected of a non-injured individual (Georgoulis et al. 2010). However, in some activities like jump landing (Pfeiffer et al. 2018) individuals with ACLr display clear differences but can be so successful at stabilising their knees during lower-level dynamic activities such as walking, that they will display little difference in their gait biomechanics (Rudolph et al. 1998; Georgoulis et al. 2003). It is therefore extremely important to conduct separate analyses for different levels of functional activity and by sub-group of copers and non-copers so that the true understanding of a lack of ACL function can be understood (Rudolph et al. 2001).

Considering biomechanical differences for those with a change in the ACL, it has been deduced that ACL injury or deficiency causes internal rotation in the knee joint that is not present beforehand (Andriacchi et al. 2006). This causes loading on areas of the knee joint that are not prepared for this task, causing a change to the cartilage homeostasis (Van Rossom 2017; Crook et al. 2021), potentially allowing for cartilage breakdown (Andriacchi et al. 2006). Interestingly, there has been systematic information to suggest that hand OA is a risk factor for the onset of knee OA (Blagojevic et al. 2010), with the links between the two very different types of OA suggesting that OA is a generalised disease (Dahaghin et al. 2005). However, this does not deduce the kinematic and kinetic factors affecting the development of OA in these individuals and relates more to identifying biomarkers for OA (Dahaghin et al. 2005).

Considering the particular link between individuals with ACLr and OA, biomechanical lower limb differences are still present after surgery (Slater et al. 2017; Blackburn et al. 2019). This is because the sagittal (Chua et al. 2016; Slater et al. 2017) and frontal kinematics are changed (Myer et al. 2011; Slater et al. 2017), along with increased muscle co-contraction (Blackburn et al. 2019; Alfayyadh et al. 2022). All these altered factors induce an abnormal loading profile and consequent cartilage wear (Slater et al. 2017) during loading with incorrect biomechanics leading to OA development after ACLr (Dewig et al. 2023). Knee alignment and its link to OA development is discussed more in section 2.1.2.3.
The main other risk factors for knee OA are discussed in more detail in section 2.1.2.2, while Figure 1 shows all risk factors influencing knee injury and development of knee OA (Lohmander et al. 2007; Wen and Lohmander 2014). This figure has been adapted by the author to reflect the more recent knowledge around ACL injuries and the influence of genetics and epigenetics (Bechaud and Forelli 2021). This figure shows mechanical areas of interest after knee injury that are pertinent to this research, including ACLr which has already been discussed in section 2.1.1, and changes in muscle strength and coordination in section 2.1.3.3 and increased joint loading in section 2.1.3.4.

![Figure 1 - OA development from ACL knee rupture](image)

Adapted figure (Wen and Lohmander 2014), reprinted by permission of SAGE Publications (Lohmander et al. 2007)

2.1.2.2 Knee OA

OA is the most common form of arthritis and affects approximately 8.75 million people in the UK alone (Versus Arthritis 2018). OA is a painful disabling disease of the whole joint including articular cartilage degradation (Loeser et al. 2012; Marks 2017).

As OA is the most prevalent arthritis in humans there is a vast concern about reducing the impact of this disease in the population, with a primary focus on prevention of development to end-stage OA (Sharma 2007). The OA that occurs after ACL injury is considered post-traumatic OA (Robbins et al. 2019), and is termed secondary as it has derived from causes such as previous trauma of the knee (Robbins et al. 2019; Staines et al. 2020), and is not to be mistaken with primary OA from naturally occurring causes (Staines et al. 2020). Non-traumatic OA has very different muscle activation patterns in comparison to post-traumatic OA individuals and it is important to divide these characteristics when studying previous literature (Robbins et al. 2019).

Of all OA types, knee OA accounts for 83% of the general OA burden (Vos et al. 2012). While many studies discuss the term knee OA and, without further discussion, assume
this to be tibiofemoral OA (Øiestad et al. 2010; Costa-Paz et al. 2019), OA in the knee can be broken down into tibiofemoral OA or patellofemoral OA, with previous studies examining the difference between the onset of both post-ACLr (Risberg et al. 2016; Vaishya et al. 2019). OA is a degenerative disease of the joints, causing high levels of pain, damage to anatomy and impairment of function that predominantly affects older adults (Marks 2017). OA can destroy not only articular cartilage and bone, but also the joint capsule, tendons, ligaments, muscles and nerves (Marks 2017). Considering the risk factors for knee OA for those aged 50 years old and older, being overweight or obese is over 8 times more likely to be the cause of new knee pain when compared to a knee injury (Silverwood et al. 2015). Critically however, the pooled odds ratios that bring together many different studies, identified a previous knee injury as the leading cause of knee OA, with being obese coming in second (Silverwood et al. 2015). Understanding the prevalence of knee OA and its association with knee injury shows there is a need for understanding the changes after knee injury and ACLr (Schrijvers et al. 2019).

Next, it is important to identify how knee OA occurs such as through malalignment and what the additional risk factors could be in the development of knee OA.

2.1.2.3 Knee Alignment and its Role in Knee OA
Section 2.1.1.2 deduced that injury of the ACL changes the structural alignment of the knee (Scarvell et al. 2005) and that ACLr cannot fully restore this alignment (Samaan et al. 2016; Ithurburn et al. 2019). Additionally, there is an association between this mal-alignment and the development of OA (Vaishya et al. 2019; Rodriguez-Merchan and Encinas-Ullan 2022). Therefore, it is important to understand how changes in knee alignment lead to the development of knee OA. It is also important to differentiate between static or unloaded situations and dynamic situations of the knee, therefore only dynamic mal-alignment is being considered in this research.

Though alignment of the knee could be considered in all three planes, it is commonly a substitute term to mean the hip-knee-ankle angle in the frontal plane, otherwise termed the varus-valgus angle of the knee (Sharma et al. 2001). In a ‘normal’ knee and considering just the lower limbs and considering this explanation as a basic overview, the line of action of the load-bearing goes in a vertical line through the hip, knee and ankle (Sharma et al. 2001). In a varus knee, the line of action of the load is medial to the centre of the knee, meaning the knees ‘point’ laterally (Sharma et al. 2001). In valgus, this concept is reversed (Sharma et al. 2001). It is popular to investigate the change in varus-valgus angle and its impact on knee joint health (Sharma et al. 2001; Sharma 2007; Van Rossom et al. 2019). A varus knee tends to affect the medial compartment health of the tibiofemoral joint, whilst a valgus knee tends to affect the lateral compartment
The external knee adduction moments (eKAMs) can be directly linked to a varus-valgus knee angle, due to the horizontal moment arm between the vertical CoM and the lateral distance to the knee which changes according to this frontal knee angle (Sharma et al. 2001). Some have therefore even gone so far as to state that eKAM is a ‘surrogate’ of medial compartment force for studying osteoarthritic mechanisms (Appleton et al. 2017).

Reasons for change in the frontal knee alignment from neutral include injury as discussed in section 2.1.1.2, where an injured ACL leads to internal rotation of the knee (Scarvell et al. 2005; Bhardwaj et al. 2018) which changes the knee in a frontal plane too. Three other factors can also change the frontal knee alignment; existing OA (Sharma et al. 2001), toe in/ out gait (Bennett et al. 2017), or step width changes (Bennett et al. 2017).

Considering these three sub-factors, it has been suggested more severe OA knees have more of a varus alignment dynamically than less severe OA counterparts indicating higher knee adduction moments are a result of mal-alignment rather than the cause of the OA wear (Mündermann et al. 2004). With the position of the toe of the weight-bearing leg, a toe-out gait increases the first peak knee adduction moment during varying speeds, while a toe-in gait reduces this first peak knee adduction moment (Khan et al. 2017). This change in the peak knee adduction moment would be linked directly to the varus-valgus angle (Hunt et al. 2008). With the latter factor, step width, it is a debated topic whether varus or valgus of the knee occurs from wider or narrower step widths and hence whether greater or lesser external adduction moments occur around the knee joint (Gerbrands et al. 2014; Bennett et al. 2017; Bowd et al. 2019). When the step width is wider, the knee could be more varus, and more acute angles for the hip and ankle in the frontal plane accordingly, and an increase in the associated eKAM. Or with a wider step width, the knee could be less varus, or less of an adducted angle, with the hip and ankle changing their angles to ‘make up the difference’ than for a narrow step width (Paquette et al. 2014). This latter theory would support the previous knowledge that a wider step width allows for a lower eKAM (Simic et al. 2011).

Next, the implications of the biomechanics in all three planes of movement together during gait are considered. Gait is discussed here as it is the most common and fundamental daily movement for an individual, and it is crucial to particularly analyse gait to understand functional limitations (Cimolin and Galli 2014) and is discussed more in section 2.1.2.5. During the gait cycle, and considering a knee’s functional range, firstly in the transverse plane, individuals with ACL deficient knees tend to rotate their knees internally during the swing phase (Georgoulis et al. 2003; Bytyqi et al. 2014) or simply
have reduced external rotation (Andriacchi and Dyrby 2005) compared to those with reconstructed knees and healthy individuals, who rotate externally (Georgoulis et al. 2003). Women, in turn, tend to have more tibial external rotation compared to males, whether that is as a control individual, or a person pre or post-ACLr surgery (Asaeda et al. 2017). Interestingly, during the stance phase, those with OA compared to control individuals have much less internal rotation but do not rotate externally (Bytyqi et al. 2014). Sagittally, the peak internal knee extension moment is much lower in a pre-operative ACLr cohort when compared to 12 months post-operation or control individuals, and at six months post-operatively the moment value still has not recovered (Asaeda et al. 2017). In fact, for individuals who have received ACLr but still display knee instability, peak knee flexion and extension points on an internal knee extension moment graph are lower when compared to their non-injured limb (Rudolph et al. 1998; Rudolph et al. 2001; Hurd and Snyder-Mackler 2007), indicating a much lower sagittal plane excursion of the knee (Rudolph et al. 2001; Hurd and Snyder-Mackler 2007) which is linked to greater co-activation (Blackburn et al. 2019). Co-activation is referred to as co-contraction in this research and is discussed more below in section 2.1.3.3.

Considering the sagittal plane, for those with OA, there is less knee flexion in the stance phase, and lower knee extension in the swing phase, suggesting less range of movement of the knee joint as a whole than their healthy counterparts (Bytyqi et al. 2014). In the frontal plane, OA individuals display much more adduction than control individuals who mostly display abduction during the gait cycle (Bytyqi et al. 2014) and women also have an increased knee valgus, otherwise known as knee abduction, throughout the entire gait cycle (Røislien et al. 2009).

A change in the kinematics of the knee joint from an ACL injury will change the loading pattern of the knee joint (Andriacchi and Dyrby 2005; Crook et al. 2021), creating abnormal loading of the cartilage (Georgoulis et al. 2010), causing cartilage strain (Sutter et al. 2018; Crook et al. 2021), lower cartilage thickness (Sutter et al. 2018), and degenerative changes in the meniscus (Andriacchi and Dyrby 2005), and as discussed before in section 2.1.2.1, can lead to osteoarthritic development much earlier than it would have without a traumatic injury (Driban et al. 2014). However, this is somewhat simplistic and does not well define the movement strategy of the ACL reconstructed knee to cause these changes in knee contact forces and is not as straightforward as might be thought, therefore supporting the need for this research. Non-copers tend to stiffen the knee joint by less knee flexion at initial contact (IC) and less knee flexion during the weight acceptance phase in the first half of the stance phase (Rudolph et al. 1998). This leads to lower peak vertical ground reaction forces (GRF(s)) in the involved
leg than the uninvolved leg in non-copers, and interestingly the same is true for copers too (Rudolph et al. 1998). Yet non-copers also stiffen the knee joint which causes higher joint contact forces (Rudolph et al. 1998). This disparity between lower peak vertical GRFs and higher joint contact forces in the involved limb is difficult to understand, and this is because it is oversimplified. An external resultant force, otherwise known as the GRF, is being compared to one set of internal force information, the joint contact information. The other structures producing internal forces that contribute to the joint contact loading results need examining, and the contracting muscles are part of this as there will be an effect from these on the resultant muscle activations (Lenhart et al. 2015a). Firstly however, the focus needs to return to knee joint contact forces and find out how contact forces can be reliably deduced when there is no data for what occurs at the joint surface, through the utilisation of musculoskeletal modelling (Lenhart et al. 2015a).

Aside from knee alignment and how changes in knee alignment can lead to the development of knee OA, there are additional risk factors for the development of knee OA as discussed next.

2.1.2.4 Obesity, Gender and Age and their Role in OA

Other contributing factors to the development of knee OA, not just previous knee injury, include obesity, gender and age (Coggon et al. 2001; Roos 2005; Blagojevic et al. 2010). The influence of obesity, gender and age on initial knee injury is discussed in section 2.1.1.4 with their link to initial knee injury.

Obesity or being overweight is known to be a risk factor for knee OA (Coggon et al. 2001; Roos 2005; Driban et al. 2014), and is the second highest risk factor for the onset of knee OA (Blagojevic et al. 2010). As with all factors mentioned here, obesity can be present with other risk factors, increasing the overall risk of the development of knee OA (Coggon et al. 2001). Fortunately, obesity-related OA is a somewhat reversible issue, as an increase in exercise to lose weight neither increases the likelihood of OA onset nor OA progression (Soutakbar et al. 2016).

Gender is the third highest risk factor for the onset of OA (Blagojevic et al. 2010). It has been found that generally older women are at increased risk of developing OA compared to their male older counterparts (Blagojevic et al. 2010), or generally, women with an ACL injury will be at an increased risk of developing more severe OA than men (Templeton 2021). There are stronger risk factors for OA onset than gender, with previous knee injury being the highest, followed by being obese or overweight (Blagojevic et al. 2010).
Age is another established factor relating to the onset of OA (Roos 2005). There is a link between older injured individuals and the development of OA more rapidly than for younger individuals (Driban et al. 2014).

As can be seen, there are many contributing factors to knee OA development. The most important note, however, is that these issues are often interlinked and difficult to analyse individually.

2.1.2.5 Functional Testing
To assess the change in the functional capabilities of the knee joint after ACLr surgery, certain tests can be conducted to gauge the ability of the individual. This research intends to investigate the subjects through functional testing. This means the subject performs movements that are part of everyday life and in a dynamic setting such as walking instead of the traditionally investigated non-everyday tests like a single-legged balance test or lying supine flexing the knee (Lee et al. 2009; Esfandiarpour et al. 2013). It is extremely important to gather information from subjects performing day-to-day functional dynamic movements like gait to establish a subject’s functional abilities (Andriacchi et al. 2004; Cimolin and Galli 2014). Gait is also one of the most studied areas in biomechanics (Schmitt et al. 2021), and has even been equated to represent all in-vivo function (Andriacchi et al. 2004), and hence examining gait is well-founded. Furthermore, analysis of gait allows for examination of changes over time and the effects of rehabilitation interventions (Cimolin and Galli 2014). Finally, atypical gait is a contributing factor to OA after ACLr (Dewig et al. 2023). If a subject is asked to perform a static test or is asked to lie supine to investigate knee range of movement, data about the subject’s muscle behaviour, strength and balance when upright would not be captured, which would not give a true reflection of the more challenging situations found in daily life (Fernandes et al. 2016). Though as gait data is often collected in a lab, this does not fully represent gait performed daily by an individual (Schmitt et al. 2021). Functional testing has been investigated previously with pre-surgery non-copers (Button et al. 2008; Samaan et al. 2016) and post-surgery non-copers (Hopper et al. 2003; Zouita Ben Moussa et al. 2009; Button et al. 2014; Samaan et al. 2016) hence it would be beneficial to investigate individuals with ACLr several years post-surgery to understand what long term effects have been caused. Understanding how the knee works during a dynamic activity like gait can be represented by dynamic knee joint stability (Schrijvers et al. 2019). More exploration of the term dynamic joint stability and its contributing factors can be found in section 2.1.3.
2.1.2.5.1 Gait Speed, Cadence, Stride Length and Gait Variability
A historical value of minimum ‘normal’ walking speed is considered as 1.2m/s (Crabtree et al. 2015), though clearly with ACLr patients or those who may have the indications of OA wear this may be a lot lower (Mündermann et al. 2004; Fukuchi et al. 2019a). Speed of gait also affects the whole body kinematics of a subject, with speed changes directly linked to pelvis mechanics which in turn are actively able to predict the step-by-step step width changes of a subject (Stimpson et al. 2017).

If a participant wants to change their speed, they can either change their cadence, which is the number of steps in a minute, or their stride length, or a combination of both (Ardestani et al. 2016). Cadence can affect joint kinematics, where cadence changes to increase speed cause lower joint moments than those who change their stride length, with the sagittal plane moments being particularly affected by longer stride lengths (Ardestani et al. 2016).

Gait variability in simplistic terms can be thought of negatively, especially in older adults (Verghese et al. 2009). They can be a prediction of falls (Verghese et al. 2009) or can be used to demonstrate changes in the nervous system (Brach et al. 2008). However, gait variability can show the robustness of an individual to remain upright even from adapting to the smallest of external stimuli (Brach et al. 2008). It can show the flexibility of the subject and can even, in some cases, demonstrate benefits, such as the ability of the individual to adapt to a high load (Ulman et al. 2022). It is therefore worth designing a data protocol that allows for a few repetitions of a movement to be collected, so a more reasonable average can be deduced from this variability (Hausdorff 2005).

2.1.2.5.2 Self-Paced Walking
To assess a subject’s walking pattern, a speed for their walking needs to be determined. If a set speed is selected to reduce the variability of the outputs, as self-paced walking is more demanding on an individual (Kao and Pierro 2021; Gupta et al. 2023), some subjects may feel rushed while others may feel that it is too slow. Speed of an individual is dependent on many factors including gender (Dommershuijsen et al. 2022), age (Souza-Júnior et al. 2022), height (Dommershuijsen et al. 2022), physical activity (Custodero et al. 2023), and knee injuries (Neri et al. 2019). Therefore, if the speed is selected, you have the potential to influence gait biomechanics in the subject (Mündermann et al. 2004) and from this, the internal loading response (Mündermann et al. 2004; Na and Buchanan 2022). However, influencing gait biomechanics with selected speeds has been contested in more recent literature suggesting that there are no differences in kinematics between self-paced and imposed speed walking (Theunissen et al. 2022). It is more beneficial to allow the subject to select their walking speed as it is
more representative of normal over ground gait than fixed treadmill walking (Gupta et al. 2023), as well as being the more recommended modality (Kao and Pierro 2021; Meinders et al. 2021; Van Bladel et al. 2022). Some studies then average this self-selected speed and then re-input this speed for the subjects (Khan et al. 2017), though to determine the full variability of an individual in this research it is important to keep the raw self-selected speeds and then group subjects that fall within certain speed categories (Meinders et al. 2021).

2.1.2.5.3 Speed Variability related to Biomechanical variables

When examining previous studies investigating speed variations and gait patterns, common variables of interest include stride length, cadence and step width, or related measurements of these variables (Vergheese et al. 2009; Ardestani et al. 2016; Item-Glatthorn et al. 2016; Da Rocha et al. 2017).

Further commonly used variables for examination of the knee during gait include joint angles (Hurd and Snyder-Mackler 2007), joint moments (Andriacchi and Dyrby 2005; Hurd and Snyder-Mackler 2007), leg (Bishop et al. 2006) and knee stiffness (Blackburn et al. 2019) and co-contraction (Hurd and Snyder-Mackler 2007; Blackburn et al. 2019). Whilst frontal plane parameters such as step width and foot rotation are interesting, they may not be for primary investigation as sagittal plane changes are the largest plane for variation as discussed in Chapter 4 (Schrijvers et al. 2019). There is little previous information to directly relate variations in speed to knee joint contact loading (de David et al. 2015; Knobel et al. 2021), demonstrating the requirement for investigation in this research, and this is discussed further in section 2.1.3.

Speed is more challenging for those with ACLd compared to their healthy counterparts (Nazary-Moghadam et al. 2019). Additionally, ACLd knees are more dynamically unstable compared to the intact leg, regardless of the speed, as they do not sense small perturbations as readily (Stergiou et al. 2004). Furthermore, one study established that normal and higher speeds perform similarly biomechanically and cause more anterior-posterior translation of the tibia relative to the femur in ACLd individuals when compared to the intact limb (Yim et al. 2015). Those with ACLd subjected to dual-task gait conditions sacrifice more of their cognitive task to focus on their gait compared to healthy individuals regardless of the speed involved (Nazary-Moghadam et al. 2019). There has been a previous association between those with knee injuries, unusual gait biomechanics and OA development in the tibiofemoral joint (Roos 2005; Blagojevic et al. 2010; Driban et al. 2014; Snoeker et al. 2020), and therefore it is beneficial to analyse speed-related studies with participants with OA. Participants with OA who had been asked to walk at self-selected speeds, and 150% of their self-selected speed, had
reduced external rotation moments and flexion moments during the beginning of stance compared to controls regardless of speed (Landry et al. 2007). This is also true for ACLr participants who have lower external rotation moments compared to controls (Stoelben et al. 2019). Increasing the speed increases the moments in all 3 planes of the knee for those with OA (Landry et al. 2007) and with ACLr (Stoelben et al. 2019). Interestingly, for OA participants, when there were biomechanical differences at lower speeds compared to healthy individuals, there were no additional changes to these altered biomechanics at faster speeds, suggesting that there is not a ‘transitional’ zone of interest that sits between normal walking speeds and running where specifically different biomechanics occur (Landry et al. 2007).

As is also known, OA participants are known to walk slower (Fenton et al. 2018), though it has been thought that this was because OA develops and then a slow gait ensues (Robbins et al. 2023). One study suggested using moderate to fast walking as a training strategy to prevent slow gait in individuals who have or are at risk of OA, suggesting slow gait occurs and then OA does (Fenton et al. 2018).

2.1.3 The Dynamic Knee
This next section argues why the over-arching interest is to identify the knee control strategy and its impact on joint loading for post-ACL reconstructed individuals, as it is very difficult to establish what occurs internally at each time step at the joint level (Lenhart et al. 2015a). This is done through a review of the literature on the biomechanical discussion around dynamic knee stability and the main factors that contribute to maintaining stability and function.

2.1.3.1 Dynamic Joint Stability
Considering the basic biomechanics of the human body, when the knee is moving, it is carrying large forces from the mass of the human body (Wikstrom et al. 2006), from muscle activations, and the momentum of walking (D'Lima et al. 2012). Therefore high forces, also known as loads, pass through the medial and lateral compartments of the tibiofemoral joints of the knee, which is a relatively unstable joint due to the lack of bony congruence (Wikstrom et al. 2006; D'Lima et al. 2012). If the knee was expected to cope with these large forces solely on passive components such as ligaments, there would be injury (Wikstrom et al. 2006). Therefore, there are also active components in the knee and lower limb at work to aid functionality and stability (Andriacchi and Dyrby 2005). It has been proposed that the words dynamic and passive are considered more representative of the knee restraints (Davis and DeLuca
This is because ACL length (Taylor et al. 2013) and force (Shelburne et al. 2004) change over the dynamic gait cycle, even if the ligament itself is not active. Therefore, dynamic joint stability is the combination of dynamic restraints, such as muscles, and passive restraints, such as ligaments, to maintain joint stability (Wikstrom et al. 2006) and is the capacity of the knee to cope with a gait challenge within its natural abilities (Schrijvers et al. 2019). Though these are two definitions, many define it differently, and there is not one clear definition of dynamic joint stability (Schrijvers et al. 2019).

Additionally, knee function statically or dynamically is very different, and gait analysis allows for the quantification of dynamic knee joint stability (Schrijvers et al. 2019). When the knee joint is subject to very large forces, there is more reliance on the dynamic restraints than the passive restraints as the latter cannot cope with very large forces (Wikstrom et al. 2006); if this cannot be maintained, an injury ensues, often a rupture of the ACL in the case of the knee joint (Erickson et al. 2016; Bhardwaj et al. 2018).

Analysis of the body in motion in a dynamic setting is the best way to gauge understanding of dynamic stability, as static analyses can have no real bearing on dynamic postural control (Wikstrom et al. 2006). The dynamic human body can be analysed through 3D motion analysis including kinematic and kinetic results though crucially, what is occurring internally in the knee cannot be fully understood (Lenhart et al. 2015a). Therefore, the implementation of musculoskeletal modelling alongside 3D motion analysis addresses this issue (Fregly et al. 2012), where subjects can be analysed without invasive procedures, to produce in-depth and accurate results as discussed in section 2.1.4.

The clinical measurements that define dynamic joint stability are many as shown in Table 1. One clear process is not used, meaning comparisons between different studies are difficult, and there isn’t even a clear validation of one method over another (Schrijvers et al. 2019).
The most common measure of dynamic stability of the knee joint is the knee flexion angle (Smale et al. 2019), reported at a usage of 25% in one literature review (Schrijvers et al. 2019). Knee flexion-extension moments are also popular at a usage of 15%, co-contraction index (CCI) at 10%, and knee joint angular stiffness at 6%, where the change in joint angles is divided by the change in joint moments (Schrijvers et al. 2019; Smale et al. 2019). These top four commonly used measures can be combined and reduced down to two of particular interest; knee stiffness, as this considers knee angles and moments, and the CCI. The CCI is not to be confused with the co-activation index, co-contraction ratio or the co-activation ratio, and the CCI calculation can slightly vary depending on which definition was used, which is discussed in section 2.1.3.3. This research uses the words co-contraction and CCI.

Dynamic stability using a co-contraction strategy stabilises the knee joint for those with ACLd, but at the cost of increasing the muscle forces and hence the load on the joint (Sharifi et al. 2017). This can risk further injury and damage, as well as reducing the forces through the lateral hamstrings and in turn, the contact forces in the lateral portion of the knee joint contact surface (Sharifi et al. 2017). If the forces are increased in the medial portion of the knee for those with ACLd, clearly the effects at the knee
joint surface need to be studied and compared to their healthy counterparts. The forces at the knee joint surfaces can be modelled, as merely collecting GRFs would not be detailed enough, therefore this is a third measure of interest (Lenhart et al. 2015a). One more measure, limb stiffness, links the three other aforementioned measures together. It takes GRFs that are used to aid the calculation of knee joint loading (Lenhart et al. 2015a), and as it is a stiffness measure, is linked to joint stiffness (Butler et al. 2003). Stiffness is also linked to co-contraction, as the more or less muscles contract, the less stiff or stiffer the joint, and hence the limb, will be (Collins et al. 2014). Selecting these four factors, joint stiffness, limb stiffness, co-contraction and joint loading can best encompass dynamic joint stability and support each parameter well. Figure 2 shows how these four factors can be combined to fully analyse the dynamic knee joint stability required for a movement.

![Figure 2: Joint stability and its relationship with co-contraction, limb stiffness, joint stiffness and joint loading](image)

Joint stability can be represented through a combination of co-contraction, joint stiffness, limb stiffness and joint loading (Lenhart et al. 2015a; Schrijvers et al. 2019). If the ACL is injured and repaired, there is the possibility that all parts of the system are functioning but the impact on joint loading is sustained as discussed earlier in sections 2.1.1.7 and 2.1.2.1. There will be a potentially negative effect on the joint if the movement is not within safe boundaries (Schrijvers et al. 2019). Therefore, it is crucial to understand joint loading in a healthy joint so that direct comparisons can be made. It is worth noting that there are independent factors that influence knee joint loading too, such as gender (Obrębska et al. 2020), age (Hafer et al. 2019), joint health (Morgenroth et al. 2014), weight (Moyer et al. 2010) and gait speed (Robbins and Maly 2009). Those
with pre-existing conditions of the knee will have altered knee joint stability parameters, which is discussed more in Chapter 4.

Normally, knee joint contact loading is either calculated from forward or inverse dynamics, taking as inputs a combination of either marker positions or EMG, with force plate data too (Kumar et al. 2013; Lenhart et al. 2015a). Therefore, the values include positional data, angles, moments, muscle forces, GRFs, joint contact size and biological constraints (Lenhart et al. 2015a). When examining Table 1 to generate all the possible parameters to calculate the knee joint contact loading, Table 2 can be generated.

| Table 2- Various parameters used to define knee joint contact loading |
| Adapted table (Schrijvers et al. 2019) |
| **Kinematics** | **EMG** |
| 2. Tibiofemoral A-P translation | 14. Muscle onset time |
| 3. Varus-valgus movement | 15. Duration of muscle activation |
| 4. Relative phase dynamics | Combination |
| 5. 3D knee angles | 16. Modelling muscle forces |
| 6. Tibial rotation | |
| 7. Knee contact point movement | |
| 8. 3D knee translations | |

| Kinetics |
| 9. Knee extensor moment |
| 10. Ground reaction forces |
| 11. Total support moment |
| 12. Knee rotational moment |

This research establishes that knee stiffness, leg stiffness, co-contraction and joint loading all combine to form dynamic knee joint stability. To that end, the following sections will explore each of these factors, beginning with an overview of stiffness and how knee and leg stiffness amongst other types of stiffness are defined.

2.1.3.2 Stiffness
2.1.3.2.1 Overview
In the most simplified sense, stiffness is the relationship between a force and the deformation of a body (Butler et al. 2003). Stiffness originates from the basis of Hooke’s law and associates deformation with a spring-like constant (Serpell et al. 2012). Stiffness is a popular measure to analyse individuals as it has been linked to both performance and injury and therefore can define the success of a movement strategy (Butler et al. 2003). The term compliance is sometimes used in place of stiffness to describe the behaviour of a muscle, a joint or a limb chain (Gottlieb 1996). Stiffness and compliance
are related and whilst compliance can be considered the ease at which something can be moved, stiffness is the difficulty for that same something to be moved, both in quantitative terms (Gottlieb 1996). Inertia is sometimes confused with stiffness, and inertia is also the ease or difficulty for something to be moved from its current situation but is instead woven within stiffness as discussed below (Johns and Wright 1962). The compliance of a system can be studied through its inertial terms considering the relationship between acceleration and force (I), viscous terms considering velocity and force (B) and elastic terms linking its location and force (K) (Johns and Wright 1962; Weiss et al. 1986). Inertial moments and hence inertial stiffness are directly related to rotational acceleration (Johns and Wright 1962) and are not discussed further in this research due to the acceleration component not being considered. Inertial moments are a much smaller component of overall moments along with viscous moments and friction moments being smaller still, with elastic moments being the largest component of overall stiffness by far (Johns and Wright 1962). Viscous moments, and in turn, viscous stiffness, are produced over the normal elastic range and at the yield stress of a plastic body (Johns and Wright 1962). Hence, viscous moments are ignored in this research, as this is not produced in normal functional tasks (Johns and Wright 1962). Frictional moments are also ignored due to their being independent of velocity, acceleration or rotational displacement and as it is difficult to obtain (Johns and Wright 1962).

Elastic stiffness, or K, which is derived from the relationship between elastic moments and rotational displacement (Johns and Wright 1962), is the main element of stiffness discussed in this research. It is defined hereon as joint stiffness, which is also morphed to represent leg stiffness too when not a rotational but linear displacement is considered (Butler et al. 2003). Additionally, the joint stiffness calculation can be adapted to represent muscle stiffness (Davis and DeLuca 1996). Muscle stiffness has not been considered in further depth in this research, as the CCI was used to primarily investigate the contribution from the muscles instead, due to the existing popularity of this measurement (Schrijvers et al. 2019). As will be seen, joint and leg stiffness are now the main stiffness measurements of interest.

Throughout this research, the early stance phase or weight acceptance phase is of particular interest. This is somewhat due to how the two stiffness calculations are formed, for example, with the change in the moment being from IC to peak flexion angle, or to peak weight acceptance which is the internal extension moment, which are both at similar points in time (Butler et al. 2003; Gustafson et al. 2019). However, it has been recommended to narrow this investigated period from the peak internal flexion moment to the peak flexion angle/internal extension moment, due to the initial
negative internal flexion moment affecting the linearity of the calculation (Dixon et al. 2010).
This discussion on stiffness in this research is based on a quantitative calculation but is related to the more clinically termed stiffness which considers a reduction in flexion or terminal extension of the joint and is categorised as either mild, moderate or severe (Blevins and Sculco 2018). Considering this more clinical definition, one study considered self-reported stiffness and compared it to quantifiable stiffness and demonstrated that the two results directly conflicted (Blevins and Sculco 2018).

Very little previous research is available investigating gait in individuals with ACL injury/ACLr or even OA and crucially, linking with either joint or limb stiffness, even though a stiffness measure has been purported to provide important information for gait (Dixon et al. 2010).

2.1.3.2.2 Joint Stiffness
The stiffness of a joint, or the resistance to rotation of a joint, is defined as the change in the applied moment divided by the change in angular rotation (Agarwal and Gottlieb 1977). Commonly in this thesis, knee joint stiffness is shortened to the term knee stiffness.

Stiffness in the joint sense includes the consideration of not only the muscles that cross that joint but the soft tissues too (Davis and DeLuca 1996; Gustafson et al. 2019). It is important to note that joint stiffness can either be passive or active (Johns and Wright 1962). Passive joint stiffness can be considered to be passive soft tissue strain including passive lengthening or shortening (Roy et al. 2011) of the muscles and other components like the tendons, skin and joint capsule (Johns and Wright 1962). Dynamic joint stiffness can be either an active eccentric, which is lengthening, or concentric which is shortening, muscle contraction (Davis and DeLuca 1996), and this is discussed further below in section 2.1.3.3. However, dynamic joint stiffness can also be from passive soft tissue strain or a combination of both active and passive situations (Davis and DeLuca 1996). Hence dynamic joint stiffness can be quite different to passive joint stiffness (Davis and DeLuca 1996).

Dynamic joint stiffness is an instantaneous measurement; it is defined as the instant slope on the joint moment versus joint angle curve for a complete functional movement and hence can appear like a line of best fit representing the movement (Davis and De Luca 1995). However passive stiffness considers a smaller component of the moment-angle graphs, only when the data is truly linear (Takahashi et al. 2018). It is understood that this research is analysing the dynamic joint stiffness or termed simply as in this thesis as the joint stiffness (Davis and DeLuca 1996; Zeni and Higginson 2009b; Chang et
Joint stiffness is therefore a beneficial easily quantifiable measurement for comparing similar functional movements, such as with gait (Davis and DeLuca 1996). Parameters such as joint stiffness are an overall important mechanism for joint stability (Needle et al. 2014).

Moment versus angle graphs can be analysed for their stiffness results, with the latter measure either being in degrees (Davis and DeLuca 1996; Frigo et al. 1996) or in radians (Weiss et al. 1986). This analysis between moments and angles is considered to be an analysis of the neuromechanical control behaviour of the whole body during gait (Frigo et al. 1996). The stiffness results can supplement and support the modelling data, to understand if there have been changes in performance as discussed earlier in section 2.1.3.1, hence demonstrating the effects from an original injury or possible future injuries (Butler et al. 2003). Figure 3 represents these moment-angle graphs well with overlaid sections of the gait cycle (Martinez-Villalpando and Herr 2009). These graphs are comprehensive in showing a healthy range for knee angle and knee moment data during a gait cycle (Martinez-Villalpando and Herr 2009). It shows, when combined, the interaction between these two measurements, from initial heel strike (HS) thorough stance flexion to maximum stance flexion (MSF) (Martinez-Villalpando and Herr 2009). It then shows through stance extension to maximum stance extension (MSE) through pre-swing to toe off (TO), into swing flexion to maximum swing flexion (MWF) to swing extension and back to heel strike (Martinez-Villalpando and Herr 2009). The initial loading component of the gait cycle is of most interest as this informs how dynamic joint stiffness is maintained in a particularly challenging part of the gait cycle; when the other leg is preparing to swing forwards (Butler et al. 2003). It can therefore be seen in this moment-angle graph how the volunteer’s dynamic joint stiffness result could be derived (Butler et al. 2003).

As previously mentioned in this section, this relatively linear region of the moment angle graph represents the initial loading response of the knee as the external knee flexion moment increases up to the point of the peak knee flexion moment, and represents the dynamic joint stiffness as shown in Figure 4 (Zeni and Higginson 2009b). However, a more recent but less common definition of knee joint stiffness is the knee angle against the knee moment during the weight acceptance phase, between the peak knee flexion moment, to whichever occurs first between the peak flexion angle or the peak extension moment (Gustafson et al. 2019). It is assumed that the peak knee flexion moment indicates the initial peak external extension moment and that the peak extension moment is the peak external flexion moment (Webster et al. 2019). Therefore, there are slightly different time periods analysed depending on which calculation is used.
Figure 3- An edited figure of knee biomechanics on one participant for level walking for (a) angle, assuming flexion angle positive and moment, assuming internal flexion moment positive over the gait cycle and (b) knee moments against knee angles representing stiffness and dampener behaviours

(Martinez-Villalpando and Herr 2009)
Figure 4- Identifying the loading response portion of the gait cycle, using the maximum external flexion moment to define the time period for the knee moments assuming the external flexion moment positive and angles, and then plotting this information against each other to produce a linear plot, assumed external flexion moment is positive

(Zeni and Higginson 2009b)

\[ K_{\text{Joint}} = \frac{\Delta M}{\Delta \theta} \]

Equation 1

(Zeni and Higginson 2009b)

Equation 1 is used to calculate the joint stiffness, where an increase in the x-axis angle defines an increase in flexion, and an increase in the y-axis moment defines an increase in the external flexion moment (Zeni and Higginson 2009b). The boundaries for this measurement are between IC and the maximum joint moment at joint angle time (Zeni and Higginson 2009b).

A greater difference in joint moments or less joint range of movement are two contributors to increased joint stiffness (McGinnis et al. 2013), which is clear as both increase the mathematic result. With increasing speeds for healthy participants there is an increase in both the heel strike and peak flexion angles in the first half of stance (Robert-Lachaine et al. 2020). For an increasing speed for external flexion moments, there appears to be a larger range in the moments in the first half of stance; the moment at heel strike is lower, but the peak moment is higher (Meinders et al. 2021). This suggests that because the knee flexion moment component increases linearly with speed, there would be an increase in joint stiffness with an increase in speed (Butler et al. 2003; Meinders et al. 2021). Joint stiffness can increase when there is an increase in
joint laxity due to injury or disease (Zeni and Higginson 2009b), which in itself can be a vicious cycle of more forces through the knee joint, and further deterioration of a condition (Zeni and Higginson 2009a). Furthermore, the contralateral limb can also be impacted when trying to avoid damaging the symptomatic limb as a compensatory strategy (Metcalfe et al. 2013). It is therefore beneficial to collect such a pertinent measure on a healthy group of volunteers to be able to compare and contrast the different results for a patient group (Webster et al. 2019). Though this research is primarily interested in those with issues with their ACLr and possible early indications of OA, there is some merit to examining stiffness information from those that already have OA or severe OA as the same biomechanical principles used in such cases may help to find changes in these ACLr cases too. Those with severe knee joint OA are known to walk with higher knee joint stiffness values, irrespective of speed, which may occur to overcome the knee instability they often experience but can exacerbate their condition (Zeni and Higginson 2009b). A recent literature review investigating the effects of normal walking on joint stiffness results between healthy and affected populations was explored (Schrijvers et al. 2019). Two studies had no difference (Collins et al. 2014; Galli et al. 2018), one had more stiffness for those with fewer symptoms, which is directly in contrast to the summary of the literature review (Gustafson et al. 2016), and for challenged walking, one study had higher joint stiffness for an affected population at pre-defined speed, self-selected and fast speeds compared to a healthy population (Zeni and Higginson 2009b). Having only four studies identified by this recent systematic literature review into walking speed and joint stiffness for healthy and injured populations, with the results of which directly conflict with one another, suggests that there is not enough known about the effects of walking speed on knee joint stiffness results for healthy and injured populations (Schrijvers et al. 2019).

The dynamic joint stiffness for the hip and ankle joints also requires consideration. In terms of the hip, passive hip stiffness can be derived more readily to address its involvement in gait and successful function of movement (Carvalhais et al. 2011; Cardoso et al. 2020; Ocarino et al. 2021). In terms of the ankle, it has been shown that elastic joint stiffness can be reliably calculated with the presence of the total joint moment regardless of whether it was produced through passive or active means (Weiss et al. 1986). This is proved by obtaining the elastic joint stiffness from the slope of the moment-angle curve by dividing the change in the moment by the change in angle (Weiss et al. 1986). From there, this result is plotted against the moment value, hence producing a new slope of the inverse of the angle, 1/angle (Weiss et al. 1986). This new slope has a consistent gradient no matter whether the results are collected at one
2.1.3.2.3 Leg Stiffness
Limb stiffness, or the term commonly used in this research, leg stiffness, is the resistance to the change in leg length after applying internal or external forces (Serpell et al. 2012) and considers the combination of several joints into one calculation (Farley and Morgenroth 1999). Leg stiffness can also be categorised by the same single linear spring principle used for muscle stiffness (Butler et al. 2003) and can describe the change of elastic deformation in the leg (Akl et al. 2020). For leg stiffness, the hip, knee, and ankle joints are each considered an individual spring (Farley and Morgenroth 1999). Leg stiffness is the recommended calculation for the lower limbs when the leg is performing walking or running, with vertical stiffness more recommended for vertically aligned activities like hopping (Butler et al. 2003). This is because the leg stiffness calculation has a component in the calculation which considers if the subject made IC with the ground and was in stance phase absorption but was not in a purely vertical scenario (Butler et al. 2003). Therefore, a component of $\Delta L$ needs to be considered where the spring is not fully in a vertical component, and so the displacement at a

recently, sometimes joint stiffness is represented by the term ‘quasi-stiffness’, and is defined as “the stiffness of a spring that best mimics the overall behaviour of a joint during a locomotion task” (Foster et al. 2020). Quasi-stiffness can therefore look at the function of the joint over a longer period of time, such as the whole stance phase (Foster et al. 2020). However, this is still observing the system for a small amount of time, and not when the system is in equilibrium (Latash and Zatsiorsky 1993). This is a more modern translation of quasi-stiffness, that was originally used to represent leg stiffness (Latash and Zatsiorsky 2016). Therefore, as limb stiffness and joint stiffness are inextricably linked theoretically through the use of Hooke’s Law, it is deducible that the term quasi-stiffness can be used for both joint and leg stiffness (Foster et al. 2020). A single recent counterargument to this suggests that joint and limb stiffness are unconnected during eccentric loading (Akl et al. 2020), though this recency means further debate is required before it can be widely accepted. It could also be that the non-linear characteristics of joint stiffness linked to linear characteristics of leg stiffness were not fully considered (Günther and Blickhan 2002).
certain angle also needs to be calculated as well as the change of displacement generally (Farley and González 1996; Sano et al. 2017).

However, when all lower leg springs are in maximum flexion, likened to each spring being in full compression, the lower leg can be assumed as one large, compressed spring with absorption of the energy (Bishop et al. 2006). It is also assumed that the subject’s centre of mass (CoM), which is the centre between two parts that have the same moment of inertia (Tesio and Rota 2019), is at its lowest vertical point during the motion (Bishop et al. 2006). This then simplifies the leg stiffness calculation to equate to a calculation for vertical stiffness, where the non-vertical components no longer require consideration (Zhao et al. 2023). Leg stiffness resolved down to vertical stiffness is also seen in other research (Farley and Morgenroth 1999; Akl et al. 2020). Additionally, leg stiffness can be represented using full dynamic equations considering springs and dampeners and can be a better representation of system dynamics, though is a full area of research in its own right (Kim and Park 2011; Yang et al. 2015).

For gait, the CoM is lowest just after the heel strike when there is the double support pre-swing, and highest at midstance (Gordon et al. 2009), whilst the peak GRF is highest at approximately maximum knee flexion in the first half of stance (Webster et al. 2019; Jiang et al. 2020). This means simplifying the point of lowest COM and highest vertical GRF does not tally to a single point in time for gait to show maximum compression of the spring (Webster et al. 2019; Jiang et al. 2020). It is still possible to simplify the spring though as it is the change in CoM throughout the stance phase (Farley and Morgenroth 1999). Assuming that the lower limb is not going through a large range of motion as is present in running (Mann and Hagy 1980), the trigonometric component of leg stiffness is no longer required and the leg stiffness can be simplified to equal the vertical stiffness (Farley and González 1996).

This is demonstrated in Figure 5, and hence leg stiffness is commonly calculated as the peak vertical GRF (F) divided by $\Delta L$, the change in vertical displacement of the CoM, where the peak vertical acceleration can be double integrated to aid the calculation, as seen in Equation 2 (Bishop et al. 2006). The change in vertical displacement in the CoM can be obtained without double integration if the CoM is known (Farley and Morgenroth 1999). The time period for this calculation is between IC and 50% of the stance phase. It’s important to note that after IC and during stance phase absorption spring compression occurs (Bishop et al. 2006), and therefore fits in well with the other variables that are being derived in the initial load-bearing response phase of gait. While the spring in Figure 5 represents the lower body and the point at the top of the spring represents the CoM of the whole body, it could be questioned whether CoM in other
literature is of the whole body or just the lower body. CoM is not mentioned to be lower body CoM in other literature (Farley and González 1996; Bishop et al. 2006; Sano et al. 2017), so it is assumed that CoM represents the whole body CoM as it does in Figure 5 and for ease of calculations.

![Conceptual model of leg stiffness represented as a spring](Bishop et al. 2006)

Figure 5 - Conceptual model of leg stiffness represented as a spring

(Bishop et al. 2006)

\[ K_{leg} = \frac{F}{\Delta L} \]

Equation 2

(Bishop et al. 2006)

It is important to understand how the leg stiffness calculation operates and what the results from it mean. Theoretically, if the leg is more extended at IC, the joints at the hip, knee and ankle are stiffer and the range of motion at each joint is less (García et al. 2023). The GRF vector will be more in line with the leg, and hence the joint moments will decrease, but there is less displacement of the leg as a spring, otherwise known as leg compression, meaning a higher leg stiffness (Farley and Morgenroth 1999). Therefore, a higher value for leg stiffness means a stiffer lower leg chain (Farley and Morgenroth 1999).

As discussed previously in the joint stiffness section 2.1.3.2.2, quasi-stiffness is a popularly used term when referring to leg stiffness (Latash and Zatsiorsky 2016), which uses one value to comprehensively represent lower leg stiffness, including the bone, cartilage, ligaments, tendon and bone (Pearson and McMahon 2013), with it suggested that quasi-stiffness does not consider elastic energy (Latash and Zatsiorsky 1993). This is in direct conflict with more recent definitions of what leg stiffness represents (Akl et al. 2020).

Leg stiffness has been defined as independent of speed but is influenced by surface conditions and therefore when calculating leg stiffness in this research, it will be
important to collect all data from one type of surface condition only to aid comparability (Ferris et al. 1998). It also appears to be important to maintain surface type for joint stiffness results too, as it was suggested that joint stiffness results changed too; both the moments and angles changed for their surface exposure (Ferris et al. 1998). It is important to note that this article was for running (Ferris et al. 1998) as it has been difficult to establish a link in walking trials for this research, demonstrating the need for research between leg stiffness and walking, particularly for individuals with ACLr. Leg stiffness is calculated by taking the difference in the movement of the CoM over a period of time (Farley and Morgenroth 1999). Whilst it may have been argued that maintaining a similar CoM most of the time during a movement like gait is better for the metabolic cost, this has been disproven (Ortega and Farley 2005). It was suggested that the more a participant tried to keep a flat CoM trajectory the more force generation was required for the continuation of gait and interestingly, that the limbs were more flexed at stance with this walking style (Ortega and Farley 2005). This suggests that to maintain a healthy gait style with lower metabolic cost a certain amount of vertical variation with less flexion is required (Ortega and Farley 2005).

Whilst greater knee flexion is acceptable in uninjured individuals and helps to attenuate load (Creaby et al. 2013), there is historically no difference in peak flexion angles for individuals with ACLr during gait (Kaur et al. 2016). Greater knee flexion in OA individuals does however occur and causes higher sagittal plane knee contact loading during walking (Creaby et al. 2013). Therefore trying to maintain a similar CoM throughout gait for those that may have early indications of OA could be particularly damaging (Creaby et al. 2013), which could be possible for some individuals with ACLr as discussed earlier in section 2.1.2.1.

Now that different stiffness concepts have been discussed, particularly joint and leg stiffness to support the concept of dynamic knee joint stability, the other two components that define dynamic knee joint stability, namely co-contraction and knee joint loading are explored.

2.1.3.3 Co-Contraction
Co-activation and co-contraction have been used interchangeably in the literature (Lewis et al. 2010; Na and Buchanan 2019), though there are perceived slightly different definitions (Kellis et al. 2003). Typically co-activation is a muscle coordination strategy through the concept of the simultaneous activation of a set of agonist and antagonist muscles (Centomo et al. 2007), whilst co-contraction is the measurement of this change, represented through the CCI (Kellis et al. 2003). For clarity, the term co-contraction has
been used in this research. It has been found that co-contraction is a useful strategy in increasing joint stiffness when there is an instability (Lewis et al. 2010).

CCI calculations can be an excellent implementation to compare subjects and can provide good reliability, though higher CCI s are not always associated with more variability between subjects (Hubley-Kozey et al. 2013). While it has been argued that gathering co-contraction information through normalised modelled muscle moments (MCCI) can produce lower co-contraction results than traditional methods calculated from EMG data, MCCI could reflect a more detailed perspective of the human body during motion as it can produce information on muscles that would be too difficult to study with surface EMG methods (Knarr et al. 2012). For ease, co-contraction will be the term used going forward in this research to represent both the muscle coordination strategy and the quantifiable measurement of this change.

Coordinated muscular activity can decrease the strain on the knee ligaments as the knee joint stability is improved (Williams et al. 2001). Understanding that co-contraction is used to aid stability of a joint, and considering a recent literature review establishing the CCI as a commonly used measurement of Dynamic Joint Stability (Schrijvers et al. 2019), 5 studies investigated the CCI for ‘normal’ gait, using very similar equations (Lewek et al. 2006; Centomo et al. 2007; Hurd and Snyder-Mackler 2007; Ramsey et al. 2007; Collins et al. 2014).

Hurd and Snyder-Mackler (2007), calculated the CCI in Equation 3 from an earlier source:

$$CCI = \frac{EMGS}{EMGL} \times (EMGS + EMGL)$$

Equation 3

(Rudolph et al. 2001)

CCI was calculated by integrating the normalised EMG data after the linear envelope was produced, where EMGS is the level of activity in the less active muscle, and EMGL is the level of activity in the more active muscle (Rudolph et al. 2001). This equation was integrated over a ‘weight acceptance period’ between 100ms before IC and until the time point of peak knee flexion (Rudolph et al. 2001). 100ms was selected to take into account any electromechanical delay (EMD) (Rudolph et al. 2001). Before analysing any EMG data, it is important to normalise it to the peak EMG of each trial, which is a common practice (Richards and Higginson 2010).
Equation 3 was used to calculate CCI in this research, but with a different EMD period as discussed in more detail in Chapter 6.

The next step is to identify the most appropriate muscle pairings from historical literature. Due to the feasibility of the Research Centre for Clinical Kinesiology (RCCK) Laboratory, 16 muscles across two legs can be collected, hence 8 per leg, so a certain amount of discernment for selected muscles is required. The historical literature is sporadic and not a well-defined field, no doubt due to the difference in the experimental data collection between studies.

Initially, studies that investigated two muscles to create a CCI, or a ‘single pairing’ were sought. Two studies examined the pairing of the vastus lateralis and gastrocnemius lateral head (Hubley-Kozey et al. 2013; Mohr et al. 2018), and two studies also examined the vastus lateralis and lateral hamstrings pairing which is assumed by the author to be lateral biceps femoris (Hubley-Kozey et al. 2013; Jones et al. 2018).

It was found that most previous literature examined individual muscles into a functional group, or a ‘grouped pairing’, to form co-contraction indices (Souissi et al. 2017). One group was the plantar flexors, which are soleus and gastrocnemius lateral head, versus the dorsiflexor of tibialis anterior (Souissi et al. 2017). Another group was the knee flexors of lateral biceps femoris, known as the short head, medial biceps femoris, known as the long head, and gastrocnemius lateral head versus the knee extensors of rectus femoris and vastus lateralis (Souissi et al. 2017).

As there were only two identified single pairings, more single pairings could be made. Discernment for the number of muscles that can be practically examined was required, and also that some muscles that lie close to one another are extremely difficult to isolate, and that there is previous precedent for a certain 8-muscle layout (Afschrift et al. 2019). One study (Richards and Higginson 2010) collected vastus lateralis and medial hamstrings to verify modelled quadriceps (rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius) and hamstrings (medial potion was long head biceps femoris, semitendinosus and semimembranosus and lateral was short head biceps femoris). Therefore, a pairing of the vastus lateralis and medial hamstrings can also be considered, assuming the medial hamstrings are defined as the medial biceps femoris as there is precedent not to collect semimembranosus and semitendinosus (Afschrift et al. 2019).

Due to the variation between different muscle pairings and different CCI calculations used amongst existing literature, it is extremely difficult to form a quantifiable understanding of previous CCI results for a healthy population let alone an injured one that can be used comparatively to the results that will be garnered in this research.
During gait however, CCIs around the knee appear to be highest during the first double support phase when compared to the single support phase, second double support phase or the swing phase, suggesting that the knee is under the most co-contraction during the IC phase of stance (Souissi et al. 2017). More examination of dynamic knee stability and co-contraction during gait is explored in Chapter 4.

Remembering that co-contraction has a role in dynamic knee joint stability (Schrijvers et al. 2019), and that co-contraction is linked to joint stiffness (Collins et al. 2014), leg stiffness (Holt et al. 1996; Gontijo et al. 2008), and joint loading (Al-Khlaifat et al. 2013), it is important to establish the link between each CCI equation and which other dynamic knee stability factor during gait it most closely influences. As the CCI is one measurement that can be used to define dynamic knee stability, it is reasonable to assume that all the CCI measures in this research will affect the dynamic knee joint stability result (Schrijvers et al. 2019).

The quadriceps and hamstring components (simplified to vastus lateralis and medial biceps femoris respectively) used in CCI One are mainly responsible for flexion and extension of the knee joint (Webster et al. 2019). These two muscles are on either side of the knee joint and will influence the knee joint stiffness as they are responsible for a change in knee angle and thus the knee joint stiffness calculation (Gray and Lewis 1918; Schrijvers et al. 2019). And where the knee joint stiffness calculation can be affected, the leg stiffness calculation can be too as the more flexed a joint becomes, the lower the CoM, and that directly changes the leg stiffness results, meaning to some extent knee and leg stiffness are linked (Butler et al. 2003). Adjustment in the knee angles affects the knee moments, as moments are derived from a force with a moment arm which changes with a change in angle, which in turn affects the knee loading results, as knee loading results are in part derived from the external moment information (Delp and Loan 2000). This same theory for changes in joint stiffness, leg stiffness and joint loading is also possible for CCI Two and CCI Three. The lateral gastrocnemius in CCI Two is considered due to the moment arm it creates around the knee joint in both sagittal and frontal planes for stabilization (Mohr et al. 2018) and considering the link between ACLr and individuals with OA as discussed in the general literature chapter (Vaishya et al. 2019), the lateral gastrocnemius muscle activation is increased for those with OA during early stance (Heiden et al. 2009). Furthermore, the vastus lateralis and gastrocnemius lateral head pairing (CCI Two) has shown no differences between a control group and the contralateral leg of a severe OA group, or even between the affected and
contralateral leg itself (Lewek et al. 2006). The vastus lateralis and lateral biceps femoris pairing (CCI Three) has previously shown no differences between a control group and the contralateral leg of an OA group either (Jones et al. 2018), or severe OA group (Lewek et al. 2006), during initial loading, though this has been challenged with higher co-contraction of this pairing being found (Metcalfe et al. 2013). That is not to say that there are no significant differences between OA affected and unaffected sides (Lewek et al. 2006). CCI Four concentrates on the ankle plantar flexors versus the dorsiflexors whilst CCI Five looks at the knee flexors versus the knee extensors (Souissi et al. 2017). As these muscles are located on either side of the knee joint (Gray and Lewis 1918), they will affect the knee joint stiffness, the leg joint stiffness, and in turn, the joint loading (Schrijvers et al. 2019).

Next, it is important to understand how each of the identified muscles discussed above works during the gait cycle without considering the co-contraction component. Throughout this thesis, the terms eccentric, concentric and isometric have been used in relation to muscle contractions. Isometric contraction is when the muscle generates force but there is not a change in length such as holding a weight in a static position and not letting it drop (Fortier et al. 2010). Isotonic contraction is when there is a contraction of the muscle to generate force causing a change in the length of the muscle, with two subcategories, isotonic eccentric when the muscle lengthens, and isotonic concentric when the muscle shortens (Fortier et al. 2010). Figure 6 shows the muscle activation pattern for a typically healthy individual during gait whilst Figure 7 demonstrates how the aforementioned muscles of interest for this research contract differently during the first half of the gait cycle.

For dynamic joint stability and knee joint loading during gait, CCI Five (gastrocnemius lateral head, lateral and medial biceps femoris versus vastus lateralis and rectus femoris) is the best indicator theoretically as it covers the whole of the stance phase with the most muscles considered (Sheffler and Chae 2015). CCI Four (gastrocnemius lateral head and soleus versus tibialis anterior) covers most of the stance phase and is the second-best indicator of dynamic joint stability (Sheffler and Chae 2015). CCI Two (gastrocnemius lateral head versus vastus lateralis) is the third best indicator covering a large proportion of the stance phase, with CCI One (vastus lateralis medial biceps femoris) covering just under the first half of the loading response, and CCI Three (vastus lateralis versus lateral biceps femoris) covering the initial loading response only (Sheffler and Chae 2015).

In terms of joint and leg stiffness, CCI Four and CCI Five feature a similar number of constraining muscles to represent stiffness and hence are both the best CCI to use, with
CCI One, CCI Two and CCI Three jointly the second best CCI to use as they all consider the same number of constraining muscles (Sheffler and Chae 2015).

**Figure 6** - Muscle activation pattern for the lower limbs during healthy gait (Sheffler and Chae 2015)

**Figure 7** - Analysis of muscles, considering the type of muscle contraction during the whole gait cycle for healthy individuals

Adapted figure (Sheffler and Chae 2015)
However, this does not seem to distinguish which CCI calculations favour the factors of dynamic joint stability namely joint stiffness, leg stiffness and joint loading (Schrijvers et al. 2019). To try and separate the different CCI calculations, the muscle activation pattern for each of the muscles during the stance phase of normal gait can be studied in Figure 7, and it can be seen that each muscle has different periods of activation (Sheffler and Chae 2015). This section will analyse how the CCI measurements are interrelated to other dynamic knee stability factors in terms of the length of activation each muscle has over the stance phase of the gait cycle and hence the contribution it could have when in a CCI calculation. The type of movement and contraction they are performing is also stated, as this can have a direct impact on the joint loading, as muscles create the highest forces when eccentrically loaded (Hortobágyi and Katch 1990; Douglas et al. 2017), due to the contribution from the calculated muscles of the model to establish the joint contact loading (Lenhart et al. 2015a), assuming that the model represents the activations correctly as explored more in Chapter 6.

Therefore, the soleus could be an important muscle during the loading response for joint loading, as it is the only muscle purely in eccentric contraction (Webster et al. 2019). As mentioned earlier, dynamic joint stability can be represented by several different measurements, though as a strategy, it comes from joint moments caused by muscle contractions initially, the same two components of which aid the ability to derive the joint loading results from the musculoskeletal model (Lenhart et al. 2015a). To this end, in terms of a motor control strategy, dynamic joint stability and joint loading should have the same preferred and least preferable CCI calculations (Lenhart et al. 2015a).

Finally, joint stiffness can be linked to the constraints on the lower leg between initial loading and mid-stance (Butler et al. 2003), roughly the period of time joint stiffness is calculated between, as seen in Figure 8 (Webster et al. 2019). The constraints on the vector during this period are the muscles of the lower leg, and it is worth remembering that the movement of the muscle is not being examined but an external moment opposing the movement (Webster et al. 2019). For example, the quadriceps is a knee extensor but an external knee flexion moment is opposing this (Webster et al. 2019). When the leg is initially loaded the constraining muscles are gluteus maximus (hip flexor moment, but not collected in this study), quadriceps (knee flexor moment, just rectus femoris and vastus lateralis are collected for the quadriceps) and the tibialis anterior (ankle plantar flexor moment) respectively (Webster et al. 2019). Towards early midstance, the constraining muscles are the quadriceps (knee flexor moment, lower than at IC) and the soleus (ankle dorsiflexor moment) (Webster et al. 2019). There is no hip muscle action required in mid-stance as the GRF vector falls
directly behind the hip joint, therefore there is no muscle activation for a hip moment required (Webster et al. 2019). There is a balance between the external moments of the body and the aforementioned muscles to dictate the maximum the hip, knee and ankle can flex and the angle and associated internal moments they can then produce (Sheffler and Chae 2015). As discussed earlier in this section, in this way the CoM vertical position is directly affected, and in turn, therefore, the leg stiffness (Butler et al. 2003). Also, leg stiffness is calculated over a similar time period as that for joint stiffness (Butler et al. 2003). Therefore, joint stiffness and leg stiffness should have the same favourable CCI calculations (Butler et al. 2003).

![Figure 8: The loading response vector (or ground reaction force vector) for IC through to mid stance of the gait cycle for a healthy individual.](Webster et al. 2019)

Next, the agonist and antagonist pairs need to be identified to be input into the CCI calculations and to hence advise which muscles need recording in the laboratory. As discussed earlier in this section, two studies discussed the vastus lateralis and gastrocnemius lateral head pairing (Hubley-Kozey et al. 2013; Mohr et al. 2018), and hence this was called CCI Two in this research. Two other studies paired vastus lateralis and the lateral hamstrings (assumed by the author to be lateral biceps femoris) (Rudolph et al. 2001; Hubley-Kozey et al. 2013; Jones et al. 2018), and this became CCI Three. Another single muscle pairing was sought, and there was the pairing of vastus lateralis and the medial hamstrings (assumed by the author to be medial biceps femoris as semimembranosus and semitendinosus were not collected), and this was called CCI One (Richards and Higginson 2010).

Then, for grouped CCI calculations, the plantar flexors of soleus and gastrocnemius lateral head versus the dorsiflexor of tibialis anterior were CCI Four, then the knee
Flexors of lateral biceps femoris (short head), medial biceps femoris (long head) and gastrocnemius lateral head versus the knee extensors of rectus femoris and vastus lateralis became CCI Five (Souissi et al. 2017).

Some of these CCI pairings were slightly adapted from previous literature, due to how many muscles could be collected on each leg at a time with the EMG equipment (Afschrift et al. 2019), and to avoid collecting ‘cross-talk’ from placing pads too close to one another (Konrad 2006). As a group of lower limb muscles was previously studied in the RCCK laboratory, this research aligned with that selection allowing data comparison with previous and future studies (Afschrift et al. 2019). Considering this historic layout only gluteus medius was collected but not used in further analyses. Figure 9 shows the EMG placements for all subjects involved in studies investigating EMG in this research whilst Table 3 shows this in tabular form.


Figure 9 - EMG Muscle Placements

Adapted figure (Daley 2017)
### Table 3 - The agonist and antagonist pairs for the five CCI measurements

<table>
<thead>
<tr>
<th>CCI One (single pairing)</th>
<th>Agonist and Antagonist Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus Lateralis</td>
<td>Medial Biceps Femoris (component of quadriceps and a knee extensor)</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td>(component of hamstrings and a knee flexor)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCI Two (single pairing)</th>
<th>Agonist and Antagonist Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus Lateralis</td>
<td>Gastrocnemius Lateral Head (component of gastrocnemius and a knee flexor)</td>
</tr>
<tr>
<td>Gastrocnemius Lateral Head</td>
<td>(component of quadriceps and a knee extensor)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCI Three (single pairing)</th>
<th>Agonist and Antagonist Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus Lateralis</td>
<td>Lateral Biceps Femoris (component of quadriceps and a knee extensor)</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td>(component of hamstrings and a knee flexor)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCI Four (grouped pairing)</th>
<th>Agonist and Antagonist Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>Tibialis Anterior (dorsiflexor)</td>
</tr>
<tr>
<td>Gastrocnemius Lateral Head</td>
<td>(plantar flexors)</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td></td>
</tr>
<tr>
<td>(components of gastrocnemius and hamstrings and knee flexors)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CCI Five (grouped pairing)</th>
<th>Agonist and Antagonist Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius Lateral Head</td>
<td>Rectus Femoris</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td>Vastus Lateralis (components of quadriceps and knee extensors)</td>
</tr>
</tbody>
</table>

Next, what these five CCIs represent in terms of functional movement is explored, remembering that CCI calculations are based on the comparison between the least and most active muscle or muscle groups (Rudolph et al. 2001; Lewek et al. 2006). CCI One’s components can both be higher post-injury, in terms of individual muscle activation (Blackburn et al. 2019), though this has been more recently challenged (Hollman et al. 2021). Increased muscle activations would mean that CCI One is higher in a patient cohort; one could be mistaken for thinking that as both muscles are more activated and the CCI calculation is in part a ratio, the CCI result would be the same, but is an incorrect assumption with how the CCI calculation is made (Rudolph et al. 2001; Lewek et al. 2006). CCI Two should be lower for patients, as quadriceps should be more contracted for a patient group and gastrocnemius should be less activated; a greater range between two activated muscles causes the overall CCI value to lower (Sharifi et al. 2018; Robbins et al. 2019). CCI Three should have a similar result to CCI One when comparing healthy participants to patient participants (Blackburn et al. 2019). The understanding that the knee is less flexed at IC for those with a knee injury and that they have less range of knee flexion (Rudolph et al. 2001), could mean that the foot and ankle are required to move through less range to prepare for IC and TO (Fong et al. 2011). This would suggest a smaller CCI value for CCI Four for a patient group, as neither plantar nor dorsiflexors would be activated as much as they would have been for a healthy population (Webster et al. 2019). Finally, CCI Five should behave similarly to CCI Two where similar muscular components are investigated, hence CCI Five should be lower for a patient population (Sharifi et al. 2018; Robbins et al. 2019).
2.1.3.4 Knee Joint Loading

Knee joint contact force loading considers the contact forces at the tibiofemoral and patellofemoral joints, though this research is interested in the tibiofemoral component (Lenhart et al. 2015a). Knee joint contact force loading is derived not only through the externally applied GRFs but also from the internal forces from the muscles which act on the joint (Steele et al. 2012; Holder et al. 2020). Knee joint contact force loading, often abbreviated to joint loading in this research, is not well investigated for individuals with an ACL injury (Gardinier 2013), and how that ACL injury modifies the loading on particularly the tibiofemoral joint (Crook et al. 2021). As it is an internal force, it is difficult to establish in a real-world situation, such as gait, with eKAM being a popular measurement used even recently as a surrogate measure of knee joint loading (Simic et al. 2011; Marouane and Shirazi-Adl 2019), which is derived through the use of force plates (Al-Khlaifat et al. 2016).

Some argue that eKAM should be considered in partnership with frontal knee angles (Kutzner et al. 2010), or in partnership with the peak absolute values of the external knee flexion moment (Walter et al. 2010). Others argue the validity of eKAM altogether, with frontal angle considered a better measure (Marouane and Shirazi-Adl 2019). Generally, it appears more recently that eKAM and tibiofemoral contact forces cannot be definitively linked (Bowd et al. 2020). With large advancements in imaging and modelling research, techniques such as musculoskeletal modelling can now be employed to establish what occurs at the joint level (Lenhart et al. 2015a), as discussed in section 2.1.4.1.

As discussed in section 2.1.3.3, the knee is exposed to the most co-contraction during the first double support phase, which encompasses the IC and loading response (Sheffler and Chae 2015). The ACL is under the most strain during heel strike when the knee is the least flexed (Englander et al. 2020). This period of time is when the vertical GRF is increasing to approach its first peak of the double hump vertical GRF graph typical in one cycle of healthy gait, with muscle forces and joint contact forces increasing in this period also (Kutzner et al. 2010; Gustafson et al. 2019; Marouane and Shirazi-Adl 2019). There are a lot of forces for this relatively unstable joint, which highlights the necessity to examine the internal joint loading of the knee during IC and the loading response phases (Perry 2010; Schrijvers et al. 2019). One study found higher compressive forces on both the medial and lateral tibiofemoral compartments during gait in those with an unreconstructed ACL compared to healthy individuals, though this information was garnered through magnetic resonance imaging (MRI) after a gait session, and hence does not examine the knee whilst in actual motion and the active contribution from the
muscles (Crook et al. 2021). Furthermore, this study outlined that the contralateral uninjured limb demonstrates similarity to an uninjured cohort (Crook et al. 2021), though others disagree (Gustafson et al. 2019). Another study suggested that ACLr improved eKAM measures for coper individuals, and ACLr was more detrimental for non-copers, though again this was deduced from MRI (Marchiori et al. 2019). The link between loading and OA can be examined, as knee joint loading studies considering osteoarthritic gait are more plentiful, and considering that altered kinematic behaviour from ACL injury leads to early onset OA (Crook et al. 2021), and ACLr does not protect from and can lead to OA onset (Vaishya et al. 2019). One study found that there were increased knee contact forces during longer walking periods over the whole of the tibiofemoral joint (Gustafson et al. 2019). Another study found that modelling the forces in osteoarthritic patients has determined more medial loading coupled with lateral offloading during the stance phase of gait compared to healthy controls (Kumar et al. 2013), though it is worth noting that the model used in that research was EMG driven and not kinematically and kinetically driven; the different types of modelling are discussed in section 2.1.4.1. Another EMG modelled investigation during gait established greater medial contact loading for those that had more structural OA progression, compared to those that maintained the level of structural OA changes present in their knees, termed “non-progressor” (Wilson et al. 2021). This suggests that, to a certain extent, medial knee contact forces generally increase as OA progression increases (Wilson et al. 2021). Unfortunately, less is known about the effect on the lateral compartment of the tibiofemoral joint in ACL-injured and reconstructed individuals and OA progression (Saxby et al. 2016a).

This research focuses on the effects of sagittal plane biomechanics on joint contact loading as ACL injury leads to more anterior translation of the tibia during the stance phase (Yim et al. 2015). However, it is important to note that changes in other planes have their effect, particularly in the frontal and coronal plane on tibiofemoral loading (Marouane and Shirazi-Adl 2019; Van Rossom et al. 2019; Hunt et al. 2021), such as an increase in varus angle directly increasing medial tibiofemoral loading (Van Rossom et al. 2019). Very little previous research can be established to ascertain how the changing of gait speed affects knee joint contact loading on the medial and lateral compartments of the tibiofemoral joint (Saxby et al. 2016a; Lenton et al. 2018), with no clear knowledge of individuals with ACLr using detailed inverse dynamic-driven musculoskeletal modelling.

To reduce detrimental knee joint contact loading in those with OA, gait retraining should be employed (Paterno and Hewett 2008). However, the impact on the surrounding
joints such as the hip and ankle when retraining the knee movements is not well understood in previous literature (Bowd et al. 2019). Due to the link between ACLr and OA development through movement changes (Vaishya et al. 2019), gait retraining of an ACLr individual may also be beneficial (Decker et al. 2004). This next section aims to understand the gait biomechanics of the other joints in the leg, namely the hip and ankle.

2.1.3.5 Biomechanics of Other Joints
As this research is primarily concerned with gait and more specifically, the IC and loading response phases, this section will focus on the hip and ankle during those scenarios. Kinematically for the hip, at IC the hip is at maximum flexion and extends until it is at its maximum extension at the end of the stance phase (Lunn et al. 2016). In the frontal plane, the hip is slightly adducted at IC and progresses towards abduction, until maximum abduction is found approximately a third of the way through the stance phase (Lunn et al. 2016). The hip moves more in the frontal plane than the knee, however in those with ACLr, there is constrained frontal hip motion, to aid stability for the more unstable knee joint (Davis et al. 2019). The hip is internally rotated at heel strike and increases slightly until approximately a third of the way through the stance phase when it continues to externally rotate (Uemura et al. 2018). The hip finally achieves a maximum of a few degrees of external rotation at the end of the stance phase (Uemura et al. 2018).

Kinetically for the hip, the GRF vector lies in front of the hip joint at IC and moves progressively anteriorly as the cycle progresses (Krebs et al. 1998). This position explains why a peak external flexion moment of the hip is found at IC and this moves to an external extension moment once the vector is positioned behind the hip joint centre during midstance (Krebs et al. 1998). In the frontal plane the hip is constantly in an adduction moment, assumed to be external, during stance (Krebs et al. 1998), though there is an ever so slight external abduction moment at heel strike (Lunn et al. 2016). This large external adduction moment during stance is due to the GRF vector continuously passing medially to the hip joint centre (Schache et al. 2007). Clear information on transverse plane moments for the hip could not be established, a detail echoed by an established paper (Krebs et al. 1998).

Kinematically for the ankle, at heel strike the ankle is slightly plantarflexed to aid the so-called heel rocker phase, to rock around the calcaneus until the foot is flat with the ground (Brockett and Chapman 2016). Then the ankle rocker phase allows the ankle to change direction, going from plantarflexion into dorsiflexion to allow forward progression of the tibia and fibula (Brockett and Chapman 2016). In terms of the frontal
plane, the ankle is normally inverted at heel strike and moves to eversion in mid-stance (Brockett and Chapman 2016). The foot is pronated during the stance phase with abduction, dorsiflexion and eversion and the foot does not rotate during the stance phase (Donatelli 1985).

Kinetically for the ankle, at IC there is a small dorsiflexor moment to control the foot hitting the ground (Brockett and Chapman 2016), and once the foot is flat, this reverses into a large plantarflexion moment, increasing until mid-stance (Webster et al. 2019). Ankle joint moments are not commonly reported in the frontal or transverse planes during gait due to the complexity of the ankle joint (Brockett and Chapman 2016). However, these changes need to also be understood for patients and at changing speeds, with this also being covered in more detail in Chapter 4. As speed increases, there are higher sagittal hip moments and frontal and transverse ankle moments for both ACLr and healthy individuals (Stoelben et al. 2019). There have also been found to be higher external rotation moments at the hip and ankle in the non-injured leg in an ACLr group when compared to healthy participants regardless of speed, suggesting some possible compensatory damage (Stoelben et al. 2019). It is accepted that for a patient cohort, there will be more variability in the results, particularly at the hip and ankle joints due to compensation of the knee injury (Davis et al. 2019). Variability is an important part of gait and while could be considered as a negative, is actually very important (Davis et al. 2019). Variability that is unchanging could be too rigid whilst more variability than optimal suggests an unstable system (Stergiou et al. 2006). While some suggest that those with a pathology have lower variability in the lower extremities (Hamill et al. 1999), some found greater hip and knee coordination variability compared to healthy individuals (Davis et al. 2019). Pain is an important factor to consider with variability and can prevent individuals from expressing some movements fully, leading once again to lower variability and a more rigid system (Davis et al. 2019). This consideration of a more rigid system for those with pain and ACLr could have links to the stiffness and co-contraction results, as both results could be found to be higher (Butler et al. 2003; Schrijvers et al. 2019).

Understanding the lower limbs and knee joint mechanistically in healthy gait brings the literature review to a point where it needs to be established what is occurring inside the human body in a quantifiable form (Lenhart et al. 2015a). This issue can be resolved through the use of complex computer models (Lenhart et al. 2015a). Hence, this research can expand on existing literature with modelling using the software packages SIMM and OpenSim using the functional movements and associated muscle activities as an input (National Center for Simulation in Rehabilitation Research 2016; Analysis 2020).
These modelled outputs can identify the impact of a subject’s movement on the internal joint loading patterns of the knee compared to a ‘healthy’ population, to identify if pre-injury movement patterns are regained or whether the movements cause abnormal loading which could be associated with OA occurrence (Andriacchi et al. 2006).

2.1.4 Modelling of the Knee Joint

2.1.4.1 Musculoskeletal Modelling including Joint Loading Modelling
It is possible to calculate the moments and angles produced by the human body and couple them with the GRFs to understand through inverse dynamics an external picture of the net forces and moments on a joint in motion (Walter et al. 2010). However, it is not possible to garner what is occurring internally in the joint since net forces and moments do not account for the contribution from the muscles (Lenhart et al. 2015a). Historically, transducers were implanted into a knee in vivo to understand the compressive force at a joint and even relatively recently too (Kutzner et al. 2010; Walter et al. 2010); this is not commonly permitted by ethical committees, and is far more invasive than research requires these days with the advancement of mathematical modelling (D’Lima et al. 2012). Therefore, as mentioned previously, modelling needs to be employed to understand muscle and ligament forces so that joint contact forces and the effect on the contact surfaces of the knee can be established (Walter et al. 2010).

Historically, the area of dynamic musculoskeletal modelling was quite fragmented, with different research groups spending large amounts of time developing their own software and validations that were not readily shared with the wider community (Delp et al. 2007). This was somewhat addressed by the creation of the SIMM software which was widely used for upper and lower extremity models (Analysis 2020) through a research article in 1990, though was not titled SIMM at that point (Delp et al. 1990; Delp et al. 2007). Hence SIMM could be driven through forward dynamics using EMG data to produce joint moments, muscle forces and kinematic information (Delp and Loan 2000; Lloyd and Besier 2003; Gardinier 2013; Kumar et al. 2013) or driven through inverse dynamics using kinematic and kinetic data to produce joint moment data (Delp and Loan 2000). Because the muscle activations are another internal situation that is not gained through external calculations, and by adding this internal information as an input when using forward dynamics, many of the difficulties in understanding this variable have in effect been removed (Delp and Loan 2000). However, SIMM had its drawbacks, as it could not produce muscle activation calculations for those that required muscle information as an output rather than an input (Delp et al. 2007). SIMM also had limited results analysis capabilities and the source code could not be accessed and manipulated by researchers so that beneficial modifications could be made (Delp et al. 2007).
At that point, the OpenSim software was created (National Center for Simulation in Rehabilitation Research 2016), which was introduced in a research article in 2007 (Delp et al. 2007). OpenSim is an open-source software, allowing for greater validation of the model used in this research, as well as other models available through the OpenSim community, and more collaboration between different research groups (Delp et al. 2007). OpenSim is a kinematic and kinetic-driven model, with it now being possible through the use of static-optimization to generate muscle activation data, and those muscle activation results can be compared to collected EMG for validation purposes (Adouni and Shirazi-Adl 2014). The lower limb model developed through OpenSim is of particular relevance to this research (Arnold et al. 2010), which utilises bony geometry from an earlier source (Delp et al. 1990).

However, there was still a need to understand joint loading and apply it to the actual physiological situation (Andriacchi et al. 2006). In terms of the knee, there were only a handful of studies that took the knee joint loading information and applied it to the physiological geometry of the knee surface (Andriacchi et al. 2006). Unfortunately, in the lower limb model from OpenSim the knee was overly simplified in terms of its degrees of freedom and its kinematic properties (Lenhart et al. 2015a).

A musculoskeletal model was therefore required that could have adaptable source code so that beneficial modifications could be made unlike the models in SIMM, and that could analyse joint loading and wasn’t overly simplified like the models in OpenSim (Hicks et al. 2015; Lenhart et al. 2015a; Van Rossom 2017). At this point, a new knee model was developed with six degrees of freedom at both the tibiofemoral and patellofemoral joints, therefore a 12-degree-of-freedom model in total (Lenhart et al. 2015a). Two static MRI scans were used to gather information from one healthy 23-year-old female individual and implemented to derive this knee joint model, with bone, ligament constraints and cartilage contact along with additionally assigned material properties (Lenhart et al. 2015a). The knee model was added to the lower limb model mentioned earlier, with the full model implemented with the software package SIMM (Analysis 2020) using the Dynamics Pipeline (Musculographics Inc 2016) and SD/FAST (PTC 2021) to form the multibody equations of motion (Lenhart et al. 2015a). This full model was run and validated initially with cadaveric data which was acceptable (Lenhart et al. 2015a). Then the full model was run to compare the results to additional functional MRIs collected from the same individual earlier; the participant was in a supine position and exposed to several types of knee movement (Lenhart et al. 2015a). Whole body gait kinematics were also recorded from the original volunteer and input to the now twice-validated full model to produce gait information that was compared to
traditionally assumed gait information (derived from passive joint behaviour) for further validation purposes (Lenhart et al. 2015a). It was shown that this full model showed calculated kinematics consistent with in vivo functional MRI data of the same participant, with the additional benefit of producing contact force, muscle and ligament data, rather than relying on data from cadaveric studies to propose in vivo assumptions (Lenhart et al. 2015a). When examining the full model’s gait results, the results differed dramatically from passive motion results derived from a one-degree-of-freedom traditional model (Lenhart et al. 2015a). This was not perceived as a negative result but a promising outcome, as there were still gait results that could be reasoned and accepted with previous literature (Lenhart et al. 2015a).

The knee model features 14 non-linear spring ligament bundles comprised of 76 elements crossing the tibiofemoral and patellofemoral joints (Lenhart et al. 2015a) whilst the lower extremity model includes 44 musculotendon actuators crossing the hip, knee and ankle joint (van Rossom et al. 2018), where the muscles are considered as a line of action between the origin and insertion and the force capacity of the muscles is deduced with a Hill-type muscle model (Thelen 2003). The knee model was originally validated in the initial research article that introduced it as discussed above (Lenhart et al. 2015a) and has been used by many others since (Meireles et al. 2017; Van Rossom et al. 2019; van der Straaten et al. 2020). The lower leg has six degrees of freedom (DOFs) at the pelvis, with 3 DOFs at the hip allowing for a ball-in-socket movement and one DOF at the ankle representing a simple hinge (Lenhart et al. 2015a). The knee model has 6 DOFs at both the patellofemoral and tibiofemoral joints (Lenhart et al. 2015a). The cartilage contact pressure of the knee is derived using an elastic foundation model, with uniform cartilage of 4mm across the tibiofemoral joint, and 7mm in the patellofemoral joint (van Rossom et al. 2018). The cartilage was defined as having an elastic modulus of 10MPa and a Poisson’s ratio of 0.45, as per previous studies (van Rossom et al. 2018). The model has higher cartilage thicknesses and an elastic modulus than the original model (Lenhart et al. 2015a).

To run the full model, a simulation workflow is implemented, the traditional pattern of which is discussed here. Initially, the generic model is scaled to the anthropometry of an individual to match closely to their dimensions and mass (Lenhart et al. 2015a). Then an inverse kinematics (IK) problem can be solved; the joint angles can be calculated from the least-squares fit between the individual’s marker trajectories and the modelled positions (Delp et al. 2007; De Groote et al. 2008). Thirdly, by combining the external forces with these calculated joint angles, the joint moments can be produced using a residual reduction algorithm (RRA) and the model kinematics are also refined to make
them more dynamically consistent (Delp et al. 2007). Then, muscle forces can be calculated to counteract the external moments through one of a few different optimization methods that can solve the muscle redundancy problem either static optimization (Anderson and Pandy 2001), computer muscle control (Delp et al. 2007) or the more recent, physiological analysis (De Groote et al. 2012; Van Rossom 2017). Finally, the knee joint contact forces can be found using a vector sum of the muscle forces and reaction forces in the knee joint (Van Rossom 2017). More detailed information on the specific simulation workflow used can be found in Chapter 3. This full model can be adapted to an individual’s geometry through scaling and shows good comparability for load-dependant kinematics, which are kinematics that occur during loading (Lenhart et al. 2015a). This full model also shows dramatically different knee kinematic results in gait to those derived from more traditional methods which showed far less range (Lenhart et al. 2015a). It is worth noting that this model is therefore different to models provided directly by OpenSim on the “OpenSim documentation site”, though are formed from the same source (National Center for Simulation in Rehabilitation Research 2016). Others sought this approach of adding a more detailed knee model of their own into the aforementioned lower limb model too (Marouane and Shirazi-Adl 2019).

This combination of knee and lower leg models is referred to above as the ‘whole model’, but generally in this research as the University of Wisconsin-Madison model or simply ‘the model’ (Lenhart et al. 2015a; van Rossom et al. 2018). The model has been supplied with permission from the University of Wisconsin-Madison through KU Leuven, who provided support and education about this model (Lenhart et al. 2015a; van Rossom et al. 2018) and can be seen below in Figure 10. The modelling and simulations for the University of Wisconsin-Madison model are undertaken in its entirety by SIMM (van Rossom et al. 2018), with visualizations possible in OpenSim (van Rossom et al. 2018). This model has become popular in the last few years and has been widely used (Van Rossom et al. 2017; Van Rossom et al. 2019; van der Straaten et al. 2020). This particular model considers the interactions between tissues and structures that other models do not and is useful in some clinical settings (Lenhart et al. 2015a; Jonkers et al. 2023; Lloyd et al. 2023).
One drawback of the University of Wisconsin-Madison model used in this research is the inability to adapt the ACL for an injured subject as it is always assumed in the model that a subject has not sustained an injury (Lenhart et al. 2015a). As this research considers a patient group, it would have been beneficial for example to have adapted the properties of the ACL to reflect the ACLr (Lenhart et al. 2015a). Additionally, for example, it is widely accepted that with medial compartment OA, the joint space on the medial compartment side would narrow, allowing for a more varus knee alignment (Nakagawa et al. 2015). There have been some recent manually added adaptations to the model to allow for pathological changes in knee alignment (Zevenbergen et al. 2018; Van Rossom et al. 2019), though this is still a developing area of research.

2.1.5 Summary of the Problem for Individuals with ACLr

ACL tears are an extremely common knee injury (Kupczik et al. 2013; Hetta and Niazi 2014; Erickson et al. 2016; Bhardwaj et al. 2018). Individuals who have torn their ACL and require reconstruction often present more complex gait movement strategies than pre-injury (Scarvell et al. 2005; Bhardwaj et al. 2018). Changes in functional movements allow for areas of the knee to be exposed to loading that are not prepared for this and cartilage changes can then occur, which can then lead to OA (Roos et al. 1995; Andriacchi et al. 2006). Poor adherence to rehabilitation means full recovery and return to pre-injury activity are limited (Colaco et al. 2009; Thomeè et al. 2015; Walker et al. 2021).

Whilst some investigations of individuals with ACLr have been conducted, few have investigated movement strategies years after surgery (Dewig et al. 2022). Additionally,
none have investigated dynamic joint stability through advanced musculoskeletal modelling encompassing knee stiffness, leg stiffness, co-contraction and joint loading for individuals with ACLr alongside traditional biomechanical measures. Clinically, this knowledge could inform post-surgery rehabilitation strategies and potentially aid in a higher return to pre-injury activity, reducing ongoing knee problems and lowering the risk of revision surgery.

This thesis aims to address the research questions through kinematic, kinetic and EMG data for individuals with ACLr approximately 6 years post-surgery.

2.2 Basis for Research Protocol
As mentioned in section 1.2, the aim was as follows:

The overarching aim is to identify the role of the injured and reconstructed ACL through the use of musculoskeletal modelling to investigate dynamic joint stability measures such as knee stiffness, leg stiffness, co-contraction and tibiofemoral knee joint loading and common biomechanical measures.

From this, three research questions were generated as discussed in Chapter 1. Then three objectives were generated as shown below:

2.3 Objectives
The three objectives relating to the three research questions are:

1. To use musculoskeletal modelling to analyse the difference between basic biomechanical variables (gait speed, knee flexion angle, knee flexion moment, ground reaction force) and those that represent dynamic knee joint stability (knee stiffness, leg stiffness, medial contact loading, lateral contact loading and co-contraction) for healthy and ACLr participants at different walking speeds.

2. To evaluate if there is a change in step width for healthy individuals between two different data collection systems, and if so, evaluate the change in the resultant kinematics and kinetics (frontal angles and moments for the hip, knee and ankle, and medial and lateral contact loading).

3. To investigate whether there is a difference in computing the co-contraction patterns of an ACLr population with a musculoskeletal model compared to that produced for a healthy population, and how these results compare to co-contraction data collected from EMG.
3. Methodology

3.1 Introduction
The methodology section is split into three main sections. Firstly, there is protocol development (section 3.2) where the main issues covering all parts of this research are discussed. Then there is a discussion about the initial feasibility study (section 3.3). Finally, there is the general research design section (section 3.4) which covers details of the research which are applicable in all the studies comprising this body of research. Specific details about the research design can be found in the methods section of each of the three research studies chapters that combine to form this thesis, namely Chapters 4, 5 and 6.

All data for this research was collected at the Research Centre for Clinical Kinesiology (RCCK) Laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University. The RCCK laboratory contains a GRAIL system (Gait Real-Time Analysis Interactive Laboratory) from the manufacturer Motek Medical (Motekforce Link. 2017) which is a motion analysis and virtual environment with an integrated treadmill and is discussed in more detail in section 3.4. The collected marker, force plate and EMG data combined with the musculoskeletal model provided by the University of Wisconsin-Madison (Lenhart et al. 2015a) and used in conjunction with K.U. Leuven (Van Rossom et al. 2019), allowed for a comprehensive analysis of both healthy and patient volunteers. Most of the data analysis in this thesis is focused on gait at different speeds as it is a common activity of daily living and can well represent all in vivo function (Andriacchi et al. 2004).

3.2 Protocol Development

3.2.1 The Gait Cycle
During this research, there is only interest in the stance phase portion of the gait cycle, as this is the time period when the knee is vertically loaded (van Rossom et al. 2018). Furthermore, early stance is of particular interest as that is when stiffness can be analysed linearly between IC and maximum knee flexion angle as discussed further in section 2.1.3.2.

3.2.2 Treadmill Experience and Familiarisation
Experience in using a treadmill and being given a reasonable time for familiarisation is extremely important for biomechanical results. However, as little as 5 minutes familiarisation has been used in some studies (Item-Glatthorn et al. 2016), whilst other studies have suggested up to 30 minutes, where by this time, sedentary and active older adults do not display any gait variability differences (Da Rocha et al. 2017). All
individuals who participated in this research verbally expressed confidence with using a treadmill, and hence a familiarisation period was not necessary, though 30 seconds at the start of the study required to get the treadmill into a steady state of motion, was available.

3.2.3 Laboratory Setup and Calibration
Before a session was conducted in the laboratory, the GRAIL system from Motek Medical (Motekforce Link. 2017) required calibration. The GRAIL system itself is discussed in greater detail in section 3.4.2.1. Calibration ensured that a 3D coordinate space can be established from 2D camera images (Franjic et al. 2016). A provided wand with auto-reflective markers on the frame allows for a dynamic calibration followed by a static calibration; the first to highlight the capture area, and the latter to define the origin and orientation of the lab coordinate system.

3.2.4 EMG Placement
EMG is a way of measuring a produced voltage between two electrodes, detected from a muscle contraction (Day 2021). The type of EMG used throughout this research is surface-level EMG, a non-invasive measure using electrodes applied to the skin surface to detect the voltages (Day 2021). It is common for there to be noise in the resultant voltage (Day 2021), whether that is from skin movement, detection of other muscles contracting at the same time, or surrounding actual noise, and this is addressed through filtering (Rose 2011), discussed later in section 3.4.3.1. EMG pads were placed over the bulk of the muscle of interest and no more than approximately one-quarter of the length of the muscle apart as per Seniam guidelines (Seniam 2021). If necessary, the skin was shaved and cleaned with alcohol wipes where the pads were being placed to ensure good conductance (Seniam 2021).

3.2.5 Marker Layout Development
An adapted Leuven marker set was used in this research due to its previous successful usage with this musculoskeletal model (Van Rossom 2017; Afschrift et al. 2019). Reflective markers were typically placed on bony landmarks of the body to allow for less soft tissue movement that would be more present in other areas of the body (Leardini et al. 2005). More markers mean that a marker set is more likely to be observed by the cameras and can aid the better reconstruction of a missing marker, but to the point where markers too close together can cause artefacts and be mis-recognised and mislabelled (Acevedo et al. 2023). The marker set needed to confidently represent every
segment of the body through the use of at least 3 markers, otherwise known as a bone or a part of the body represented as one rigid body (Zander et al. 2022).

The Leuven marker set (Afschrift et al. 2019) is comprised of an extended Plug-in Gait model (Vicon Motion Systems Ltd n.d.). In this research, the Leuven marker set was extended by adding markers to the left and right transverse processes of the 10\textsuperscript{th} thoracic vertebrae, the left and right iliac crests (mid-way between the ASIS (Anterior Superior Iliac Crest) and PSIS (Posterior Superior Iliac Crest)), and the left and right greater trochanters. The latter two pairs were added especially for recognition during ‘deep’ movements by the participant where ASIS markers were likely to disappear to aid recalculation of the ASIS positions. Slightly modifying a marker set that has been used frequently before is common in research, such as for those that adapt the Helen Hayes marker set (Knarr et al. 2012). The following Figure 11 represents the marker layout in greater detail. Please see Appendix A for further details on the marker protocol.
3.2.6 Subject Preparation
Each subject familiarised themself with the Participant Information Sheet. Each subject wore lycra cycling shorts, sports trainers and a racer-back t-shirt to reduce the risk of markers being occluded by fabric. If required, durapore or micropore was used to tape an individual’s clothes to prevent further obstruction of markers, or to tape EMG wires against the skin to prevent the tangling of wires.

Additional measures specific to the RCCK laboratory risk assessment were also followed; the participant wore a harness attached to the ceiling to prevent a full fall and was assisted on and off the treadmill. During self-pacing walking, participants were
encouraged to look forward when communicating with the laboratory operator (the author, who sat directly behind the individual) to maintain safety.

Maximum Voluntary Contractions (MVCs) were taken from each subject through EMG voltages to obtain the maximum effort a subject can perform for a certain muscle (Al-Qaisi and Aghazadeh 2015), as can be seen in Figure 12. They are used as a benchmark, or a mark of 100% so that the effort of a specific muscle during a specific movement can be normalised to a percentage form and in turn, one subject can then be discussed against another in percentage form (Kukla et al. 2018). MVCs are often taken at the start of a laboratory session, when the subject is most rested, in the hope that the subject can produce the largest MVC result before any residual effect of fatigue (Konrad 2006). To take a successful MVC, the subject must perform an isolated movement against a fixed resistance to produce an isometric contraction, with the maximum EMG voltage from this movement forming the basis of 100% (Al-Qaisi and Aghazadeh 2015). To combat the effects of fatigue on the data, the subjects were given regular breaks to rest.

![Figure 12: Performing an MVC on a volunteer](image)

3.2.7 Data Processing
The data processing workflow for each study is discussed in more detail in the specific chapters, with even more detail on the script development in the Appendices.

3.2.8 Validation of Software Code and Modelling
The development of the workflow is iterative to ensure that the process is robust and produces valid results, with much support for learning the process coming from K.U. Leuven (Van Rossom et al. 2017). To fully validate the model, dynamic and kinematic
considerations are required (Van Rossom 2017). However, as the contact force results cannot be compared with in vivo measured contact forces during the same movements, dynamic validation of the model this way is not possible (Van Rossom 2017).

Alternatively, the model has been dynamically validated previously based on input data provided by the Grand Challenge Competition, where the results of the model were compared to data from instrumented knee implants (Fregly et al. 2012; Smith et al. 2016; Van Rossom 2017). The model produced a total knee contact force root mean square error of 0.33 body weight meaning the model results were very acceptable to use in this situation (Smith et al. 2016). Kinematically, once again it is not possible to confirm the modelled kinematics with in vivo measured kinematics during the same movements, such as with functional MRI (Van Rossom 2017). The knee flexion angle was specified in the model, but the secondary kinematics such as the knee varus/valgus and internal/external rotation were deduced with an optimization formula called the COMAK which is discussed in more detail in section 3.4.3.1.2. These secondary kinematics evolved from the contact force, muscle force and ligament force data (Van Rossom 2017). Therefore, as the force results were already validated, and the kinematic results were confirmed with in vivo kinematics from functional MRI (Lenhart et al. 2015a), the kinematic results can be accepted as valid.

Then it was required to validate the results specific to this research. For example, initially, the model demonstrated almost complete medial compartment offloading in the first half of stance. This behaviour was not supported by a primary examination of the literature (van Rossom et al. 2018), and hence, evidence was required to ensure the workflow was producing the correct results. K.U. Leuven supplied data to run through the workflow and then compared the results to the values obtained through a workflow in K.U. Leuven, and then these issues were finally resolved, with there being a small orientation and conversion issue.

Further validation was required when in mid-2018, the left leg model was supplied to complement the pre-existing right leg model, which can be seen in Figure 13. All Matlab scripts required adjusting and validation between the left leg and right leg models was required.
All healthy subjects were analysed with the right leg model, and patients were analysed depending on which leg received the ACLr; if both legs received ACLr, it was the leg that had been analysed previously (Letchford 2015). Figure 14 demonstrates the right (injured) leg angles for the 4 walking scenarios, whilst Figure 15 demonstrates the left side, whilst Figure 16 and Figure 17 represent the same but for moments instead. Even though these four figures are for a patient individual walking 9 steps at four different speeds, it can be seen that the angles and moment results are extremely similar and hence, is a simple check to ensure that the outcomes from two separate models (left leg and right leg) are comparable.
Figure 14 - Flexion angles for P06 right knee modelling for the 4 walking speeds

Figure 15 - Flexion angles for P06 left knee modelling for the 4 walking speeds
Figure 16- Internal flexion moments for P06 right knee modelling for the 4 walking speeds

Figure 17- Internal flexion moments for P06 left knee modelling for the 4 walking speeds

Figure 18 shows the individual steps for healthy individual P11 with the highest pressure on the tibiofemoral joint during slow, normal, fast and very fast walking pictorially.
There are 9 stance phases per walking speed, and this slight variation in colours shows the normal variation in walking. Additionally, the consistent change in colours between walking speeds shows the change in loading with increased speed, culminating with more red (or higher loading over approximately 12Mpa on the two condyles) seen at the very fast walking speed. This pictorial form is a beneficial method to check patterns visually during processing.

![Figure 18 - Pictorial of P11 slow, normal, fast and very fast walking maximum pressures on the tibiofemoral joint](image)

### 3.2.9 Result Normalisation

Normalisation is required to compare results between different individuals. It is common to normalise produced moment values to the kilogram mass of the subject, which this research continues (Kuster et al. 1995; Moisio et al. 2003). This research also normalises leg stiffness and knee stiffness by mass of the subject, which is common (Dixon et al. 2010; Smale et al. 2019).
Results could also be normalised through division by body weight, which is mass in kilograms multiplied by gravity in m/s². Total mean contact loading for the medial and lateral compartments was normalised by the commonly used body weight for transferability of data between chapters (Stickley et al. 2018).

3.3 Feasibility Study
An initial feasibility study was conducted at the start of this research. This established reasonable lengths of time for participants in the laboratory without causing fatigue and to aid knowledge for further study design. It also aided learning the post-processing stage, from immediately after data capture to the results stage, and included learning on musculoskeletal modelling.

All data collection occurred in the RCCK laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University, Cardiff, Wales in November 2015. Recruitment for the feasibility study involving healthy individuals was conducted in line with Cardiff University Ethical procedures and were recruited in association with another research project titled (not investigated by the author) “slip perturbations during gait to identify balance control strategies and to validate predictive simulations of human gait” (Afschrift et al. 2019).

14 healthy subjects walked for three minutes on the treadmill. 4 participants were processed for deeper analysis as this was primarily for feasibility and learning while the additional 10 were not processed.

3.4 General Research Design
As mentioned previously, there are three key studies comprising this research. The general methods cover all studies as discussed below and study-specific methods are discussed in each individual chapter.

3.4.1 Study Design
Each study design, sample size and methods of assessment are discussed in its individual chapter. However, inclusion and exclusion criteria were commonly the same and are mentioned in section 3.4.1.1.

3.4.1.1 Inclusion and Exclusion Criteria
The inclusion and exclusion criteria were identical for all the research projects, though differed where patients were involved in Chapter 4. Patients had to be part of a pre-existing cohort (Letchford 2015) and hence would have some injury, function and pain
issues, with an exclusion criteria of ceasing communication with a previously interested participant if there was a lack of engagement.

The inclusion and exclusion criteria for these studies were as follows:

*Inclusion*
- Between 18 and 65 years old
- In general good health for the last six months

*Exclusion*
People who:
- Do not understand verbal instructions given in English
- Do more than four hours of sporting activities a week
- Are unable to maintain a single-leg balance for approximately 30 seconds
- Do not feel able to maintain a brisk walking pace for three minutes
- Have neurological pathologies, medication or other medical reasons that would affect balance and gait
- Have consumed stimulants, have uncorrected vision or low blood pressure that would be affected by these tests
- Have chronic illnesses or pain that would be exacerbated by the testing protocol
- Have sustained a musculoskeletal injury in the last six months (if it would be exacerbated or cause pain from the required tests)
- Have skin allergies or conditions that would be affected by the use of medical double-sided tape, EMG/ electrocardiography (ECG) pads, micropore/ durapore medical tape or alcohol wipes.

3.4.2 Data Collection
3.4.2.1 Equipment
All the data collection for this research was conducted in the RCCK laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University. Most of the data collection occurred with the GRAIL equipment from Motekforce Link (Motekforce Link, 2017), whilst a small portion of the research used an over ground system in the same laboratory detailed further on in this section.

The GRAIL is arranged as an instrumented treadmill, a curved screen and surround sound for an immersive experience for the volunteer, 10 Vicon T20 camera video system (Vicon Motion Systems Ltd) and integrated software packages D-Flow (Motekforce Link, 2017) and Vicon Nexus (Vicon Motion Systems Ltd). There are two separate treadmill
belts, one for each foot, to simulate slips or stumbles by desynchronising the speeds between the two belts, which was not manipulated to reduce the number of variables. Additionally, there are two force plates, again one for each foot, so that forces from foot initial contact (or IC) and the foot toe off (TO) can be collected at a frequency of 1000Hz, or 1000 samples per second (Beckham et al. 2014; Payton and Burden 2017). 12 infrared cameras gather the locations of the auto-reflective markers within the three-dimensional capture space at a sampling frequency of 200Hz (Payton and Burden 2017). The marker data is managed by the software package Vicon and voltage values of the force plate information are also available through Vicon (Vicon Motion Systems Ltd n.d.). The visualisations on the screen and floor are manipulated using four projectors and the projected images, treadmill manipulations, feedback to the subject and any similar interactions are managed by the software package D-flow (Motekforce Link. 2017), which links the system together and is crucial to maximizing the abilities of the system, as well as collecting the instantaneous treadmill speed.

In addition to the general setup were two Bortec Octopus 8-channel EMG systems (Bortec Biomedical Ltd 2002) at 1000Hz, allowing up to 8 muscles to be recorded on each side of the body, or 16 in total.

Furthermore, a wired trigger button was used to aid post-processing; it could produce an impulse in both the Vicon and D-flow data, thus allowing aligning and syncing between the two data sets to occur as D-flow is unable to recognise the start time of a captured file in Vicon. This was also required as the instantaneous speed of the treadmill in D-flow needed to be synced with the marker and force plate data in Vicon.

D-flow collects data at an approximate sample frequency of 300Hz. A handheld trigger button is used to produce a voltage impulse in D-flow; the slight variances in the sampling frequency allow for misinterpretation of trial data timestamps. Therefore, a voltage mark allows for alignment between the EMG and D-flow data.

The over ground system available in the RCCK laboratory comprises two Kistler force plates (Kistler Instruments Ltd 2021) sampled at 1000Hz and 8 Vicon MX cameras (Vicon Motion Systems Ltd n.d.) sampled at 200Hz, which are all maintained through the Vicon software package (Vicon Motion Systems Ltd n.d.).

3.4.2.2 Study Protocol
The study protocol is discussed in each individual chapter.

3.4.2.3 Vicon and D-Flow
Each recording made within Vicon is comprised of five different files. These are also analysed within Vicon to provide detailed marker and force plate information during the
recording. Markers are ‘reconstructed’, the Vicon term to generate a 3D image of the markers in the file, and the Vicon Skeleton Template (.vst) is applied. This is the same template file utilised during collection, and for this research, an extension to the Leuven marker set template (.vst) was created by the author as discussed in section 3.2.5 (Afschrift et al. 2019). Once applied, the data session identifies the joints and parameters of the individual and becomes a .vsk file. A Functional Skeleton Calibration (a Vicon term for calibrating a functional movement with the skeleton template) can be applied to the recording using the vsk information, and a .c3d file is created. This .c3d file is then analysed in the Matlab software.

Considering D-flow, a .txt file was saved for each recording. This file contains the treadmill speed at any moment and a second copy of the force plate and EMG voltages that would be present as raw voltages within the .c3d file. The D-flow file is not heavily used within the research; its primary reason for use is to synchronise the data to obtain the average speeds for the participant at any given walking category. Typically Matlab is used to calculate speeds by analysing the horizontal displacement of markers, though these are more difficult with a treadmill as the participant does not considerably move horizontally forwards in the space.

3.4.2.4 OpenSim, SIMM and The University of Wisconsin-Madison Model

Once all the necessary data had been collected, two other pieces of software were utilised to output the results; namely Matlab (The Mathworks Inc 2017) and SIMM (Lenhart et al. 2015a). Matlab is discussed in section 3.4.3.1.1.

SIMM uses the raw data and The University of Wisconsin-Madison Model (Lenhart et al. 2015a). A 3D musculoskeletal knee model developed with SIMM was adapted for each individual through the use of scaling and was used to estimate knee joint contact forces and muscle forces (Lenhart et al. 2015a). This in turn was added into a generic lower right or left leg model developed from OpenSim (Arnold et al. 2010). These two models in combination are referred to by the author as the University of Wisconsin-Madison model as discussed in section 2.1.4. The whole modelling and simulations for these combined models were performed by SIMM (van Rossom et al. 2018; Analysis 2020). The OpenSim software (National Center for Simulation in Rehabilitation Research 2016) was used for the visualisation of results only to ensure a final check that the model was performing its calculations correctly. Further details on the modelling of the knee are mentioned in the literature section 2.1.4, whilst details of the workflow are discussed in section 3.4.3.
At this point, there are three components of the essentially raw data: marker position information, force plate data and EMG data. Section 3.4.3 below discusses the order in which collected data goes through different processes into understandable outputs.

### 3.4.3 Data Processing Workflow

The calculation section is discussed in each particular research study chapter.

#### 3.4.3.1 Matlab and Data Analysis

##### 3.4.3.1.1 Matlab

Matlab can quickly manipulate mathematical calculations once executable scripts are written, while SIMM (Software for Interactive Musculoskeletal Modeling) aids in the modelling and analysis of three-dimensional musculoskeletal systems in dynamic situations (Affairs 2021).

The two pieces of software interlink and results can only be obtained through a workflow featuring multiple steps of both software. The outcomes are calculations of angles and moments around a joint, modelled muscle forces (of which muscle activations are of interest in this research as they can be compared to collected EMG), and joint contact forces. It is worth noting that the collected EMG values are not added to the model, and the muscle activations that are calculated come from other generated information as discussed in section 3.4.3.1.2. The specific model used within the SIMM software is a model developed by the University of Wisconsin-Madison, and modelled data for the right leg only (Lenhart et al. 2015a), until the left leg became available in 2018. Figure 19 shows the relationship between the software packages.

![Software Workflow](image)

**Figure 19: Software Workflow**

During the data processing step, and once the raw data from Vicon has been exported, there are various stages that Matlab has to complete before it is available for SIMM. Firstly, the data is converted from a .c3d format to a .mat format, the default format for working with data in Matlab and the data is arranged into a different alignment. The data is then split into ‘events’. Every time instant where a mass application to a treadmill
belt starts and discontinues was recorded as IC and TO respectively, and hence created the boundaries for an ‘event’. Eventing identifies the correct analysis period, as the mathematical calculations can only be applied when there is force plate data. Other anomalies were removed such as if forces were applied to the wrong treadmill belt during walking, as they can cause incorrect results during the modelling stage. Finally, executable scripts for the SIMM files are generated through Matlab, focussing only on the useful steps and only between the points of mass application.

3.4.3.1.2 Simulation Workflow and Executable Files
Executable scripts can be run to produce detailed biomechanical results on each individual. During their first stage, they run three steps, the scale, inverse kinematics and muscle force distribution (MFD) steps (Lenhart et al. 2015a; Lenhart et al. 2015b). Initially, the scale step takes the generic model markers and best scales it to fit the measured marker positions of the individual from a calibration trial and also includes the subject’s mass. Then, the inverse kinematics step calculates the subject’s joint angles through the period of movement, namely hip translation and rotation, knee flexion angle, and ankle angle, using a global optimization method that reduces the weighted sum of the squared differences between the collected and modelled marker positions (Lu and O’Connor 1999; Van Rossom 2017; Smith et al. 2018). No force plate information is used in this step. Thirdly, the concurrent optimization of muscle forces and kinematics algorithm (COMAK) calculates the muscle forces (termed as the MFD step) needed to generate the measured accelerations in several primary degrees of freedom of the lower limb joints, namely hip flexion/ adduction/ rotation, knee flexion, and ankle flexion (van Rossom et al. 2018) from the output from the IK step. The COMAK algorithm calculates the secondary knee kinematics whilst reducing the weighted sum of the squared muscle activations and contact energy (Smith et al. 2018; van Rossom et al. 2018). These results require an examination to ensure that the weighted sum of the squared muscle activation is less than an error tolerance of 1.0 so that the model appears successful (Smith et al. 2018; van Rossom et al. 2018). Those frames where the result is more than 1.0 are listed at the end of the calculations as those that do not follow convergence. It is worth noting that the secondary knee kinematics for the tibiofemoral and patellofemoral degrees-of-freedom evolve as a function of the muscle forces, ligament forces, and contact pressures (Smith et al. 2018; van Rossom et al. 2018) and hence the COMAK algorithm is also involved in the second batch of executable scripts as discussed in the next paragraph. The results from the MFD step are of particular interest in this research, as the modelled muscle activations (expressed as
voltages and in percent of activation between 0 and 1) can be compared to collected EMG.

A second stage of executable scripts that were produced by Matlab for SIMM is concerned with running the inverse dynamics and contacts steps (Lenhart et al. 2015a). The inverse dynamics step allows for the calculation of moments and forces during certain movements and requires input from the inverse kinematic results. As the MFD results are derived from the inverse kinematics results, they can be used as an input instead; hence the COMAK algorithm is also involved in this step. The inverse dynamics step produces the moments from pre-calculated angles coupled with the mass of the portions of the musculoskeletal model. Finally, the script calculates the contacts step. The MFD input is taken again and it produces contact pressure and contact area, and hence the resultant force amplitude and point of application information for the tibiofemoral joint and patellofemoral joint are then deduced (van Rossom et al. 2018). This stage can also calculate the cartilage contact pressure through utilising inputs of cartilage thickness, elastic modulus, and Poisson’s ratio mentioned earlier in section 2.1.4.1 of the literature.

3.4.3.1.3 Data Analysis

Once the first stage of scripts has produced data, it is imported back into Matlab for data analysis, which is discussed in more detail in Appendices B, C and D. This section features the universal steps used in the data analysis stage.

Firstly, the frames that did not follow convergence are removed so that only viable data is analysed. All calculated angles for one subject are imported. As all steps have different numbers of frames the data is time normalised, or ‘splined’, so that all steps are the same length and a mean can be deduced.

The same process in the aforementioned paragraph is used to analyse the subject moments. All moment values are expressed as Newton metres per kilogram (Nm/kg) to normalise for varying masses, as moment values are affected by mass (Moisio et al. 2003). Additionally, a similar process is also used to analyse contact forces. During this stage contact forces are expressed as lateral, medial and total forces. All values are expressed as Newton’s per bodyweight (N/BW), a common representation of this data (Stickley et al. 2018). Result normalisation is also discussed in section 3.2.9.

Other calculations that were obtained in Matlab included leg and knee stiffness, confirmation of the syncing patterns between the D-flow and Vicon sampling rates, CCIs analysis and filtering of the EMG data to remove noise.

It is important at this point to discuss filtering for both GRFs and EMG data. Filtering for all studies kept the same protocol to aid clearer comparisons in this research. GRFs were
filtered with a 4th-order low-pass Butterworth filter at a cut-off frequency of 6Hz, and marker data was filtered with a 2nd-order low-pass Butterworth filter with a cut-off frequency of 10Hz, modified from an earlier source (Afschrift et al. 2019).

Normally for EMG data, a bandpass filter is applied initially to remove the high and low signals, followed by rectification and a low pass filter (Rose 2011). The result should display only in the positive y direction, which is called full wave rectification, and should look like an envelope or outline of the original signal (Rose 2011). However, the EMG data was also run through a Notch filter, as elimination of interference was required (Zschorlich 1989), with the signal power examined for quality through another script from an earlier source (Afschrift et al. 2019). Some do not recommend this step, due to it destroying some signal power (Konrad 2006; Day 2021). Hence, the EMG data was passed through a second-order 50Hz Notch filter, known as a band stop filter, a fourth-order Bandpass filter of 20-400Hz, and then a fourth-order 10Hz low pass Butterworth filter through a script modified from an earlier source (Afschrift et al. 2019).
4. The Relationship between Joint Stability and Contact Loading during Four Walking Speeds for Healthy Volunteers and a Post-ACLR Patient Group

4.1 Introduction and Additional Literature Review

The purpose of this study is to gather biomechanical measures that define dynamic knee joint stability at different walking speeds to establish how joint stability adapts and how resultant knee contact loading is affected. This information can then be used as a comparator to how someone with ACL injury differs in their gait at different speeds and possibly predict the resultant effects, such as signs of mal-adaptive movement. This literature review investigates additional literature to that of the main literature chapter specifically considering the effects of walking speed and knee injury on dynamic joint stability parameters and how this may differ for uninjured individuals. Due to some lack of biomechanical literature to represent individuals with ACLr fully (Knobel et al. 2021), there have been occasions in this additional literature review where OA individuals are discussed instead. As discussed in Chapter 2, this is because even after ACLr, knee function can continue to be sub-optimal (Samaan et al. 2016; Ithurburn et al. 2019) and there is an increased likelihood of OA in ACL deficient knees (Andriacchi et al. 2006; Fox et al. 2018).

4.1.1 The Effect of Walking Speed

The ability to self-select speeds by the individual is pertinent to this research. Knee health and the link to speed can however be difficult to understand and literature produces a causality cycle, as slower speeds can indicate poor knee health, and poor knee health (such as OA) causes a decline in gait speed (Fenton et al. 2018; Knobel et al. 2021).

As previously mentioned in section 2.1.2.5.1, certain biomechanical parameters vary depending on the walking speed (Zeni and Higginson 2009a; Chung and Wang 2010; Ardestani et al. 2016). However, when examining speed itself in more detail, it is debatable whether there are biomechanical differences between walking speeds which are fixed or self-selected; the latter is where an individual chooses their gait speed whilst moving (Theunissen et al. 2022). If a walking speed is fixed, the potential for between step biomechanical and electromyographic variability, a commonly expected outcome, can be lost (Kuster et al. 1995; Keene et al. 2016). If walking speed is variable, it is hard to group participants for further analyses. However it is not impossible; there is an interesting method combining self-selected speeds and biomechanical outcomes (in this example, step width), where linear regression is used to extrapolate the
biomechanical data at unknown speeds (Keene et al. 2016). This provides the ability to compare participants at the same speed (Keene et al. 2016), and where gait speed was found to be linked to knee health (White et al. 2013).

Certain other parameters about the participant can also affect the gait speed in addition to knee health, such as their age and their level of activity. Sedentary older healthy individuals who self-select their speeds produce lower average treadmill walking speeds and in turn different gait kinematic results compared to their active older healthy counterparts (Da Rocha et al. 2017). Slower self-selected gait speeds in older individuals also seem to be linked to a higher risk of falling; symptomatic lower limb OA increases the risk of falls and in turn, there are further medical implications (Doré et al. 2015) and a greater stride length variability (Verghese et al. 2009).

Whilst slower gait speeds are often produced by participants with certain characteristics, caution is required when investigating step length, cadence and step time at slow speeds (suggested to be between 2km/h and 3km/h or less) due to the poor reliability on the reproducibility on different days of these gait parameters (Item-Glatthorn et al. 2016). This can however be viewed positively as there is good between-day reproducibility for certain gait parameters for higher speeds (Item-Glatthorn et al. 2016). This suggests that a subject is not highly influenced by factors between treadmill sessions despite a relatively short familiarisation period and laboratory-defined steady-state speeds (Item-Glatthorn et al. 2016).

As previously mentioned in this section, as gait speed is a good indicator of knee health, gathering certain knee parameters at different speeds could suggest the effect speed has on knee loading and whether healthy knee behaviours are occurring. The pattern of the internal loading in the joints adjusts according to different speeds (Montefiori et al. 2018), though how this occurs is currently not understood. For background, as knee angles and moments are popularly considered parameters, the sagittal plane (the most popular plane to consider for knee kinematics (Schrijvers et al. 2019) and different speeds could first be examined. Gait parameters such as moment-angle graphs are known to display the same general pattern but are considered non-linear at different speeds (Fukuchi et al. 2019b), though there are strong indications at the knee that the components of flexion-extension overlay each other as the speed increases (Frigo et al. 1996). The medial compartment load of the knee is closely linked to the peak external knee adduction moment during gait, and even more closely linked to the peak internal extensor moment at the knee (Aaboe et al. 2008).
An incredibly fruitful source of information to relate this study’s data to is shown above in Figure 20, with a focus on the knee extension-flexion angle and knee flexion-extension moment during the first half of the gait cycle being of particular interest. Though it was not stated in the study, it can be observed from the extension-flexion graph that internal moments are being considered, as external moments would produce two peaks in the opposite direction on the y-axis to the angles’ figures. As speed increases from the slowest speed V1 to the fastest speed V8, the first peak knee flexion angle and first peak knee extension internal moment increases (Fukuchi et al. 2019b). The first knee extension angle and the first peak knee flexion internal moment increases also, though not to the same magnitude as that of the first peak knee flexion angle/extension internal moment (Fukuchi et al. 2019b). This suggests that greater flexion angles and moments occur in the first half of stance with an increase in speed, which can suggest that the knee is under more stress to perform and work to stabilise. This is not the only study to draw the same conclusions for increasing speeds with sagittal knee angles and moments (Lelas et al. 2003; Chehab et al. 2017; Stansfield et al. 2018), though occasionally the first knee flexion angle is found to be the opposite of above, lower for increasing speeds, but still the same effect for the second knee flexion angle (Guo et al. 2006; Hanlon and Anderson 2006). This suggests that there is less flexion in individuals during the first half of stance to possibly cope with the increased speed (and to aid stability) but that flexion adjustments to the increase in speed will have to occur and do so later on in the gait cycle.

Next, the frontal and transverse planes with changing walking speeds can be considered. Figure 21 demonstrates how walking speed changes the angles on each knee plane, with each walking speed repeated 3 times. As can be seen, with an increase in speed, the
flexion peaks generally increase with speed, adduction and external rotation generally decrease with speed, and tip into more abduction and internal rotation respectively with that increase in speed. It is of note, that one study considering step ascent and descent showed no difference for frontal or transverse data between healthy and patient individuals, suggesting that while speed has an influence on knee angles and moments in all planes, an injury’s effect may be more limited to the sagittal plane (Kaur et al. 2020).

![Figure 21](image)

*Figure 21*- Mean knee kinematics for flexion, adduction and external rotation angles for 3 repetitions at four speeds (Robert-Lachaine et al. 2020)

4.1.1.1 Hip and Ankle Biomechanics

Whilst the knee is the primary interest for this research, the hip and ankle require investigation to support and fully understand the actions at the knee. Continuing with the consideration of the sagittal plane and considering the hip flexion-extension angles and moments, the higher the speed, at IC, the hip flexion angle is higher, and a larger range of subsequent hip extension is found (Chehab et al. 2017; Fukuchi et al. 2019b). This coincides with the hip internal flexion-extension moments,
where the first peak extension internal moment increases, and the subsequent range of hip flexion internal moment increases (Chehab et al. 2017; Fukuchi et al. 2019b).

In terms of ankle plantarflexion-dorsiflexion angles and moments, the first peak plantarflexion angle decreases slightly as speed increases, and the subsequent dorsiflexion range increases (Chehab et al. 2017; Fukuchi et al. 2019b). There is a slight decrease in the internal ankle dorsiflexion internal moment as the speeds increase, and there is a larger range of a subsequent plantarflexion internal moment as speed increases (Chehab et al. 2017; Fukuchi et al. 2019b). There are also self-reported differences in the function of the ankle after an ACLr (Hoch et al. 2019).

The frontal plane of hip abduction-adduction and ankle inversion-eversion and the transverse plane of hip internal-external rotation and ankle abduction-adduction have not been discussed in further detail due to the focus on the knee. Some discussion of these factors is available in the three research chapters.

This section has discussed the effect of walking speed on a range of biomechanical parameters for not only the knee but for the hip and ankle too. The next section discusses the background of ACL injury.

4.1.2 ACL Injury Background

As touched upon in the general literature review in Chapter 2, ACL injury is common, particularly in sport (NHS 2020) and ACL rupture can cause instability of the knee, causing it to give way (Rudolph et al. 1998). It disturbs the sense of balance, also termed postural control (Herrington et al. 2009), proprioception (Relph et al. 2014), kinematics and kinetics (Andriacchi et al. 2006; Hurd and Snyder-Mackler 2007; Georgoulis et al. 2010; Esfandiarpour et al. 2013) and can change the interaction of the muscles contracting around the knee joint (Hurd and Snyder-Mackler 2007). However, disturbed kinematics and kinetics between healthy and ACL-deficient individuals have been challenged, with one study showing no differences during level walking at a fixed speed (Kuster et al. 1995). All these aforementioned principles can contribute to dynamic knee stability, though as established in this research, four main factors comprising knee stiffness, leg stiffness, co-contraction and loading are of particular interest in this work. Therefore, due to such a strong link between ACL injury and its associated altered muscle activations, kinematics, and kinetics, and with the knowledge that an ACLr still provides altered knee function, further muscular, kinematic and kinetic data is required from a patient cohort. This could establish the full scale of the problem and what happens to the knee joint surface loading. This can be summarized with "the more comprehensive approach under-taken by the musculoskeletal model to estimate joint loading, including use of frontal and sagittal plane kinetics along with co-contraction
estimates . . . may provide enhanced insight into the development of OA as compared to kinetic measures alone” (Wellsandt et al. 2014). Studying a daily-used functional movement like gait is an excellent way to integrate all these queries.

4.1.3 The Dynamic Knee
4.1.3.1 Dynamic Joint Stability
As previously mentioned in Chapter 2, the movement of the knee and the pattern of gait adjust constantly to maintain stability of the knee joint and the body system as a whole. Dynamic joint stability is a term often used to discuss the instability, or stability, of the knee during daily activities, but is without a clear definition (Schrijvers et al. 2019). Dynamic joint stability is a robust concept to use as a reference to compare healthy data to those with knee issues such as an ACLd to understand the extent of an issue on the knee joint.

Returning to Figure 22 and the variables of interest that this research defines to incorporate dynamic knee joint stability, namely co-contraction, joint stiffness, leg stiffness and joint loading. Figure 23 demonstrates the assumption between the increase of speed and dynamic knee joint stability parameters based on the knowledge established from the literature.

![Figure 22: Joint stability and its relationship with co-contraction, leg stiffness, joint stiffness and joint loading](image)

*Figure 22: Joint stability and its relationship with co-contraction, leg stiffness, joint stiffness and joint loading*
In one study, individuals with ACL deficient or ACLr seem to perform movements with similar amounts of dynamic control to healthy individuals (defined to be a combination of joint stiffness, Knee Joint Center Excursion and Knee Joint Center Boundary) (Smale et al. 2019). However, ACL-deficient individuals or individuals with ACLr have a lower amount of control quantitatively in some movements compared to an individual’s perception of the movement (Smale et al. 2019). This poor correlation between performed and self-reported values has been noted elsewhere when knee joint stiffness was investigated (Dixon et al. 2010). Also, many ACL deficient individuals or individuals with ACLr complain of a ‘wobbly’ knee. In the previously mentioned study, it was attempted to qualitatively define this ‘wobble’, though no significant findings were found when using Knee Joint Center Excursion (KJCE) as a measurement of dynamic joint stability (Smale et al. 2019). Hence, there is a big difference between ACL deficient and ACLr participants’ perception of dynamic joint stability and actual measured dynamic joint stability. It is well established that those with pre-existing conditions of the knee, such as ACL deficiency (ACLD) or ACLr, have considerably different kinematics and kinetics (Oberländer et al. 2012; Sharifi et al. 2017; Smale et al. 2019), and have a loss in dynamic joint stability (Oberländer et al. 2012; Schrijvers et al. 2019), though it is worth noting as discussed above that this dynamic control in high functioning individuals can be similar to that of those that are uninjured (Smale et al. 2019).

4.1.3.2 Joint and Leg Stiffness
A recent literature review showed that during walking for healthy and knee OA individuals (Schrijvers et al. 2019), joint stiffness did not change (Collins et al. 2014; Galli et al. 2018), did increase for those with less symptoms (Gustafson et al. 2016), and did increase for affected individuals in a challenged state (Zeni and Higginson 2009b). No such comprehensive literature review could be found for measures of dynamic joint stability in individuals with ACLr. Additionally, for those with unilateral OA, dynamic...
knee joint stiffness was consistently higher in early stance phase, or weight acceptance, compared to the unaffected limb (Gustafson et al. 2019), a result that was also supported between OA participants and controls. Those with severe knee joint OA also have higher knee joint stiffness irrespective of speed (Zeni and Higginson 2009b). In agreement with these results, post-ACL rupture non-copers tend to have higher co-contraction and less joint movement to ensure a stiffer knee strategy for stability purposes (Hurd and Snyder-Mackler 2007). These are clearly all slightly conflicting results when it comes to considering gait for healthy individuals and individuals with ACLr and their resulting joint stiffness, though more often stiffness is higher for OA individuals when compared to healthy individuals; thus, suggesting a beneficial measurement when analysing a post-injury group and to identify pre-cursors to OA development. Furthermore, individuals with ACL deficit or ACLr performing side cutting or hopping movements display equal or lower joint stiffness to their control counterparts (Smale et al. 2019), though these are clearly very different movements, and lower stiffness results could show the boundary of control these ACL deficit or ACLr subjects have on the stability of their knee during extremely challenging movements. Notably, it has been suggested that joint stiffness is not related to knee joint contact forces (Dixon et al. 2010; Gustafson et al. 2019), with one study modelling these knee joint contact forces (Gustafson et al. 2019), whilst the other used the rate of loading of the GRFs as a marker for the knee joint contact forces (Dixon et al. 2010).

A previous study combining both leg and joint stiffnesses in hopping suggests changes in leg stiffness are primarily affected by ankle joint stiffness, with no notable influence from hip or knee joint stiffnesses (Farley and Morgenroth 1999), meaning it is important to explore both joint stiffness and knee stiffness. An interesting theory behind this result was that if leg stiffness as a system is deemed to be a link of several joint springs, the least stiff spring will react to a force first and influence the system, though this was not supported by the results (Farley and Morgenroth 1999). While this is not a gait study, it helps to understand the relationship between the leg at either contact or push-off phases.

There are few leg or limb stiffness articles for ACL injury or individuals with ACLr. One study examining vertical jump abilities in ACLr and healthy individuals did observe some general right leg dominance on lower leg stiffness, however only for healthy adolescents, and not for individuals with ACLr (Jordan et al. 2018).

4.1.3.3 Speed and Stiffness
It is important to establish the effect speed may have on the knee and leg stiffness results as there is not a lot of previous literature discussing the interlink between speed
and stiffness. Whilst walking and running are very different activities, one very comprehensive study was found to compare the joint stiffness results for the hip, knee and ankle at different walking and running speeds (Jin and Hahn 2018), and hence this section has been created to link stiffness to speed (when other principles of dynamic knee stability have not been linked to speed so clearly in this way previously). It was found that the stiffness of the knee was greater than the stiffness of the hip at different walking speeds (Jin and Hahn 2018). However, a correlation between joint stiffness with increase in speed was found for the hip, but not for the knee or ankle during walking as shown in Figure 24 (Jin and Hahn 2018).

However, with running, all joint stiffnesses increased with an increase in speed as shown in Figure 25 (Jin and Hahn 2018). The study indicates how the knee stiffness results are lower than the other joints, suggesting the knee joint takes more of a dampener behaviour and becomes a coordinating joint between the other two joints (Jin and Hahn 2018).

If the speeds of these two movements are studied, the two graphs in Figure 24 and Figure 25 could potentially be aligned with walking on the left, and a continuation to running on the right. Please note that walking and running make fundamentally different stiffness results for a joint even at a similar speed, though for the knee, the results are the same (Jin and Hahn 2018). The knee joint appears to be very stiff for very slow speeds, then decreases in stiffness parabolically to mid-range walking speeds, before increasing again for faster walking speeds, and then increasing from that point linearly for running speeds. A knee joint line of best fit is not present in Figure 24, as only linear lines were considered. Therefore, there are opportunities to consider the data to be more complex and behave in a parabolic fashion.
As discussed in this section, for healthy individuals there is not a strong link between walking speeds and joint or leg stiffness. However, there is some research considering changes in speed and those with OA, and due to the links in the literature between ACLr and OA development (Roos 2016), is investigated here.

Those with knee OA and instability tend to walk slower with lower knee joint stiffnesses as a result (Zeni and Higginson 2009a; Gustafson et al. 2016), though while the lower stiffness results for those in the injured population were significant in one study, no actual significant link between stiffness and speeds could be established, and the effects of gender, shoe type and self-reported stiffness needed further investigation (Gustafson et al. 2016). Changes in biomechanics are related to participants with OA choosing slower speeds rather than OA progression itself, though knee range of movement is less overall compared to healthy individuals (Zeni and Higginson 2009a). This smaller range
of joint movement suggests a stiffer joint considering the joint stiffness calculation. Furthermore, subjects with severe knee OA have been shown to walk with greater dynamic knee joint stiffness than their moderate knee OA and healthy counterparts, regardless of the speed (Zeni and Higginson 2009b). Also, it has been shown that all subjects, whether with healthy knees, or moderate or severe OA, have larger knee joint dynamic stiffness results as the speed increases (Zeni and Higginson 2009b).

4.1.3.4 Co-Contraction
Co-contraction is a good way to understand how muscles work together to produce internal forces around the knee joint to counteract the destabilising moments originating from the GRFs and lever arm combination (Lewis et al. 2010). Certain muscles can be paired or grouped so that the percentage of their combined activation (in volts) can be found and compared to those without an ACLr. It is worth noting that excessive co-contraction has been linked to mechanical wear of the knee and post-traumatic OA (Blackburn et al. 2019).

Firstly, it is important to understand how muscle activations can change in ACL-injured individuals. Early research suggested that non-copers had avoidance of contracting the quadriceps, with lower quadriceps contraction lowering the internal knee extension moment (Berchuck et al. 1990). More recently it has been found however that copers and non-copers have higher levels of knee extensor muscle activation than uninjured individuals, compared to what would be expected (Rudolph et al. 2001). At first glance, this does not support the lower internal extension moments mentioned in non-copers. The extensor muscle activation was defined as quadriceps femoris activations (Rudolph et al. 2001). Quadriceps femoris traditionally encompasses the rectus femoris and three vastus muscles, but can be simplified to just include the vastus lateralis (Rudolph et al. 2001), or additionally include the vastus medialis (Blackburn et al. 2019), or also include the vastus intermedius and rectus femoris (Souissi et al. 2017). It has been suggested that these individuals tend to have a much greater relative contribution from their knee flexors, specifically the hamstrings (biceps femoris, and medial hamstrings as semitendinosus and semimembranosus) (Blackburn et al. 2019), in comparison to the quadriceps (Rudolph et al. 2001). When the knee is at its most flexed for non-coper individuals, remembering that it has less range than for healthy individuals, the plantar flexors are more activated to increase power absorption (Rudolph et al. 1998) and as the body progresses over the stance limb, to limit the anterior tibial translation (Perry and Davids 1992; Rudolph et al. 1998). While the comparison between injured and uninjured legs is not being analysed in this research, it is interesting to note that previous discrepancies between muscles in each leg have been identified, specifically that the
injured leg tends to have higher muscle activity for the hamstrings and less for the vastus lateralis, for females only (Mohr et al. 2019).

Now considering what these changes in muscle activations mean on co-contraction results, people with ACLr have greater co-contraction, particularly for the quadriceps and hamstrings, when compared to the uninjured limb and non-injured individuals (Blackburn et al. 2019). Also, non-copers tend to demonstrate higher muscle co-contraction when compared to the uninjured limb (Hurd and Snyder-Mackler 2007). In contrast, lower co-contraction levels in the quadriceps and hamstrings with slightly more activity in the quadriceps cause a small extension moment during heel strike when there is more joint flexion but less of a posterior tibial slope, which improves the dynamic stability of those that are ACL deficient (Sharifi et al. 2018). Importantly, this does not consider co-contraction in the gastrocnemius as this deteriorates stability (Sharifi et al. 2018). Expanding on the issue of gastrocnemius, lower gastrocnemius amplitudes are present in those with post-traumatic OA compared to healthy individuals (Robbins et al. 2019).

Whilst speed and co-contraction with individuals with ACLr (Blackburn et al. 2019; Knobel et al. 2021) and individuals with ACLd have been studied previously (Rudolph et al. 2001; Hurd and Snyder-Mackler 2007), different speeds with co-contraction have not been investigated previously.

4.1.3.5 Knee Loading
Internal loading of the joints is of specific interest to establish the health of the joints, as kinematic a positional shift in loading of the knee can sometimes cause loading of a part of the knee not conditioned to be used in such a manner (Andriacchi and Mündermann 2006), with abnormal loading leading to cartilage degeneration (Kumar et al. 2013). The early stance phase is of particular interest in this research, as that is when the healthy knee is most loaded and required to stabilise (to prevent hypermobility and the possibility of ‘giving way’ (Sharifi and Shirazi-Adl 2021)) and also dampen (a concept introduced earlier when considering the lower leg as a series of springs and dampeners).

There is minimal existing literature on knee loading data at different speeds for healthy individuals and individuals with ACLr as mentioned briefly in the general literature review (de David et al. 2015; Knobel et al. 2021). This research therefore uses the established link between individuals with ACLr and those with OA to attempt to expand the relevant literature that can be examined about knee loading (Andriacchi and Mündermann 2006; Dewig et al. 2022). Several studies have sought to establish knee forces as a measurement to investigate the impact of OA on walking (Richards and Higginson 2010; Roberts et al. 2017; Gustafson et al. 2019). These knee forces can be
knee contact forces (KCF), the same as knee joint contact forces discussed in this research, or joint reaction forces (JRF), the sum of the external joint force and the forces of all the muscles spanning the knee, which appears to be a similar concept to the KCF (Thewlis et al. 2015). Knee forces are also used to investigate the impact on the length of time walking (Farrokhi et al. 2017; Gustafson et al. 2019), in conjunction with joint stiffness calculations (Gustafson et al. 2019), and modelled muscle forces (Richards and Higginson 2010; Gustafson et al. 2019). One study that did not use KCFs, but JRFs, managed to subdivide the participants into 3 categories of ability (Roberts et al. 2017). There were Biphasic who had large excursions in the sagittal knee moment curve, and Counter-Rotator, who were similar to biphasic individuals, but with a lower peak flexion moment (Roberts et al. 2017). Thirdly there was Flexor, where the moment curves cross from extension in the loading response to flexion for the rest of stance (Roberts et al. 2017).

Following on from the previous discussion, unusual gait in individuals with a reconstructed ACL can lead to firstly, post-traumatic OA and secondly, further ACL injury (Pfeiffer et al. 2018), although it has been argued by some that as little as 4 years post-ACLr, there are no differences between the non-injured leg (or control individuals) and the reconstructed leg kinetically (Stoelben et al. 2019). Interestingly, when investigating participants with ACL injury, a common study exclusion is the bilateral knee involvement (Hurd and Snyder-Mackler 2007).

During walking for individuals with knee OA there is an increased result in simulated muscle forces and the knee joint contact forces in early stance for both the affected and contralateral limbs (Gustafson et al. 2019). Interestingly, these two variables had increased peaks in late stance on the contralateral limb (Gustafson et al. 2019), suggesting a compensatory strategy, which agrees with what was discussed previously stating that the GRF is higher on the contralateral limb (Rudolph et al. 1998).
4.2 Methods and Data Processing

4.2.1 Introduction

To determine the changes that may be occurring for patients who are post-ACLr surgery and possible indicators for wear of the joint and OA, a dataset of healthy joint stability and contact loading parameters are also required. This study aimed to collect marker, force plate and EMG data from healthy and patient subjects and input this information into the musculoskeletal model in the SIMM software so that internal knee information, namely modelled joint contact loading, could be found, which would be otherwise difficult to gather in vivo. Joint contact loading is expressed in three ways; lateral condyle loading, medial condyle loading and total knee joint loading (the sum of lateral and medial loading).

The surface EMG data collected was then analysed with co-contraction indices discussed previously to deduce its relationship with the changes in joint contact loading and knee and leg stiffness over the four different speeds.

4.2.2 Study Design

This study was a quantitative, cross-sectional, observational, analytical study (Centre for Evidence-Based Medicine 2021), and considers a group of people with an ACLr matched to healthy individuals. However, this study is potentially also a cohort study, or longitudinal study (Centre for Evidence-Based Medicine 2021), as some of the results were used in comparison to some of the previous findings established by the previous researcher involved with the same patient group, Dr. Robert Letchford (Letchford 2015). All data was collected in the RCCK laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University, Cardiff, Wales.

Data from healthy individuals was collected between September and October 2016 in collaboration with three MSc project students, namely, Fatma Alothman, Alaa Kattan and Riccardo Pozzoli. Ethical approval was granted by the School of Healthcare Sciences Research Ethics Committee at Cardiff University in July 2016 under the project title “Investigation of lower body and spinal functional movements and proprioception in a healthy population to understand movement strategies and accuracies”, with recruitment available from the Cardiff University staff and student cohort. All participants provided written informed consent, and data was stored, and participant safety was conducted in line with approved documentation from the Research Ethics Committee.

Data from individuals with ACLr was collected between March and May 2018 with two research assistants provided by the Arthritis Research UK Centre (ARUK) for Biomechanics and Bioengineering at Cardiff University, namely Penny Farthing and
Natasha Williams. Ethical approval was granted by the ARUK for Biomechanics and Bioengineering in March 2018, after the initial application was started in approximately January 2017, and this large time delay was beyond the researcher’s control. No title was required for this research as it was covered under the general scope of the contract with the Research Ethics Committee (associated with Cardiff University) Reference: 10/MRE09/28 and Aneurin Bevan Research and Development Reference: 1238/14. Recruitment was available from a pre-existing patient cohort (Letchford 2015) from Aneurin Bevan University Health Board, and recruitment followed the relevant ARUK protocols at the time. All participants were provided with written informed consent and data was stored, and participant safety was conducted, in line with approved documentation and ARUK protocols.

4.2.2.1 Sample Size
A sample size of at least 19 was required statistically, with the calculation discussed in section 4.2.6. In terms of healthy individuals, 46 participants (26 males, 20 females, an average age of 28 years) provided written informed consent and participated in this research. This number was achieved due to this study encompassing the requirements for three other MSc students where a minimum of 34 subjects were required. Once removing those that were inapplicable for analysis for this particular study, a final sample size of 30 was identified (18 males, 12 females, an average age of 26 years). Due to the healthy participant data being collected before the patient participant data, it was unknown how the healthy data would match the characteristics of the patient data. In the interests of avoiding selection bias, there were very few inclusion and exclusion criteria for the individuals who participated. Furthermore, many more participants were collected than were needed to match the patient group, so that a subgroup that best matched the key characteristics (demographics) of the patient group could be sought. Therefore, in the interests of data matching to the patient group, 9 healthy participants (6 males, 3 females, average age 30 years) were analysed in depth. In terms of patient individuals, the participants for this research were sourced from an earlier research project containing 74 participants who had all sustained ACL injuries and required ACLr (Letchford 2015). The 74 participants in the patient group had an average age of 30.22 years (with a standard deviation of 8.84 years), 63 were male (or 85%), while 11 were female (15%), an average height of 1.77m (standard deviation 0.07m), and a mass of 85.9kg (standard deviation 17.29kg) (Letchford 2015). These historic patients were all highly symptomatic non-copers pre-surgery, and one year post-ACLr still failed to recover to anywhere near pre-injury ability (Letchford 2015). The research suggested more understanding was needed into useful
rehabilitation strategies that would be more engaging for the patient, and easily measurable for the clinician in terms of performance and strategy (Letchford 2015). All participants were contacted to ask if they wanted to take part (with the aid of Dr Robert Letchford) but due to the active and mobile nature of these participants, and despite determined communication, 53% gave no active communication to any attempted contact, 21% declined, 14% gave an active response but no further engagement could be sustained, and 12% participated. Therefore 9 post-ACLr participants were recruited (6 males, 3 females, average age 35 years). The 9 patients directly influenced the sample taken from the healthy group, in the interests of matching mass, height, and gender between groups. The 9 patient participants who engaged in this research had an average age of 35 (S.D. 10.16 years). 6 were male (67%), and 3 were female (33%). There was an average height of 1.73m (S.D. 0.10m, however, the height was based on 8 adults as one did not disclose their height). Finally, there was an average mass of 84.14kg (S.D. 21.67kg).

4.2.2.2 Inclusion and Exclusion Criteria
The inclusion and exclusion criteria for this study are discussed in Chapter 3.

4.2.2.3 Methods of Assessment
Kinematic, kinetic and EMG data were collected from each individual for the initial EMG MVCs and for the walking session.

**EMG MVCs:** This test collected the maximum that a muscle could be activated in a relatively static activity to compare with activation values during any of the movements (Konrad 2006). The subject was asked to perform a certain movement once with applied resistance to activate a certain muscle (Konrad 2006). For example, for the tibialis anterior the subject was asked to stand and hold the edge of the plinth for stability and raise their toes while a data collector applied downward force to ensure the muscle was activated maximally. The voltage produced by each particular muscle was then recorded. More information on the MVC testing protocol can be seen in Figure 4, with these particular tests being influenced by historical MVC testing methods (Konrad 2006; Meldrum et al. 2007; Reid 2012).
Walking session: The subject was asked to walk at four self-paced speeds (slow, normal, fast and very fast) for 30 seconds each. An initial 30 seconds allowed the subject to experience the treadmill, and 15 seconds was allowed for an increase between each speed, and 15 seconds for the decrease to a stop, which equated to 3 minutes 30 seconds.
4.2.3 Data Collection
The Equipment, Vicon, D-Flow, OpenSim, SIMM and The University of Wisconsin-Madison Model sections are discussed in Chapter 3.

4.2.3.1 Study Protocol
Each subject was prepared with 57 passive reflective markers as per the marker layout protocol defined in the general methods chapter. Each subject was asked to walk on the GRAIL equipment from Motekforce Link (Motekforce Link. 2017), and 16 channels of EMG were attached, also as previously mentioned in section 3.2.4. The participants wore a safety harness which was attached to the ceiling via safety ropes. An initial functional calibration trial was produced for every subject, a still of which is shown in Figure 26. It was quickly identified that a functional calibration was much more effective than the traditional static ‘t-pose’ to gap-fill missing markers during movement.

![Figure 26 - A still of the functional calibration process in the software package Vicon](image)

Each participant was asked to walk at four self-paced speeds (slow, normal, fast and very fast) of 30 seconds each, with an initial 30 seconds to experience the treadmill, 15 seconds to allow for an increase between each speed, and 15 seconds for the decrease to a stop, which equated to 3 minutes 30 seconds. This gait protocol is shown in Figure 27. Slow was defined to each participant as going for a gentle walk, normal as walking to the shops, fast as being late for an appointment and very fast as the fastest they could walk without breaking into a run. 9 steps were sampled from the middle of each walking speed for each participant for analysis (there could be dozens of steps in some of these walking categories, clearly dependent on each subject and the speed they were producing).
The author created a D-Flow program for the gait trial to allow the volunteer to control the treadmill through a self-paced situation. An average of the pelvis markers combined with the volunteer’s position on the treadmill so that they could speed up or slow down the treadmill. Projections informed the volunteers to change their speed at certain intervals and finally informed the participants to come to a stop. Visual information was used to inform the participant during data collection to reduce the issue of bias from communication between the individual and the researcher. Support for the design of the D-flow application came directly from the manufacturer (Motekforce Link. 2017).

![Diagram of walking protocol](image)

**Figure 27**: The walking protocol for each participant

The Data Processing Workflow below discusses the order in which collected data goes through Vicon and the model into understandable outputs.

4.2.4 Data Processing Workflow

4.2.4.1 Calculations

This research often used the mathematical method of cubic splining in Matlab to ensure that data results can be directly compared even when shorter or longer time frames occurred for an individual result. Splining ensures the results for a certain period are placed over a set x-axis, in this case, 0-100 of the stance phase, and each result is shaped so that it fits this x-axis.

When analysing the modelled loading on the tibiofemoral joint, forces or pressures could be analysed. From the way the model is constructed, forces are derived from pressure results (van Rossom et al. 2018). Whilst pressures at the joint surface are discussed in other research using the same modelling framework, they also discuss joint contact forces (Van Rossom et al. 2017; van Rossom et al. 2018). Forces are more
pertinent than pressures as it is more discussed by previous authors when utilising the University of Wisconsin-Madison model, and hence, is a better measure for comparison (Meireles et al. 2017; van Rossom et al. 2018; Van Rossom et al. 2019).

Additionally, forces can be discussed in terms of both the peak and as total of all values, and similar analysis techniques have been discussed with EMG activations too (Whiteley et al. 2021). Both have merit as they both discuss the burden on the system; the ‘worst case’ maximum, and also the ‘constant’ exposure. To analyse the ‘constant’ exposure, the average force can be taken (van Rossom et al. 2018), but there has been precedent for the total contact forces over the stance phase for either the medial or lateral portion of the tibiofemoral joint (Saxby et al. 2016b). There has also been a precedent to take the total for the whole of the tibiofemoral joint (Lenton et al. 2018) so that a certain component can be compared to its effect on the total loading in percent, such as the contribution of the muscles (Saxby et al. 2016b). This chapter takes the total (through integration) of the contact forces for the first half of stance in all individuals. The possible confounding factor of different time periods between heel strike and the peak force is avoided by integrating the total graph between heel strike and a set 50% of the gait cycle.

The appropriate time period to analyse joint stiffness used in this research was the commonly used time period from the IC to the point where the maximum knee flexion angle time point is overlaid onto the moment curve to identify the linear portion of the moment data (Zeni and Higginson 2009b). Further information for the calculations for knee stiffness and leg stiffness is mentioned in Chapter 2, while specific details on all calculations are also in the Matlab code outlined in Appendix B.

4.2.4.2 Matlab and Data Analysis

Figure 28 shows the data processing and analysis workflow. Please see Appendix B and D for more details of these steps and examples of code and outputs.

Once the data had been produced, it needed to be analysed in a way that could be compared to other research studies. Firstly, the knee flexion angles and moments could be plotted for the whole of the stance phase and then plotted against each other to establish some stiffness analyses (Martinez-Villalpando and Herr 2009). Then the forces at the knee joint could be collected and plotted over the stance phase for both the medial and lateral portions of the tibiofemoral joint.

After the initial analysis of the results, the actual performed speed rather than the speed category was used in certain plots. This is because plotting in this way takes into account an individual’s interpretation of a certain speed category (for example one individual may walk much slower than another at a requested ‘normal’ speed), as speed is known...
to change the biomechanical data (Zeni and Higginson 2009a; Chung and Wang 2010; Ardestani et al. 2016). Therefore, the actual speed is plotted to be able to tease the biomechanical information out from the data more clearly.

When scatter graphs were required for the analysis, a line of best fit was required so that general trends could be understood. When the data naturally forms a line, a linear line of best fit is recommended, also known as a 1st order polynomial, whereas when the data fluctuates, a polynomial line of best fit is recommended (Microsoft 2021). The order of the polynomial required depends on the number of directional changes in the data (times the data peaks or troughs), with the order number being one more than the number of directional changes (Microsoft 2021). Typically, a 2nd order line of best fit was selected for this study (otherwise known as a quadratic polynomial), as the data did not change direction more than once (Microsoft 2021).
Figure 28 - Data Processing and Analysis Workflow for the Healthy and Patient Individuals Study (green represents stages where comparisons are made)
4.2.5 Ethics, Data and Safety
Recruitment for data collection on healthy individuals was conducted in line with Cardiff University's Ethical procedures. The application to the School of Healthcare Sciences Research Ethics Committee at Cardiff University began in May 2016 with permission being granted by Cardiff University to conduct the study in late July 2016. Subject data was collected between September and October 2016.
The application for patient research began in approximately January 2017. Due to a lack of existing links between the Aneurin Bevan University Health Board and the Arthritis Research UK Centre (ARUK) for Biomechanics and Bioengineering at Cardiff University (who received the application to conduct this research at the University), the project was granted in March 2018. Data collection occurred between March and May 2018 with the aid of two research assistants provided by ARUK. No title for this research was required as it fell under the general scope of the contract and all ARUK informed consent and data management policies, along with other policies were adhered to (in a similar manner as they had for the step width study discussed in Chapter 5).
All subjects completed written Participant Questionnaires outlining their height and mass (though the latter is also collected from the force plate information). These were stored in lockable filing cabinets located within the RCCK laboratory, which itself is only accessible by staff and PhD students. Data collected electronically was coded simply with each subject being assigned a number in ascending order.
The same safety policies were adhered to for both data collection periods as required by the RCCK laboratory user agreement contract. Most notably all subjects were required to wear a harness attaching them to the ceiling and to prevent injury in the case of a fall whilst the treadmill was moving. Additionally, the subjects were required to be supervised at all times whilst in the laboratory.

4.2.6 Statistics
To derive the sample size \((k)\) for this study, for many clinical reliability studies \(p_0\) of 0.6 is a recommended minimum, and \(p_1\) of 0.8 for the intraclass coefficient (Shoukri et al. 2004). Optimal alpha \((\alpha)\) and beta \((\beta)\) values are recommended as 0.05 and 0.20 respectively (Walter et al. 1998). Selecting the number of replications per subject as 10 derives a sample size of 18.5 or rounded up to the nearest volunteer of 19 (Walter et al. 1998). Importantly, a low sample size can undermine the internal and external validity of the research (Faber and Fonseca 2014). However, where statistical significance is found with a low sample size, it has overcome the possibility of a false negative result, known as a Type II error (Peterson and Foley 2021).
The software package SPSS was used for statistical testing and to examine the assumptions that the data fits normality and equal variance. Initially, normality was inspected for all data with the Shapiro-Wilk Test (Shapiro and Wilk 1965). Typically, if normality is found through $p < 0.05$, parametric tests such as t-tests or two-way repeated measure ANOVAs can occur. If normality is violated, non-parametric tests are often utilised (Nahm 2016). The non-parametric Friedman test can examine the within-subject effects for either healthy or patient participants, and the non-parametric Mann-Whitney U can examine the between-subject effects for either slow, normal, fast or very fast walking (Nahm 2016). However, the Friedman test and Mann-Whitney U test clearly cannot examine within- and between-subject information in one test. There is a non-parametric proposed alternative to the two-way repeated measures ANOVA, the Scheirer-Ray-Hare test (Scheirer et al. 1976), but it is not well documented and is less likely to find significance than other tests (Mangiafico 2016). Instead, an Aligned Rank Transform of any non-parametric data occurred, so that the ANOVA procedure could be used (Wobbrock et al. 2011). The Aligned Rank Transform (ART) method is known to be a robust and powerful method (Mansouri 1998) and was used when a mixture of parametric and non-parametric data was present so that the data could be examined in a unified manner (Conover and Iman 1981; Mansouri 1998; Leys and Schumann 2010; Wobbrock et al. 2011).

For the healthy and patient participant characteristics, t-tests were used. An ANOVA was implemented for all the biomechanical results and selected over a t-test as three or more mean speeds were compared (Mishra et al. 2019). The ANOVA was two-way as two categorically independent variables of healthy and patient individuals were examined and was a repeated measures condition as the same group was being analysed at more than two different speeds (Mishra et al. 2019).

For all the biomechanical results, a Mauchly Test of Sphericity was performed to examine equal variance, where statistical significance was found if $p > 0.1$ (Mauchly 1940). Depending on the results, either a repeated-measures ANOVA with Sphericity Assumed or a repeated-measures ANOVA with Greenhouse-Geisser was performed (if the Mauchly’s Test of Sphericity was statistically significant or not respectively). As the ANOVA considered multiple group comparisons, each group was the walking category of slow, normal, fast or very fast (Leon et al. 1998). Statistical significance for the ANOVA was assumed to be $p < 0.05$.

Finally, linear regression was performed to examine the link between the loading results and the CCI calculations, and for the loading results and the stiffness results (the link between the CCI and stiffness results could not be established in this manner as they
were both ‘input’ variables. Linear regression considers continuous data (and hence was relevant when considering data over a continuous speed and not by speed groups) and so was used to complement and aid analysis of the data to a greater depth. The regression degrees of freedom (df) represent the number of independent variables being analysed (always 1 in this research), whilst the residual df considers the total number of rows in the data minus the variables being estimated (the dependent variable and the constant) (Camp 2019).

A commonly used analysis, $R^2$, was also employed across the linear regression results and where graphs displayed lines of best fit. The $R^2$ value determines what percentage of the variability is accounted for by the variables in the model, where an $R^2$ of 0.034 means 3.4% (Regents 2021). In other words, the $R^2$ considers what variations of the dependable variable are influenced by the independent variable (Inn 2020), and is commonly used in regression analyses (Kissell and Poserina 2017). An $R^2$ of 1 would mean that all the variation in the dependent $y$ value is accounted for in the independent $x$ values and the line of best fit sits with the data well (University 2016; Mark and Workman 2018). An $R^2$ of 0 would mean that none of the variation in the $y$ values is accounted for in the $x$ values and the line of best fit does not sit with the data well (University 2016; Mark and Workman 2018). If a line of best fit is applied to the graph, the line needs to produce the smallest distance (the residual) from each of the observed data points to the fitted value (Frost 2023). Therefore, the nearer to 1 that the $R^2$ is, the better the line of best fit or the linear regression is. The F-test is also used in regression analysis and determines the significance of an $R^2$ change (Inn 2020). The value required for $R^2$ to be acceptable is debated, though in sports research under 0.2 has been suggested that a model is less predictable, between 0.2 and 0.4 suggests a model that has a good fit, and above 0.4 suggests an excellent model (Kissell and Poserina 2017). The F-test is used to understand the ratio of variances and is defined as the explained variance divided by the unexplained variance (Kissell and Poserina 2017). Historically if the F change is greater than 2.5 then the null hypothesis can be rejected (Kissell and Poserina 2017). The significant F change comes from the F-test and if statistically significant, implies that the variable analysed significantly improved the model prediction (Inn 2020). $R^2$ was also reported in the graphs where a line of best fit was applied to the data, with the term ‘adjusted $R^2$’ used for all the second-order line of best-fit graphs to consider the increased number of variables (or coefficients) used in the fit of the line. This is because the $R^2$ increases when there is an increasing amount of independent variables, and an adjusted $R^2$ therefore only increases when the new independent variable improves the model or only decreases when the new independent
variable does not affect the model (Inn 2020). If a negative adjusted $R^2$ was established in this thesis, it was marked accordingly and reported as zero, as happens in certain mathematical software (Frost 2023).

4.2.7 Research Hypotheses
Hypothesis 1 H1: There is a difference between basic biomechanical variables (gait speed, knee flexion angle, knee flexion moment, ground reaction force) and those that represent dynamic knee joint stability (knee stiffness, leg stiffness, medial contact loading, lateral contact loading and co-contraction) for healthy and ACLr participants at different walking speeds.

With the null hypothesis (H0) of:
Hypothesis 1 H0: There is no difference between basic biomechanical variables (gait speed, knee flexion angle, knee flexion moment, ground reaction force) and those that represent dynamic knee joint stability (knee stiffness, leg stiffness, medial contact loading, lateral contact loading and co-contraction) for healthy and ACLr participants at different walking speeds.
4.3 Results
Table 5 represents the subject characteristics for this study. Healthy individuals were matched as closely as possible to the average of the patient group in terms of gender, age, mass and height. Six patients had a left knee ACLr and one had a right knee ACLr. A final two participants had both sides reconstructed, the 2nd side left knee of interest in one case, and the 1st side left knee of interest in the other case.
As there was a small sample of participants, a Shapiro-Wilk test for normality was taken to establish if the data were normally distributed (Ghasemi and Zahediasl 2012). Shapiro-Wilk is treated in the opposite way to other statistical tests, as the null assumes a normal distribution. Therefore, as the Shapiro-Wilk test returned significance values for healthy and patient data of above 0.05 for age (p= 0.088 and p= 0.274) and modelled mass (p= 0.144 and p= 0.545), the null cannot be rejected, and it can be assumed that these variables are normally distributed. Additionally, height (p= 0.202 and p= 0.645) and time since operation (p= 0.334 for patients only) were also assumed to be normally distributed. Lab-collected mass was not gained for patients (through the use of a weighing scale), as the modelled mass derived from the force plates is more accurate. There was no statistical significance for the comparison between the subject characteristics for the patient and healthy individuals using a two-tailed t-test, suggesting that the two groups are not statistically different for their subject characteristics and hence have been matched well. Examining the differences between the groups visually, the gender, age, modelled mass and height are very similar, and considering exact matching is not possible, they successfully complement each other. In this results section, unless explicitly written otherwise, all t-tests are independent with equal variance assumed, and all ANOVAs are repeated measures.
Table 5 - Subject characteristics for healthy and patient groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient</th>
<th>Healthy</th>
<th>Patient</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Shapiro-Wilk</td>
<td>t-test (two-tailed)</td>
<td></td>
</tr>
<tr>
<td>Sample Size</td>
<td>9 adults</td>
<td>9 adults</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td>67% (6) male, 33% (3) female</td>
<td>67% (6) male, 33% (3) female</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 (±10.16)</td>
<td>30 (±5.83)</td>
<td>p = 0.274</td>
<td>p = 0.088</td>
</tr>
<tr>
<td>Lab collected mass (kg)</td>
<td>-</td>
<td>79.17 (±14.71)</td>
<td>-</td>
<td>p = 0.438</td>
</tr>
<tr>
<td>Modelled mass (kg)</td>
<td>84.14 (±21.67)</td>
<td>80.67 (±12.90)</td>
<td>p = 0.545</td>
<td>p = 0.144</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.6 (±10.19)†</td>
<td>178.3 (±6.52)</td>
<td>p = 0.645†</td>
<td>p = 0.202</td>
</tr>
<tr>
<td>Time Since Operation (years)</td>
<td>6.25 (±) 0.70</td>
<td>-</td>
<td>p = 0.334</td>
<td>-</td>
</tr>
</tbody>
</table>

† Height results based on 8 adults, as one did not disclose their height.
* Statistically significant

Table 6 shows the Shapiro-Wilk test for normality on the biomechanical variables. If p > 0.05, then statistical significance was not found, and the data was assumed to be normally distributed. This then led to the data being examined with an ANOVA. If p < 0.05, the data was assumed to be not normally distributed and the ART method was utilised before examination with an ANOVA (Conover and Iman 1981; Mansouri 1998; Leys and Schumann 2010).

Statistical significance was found for average speed (healthy slow p = 0.035), and peak knee flexion angle (patient slow p < 0.001, patient fast p < 0.042). It was also found for peak internal knee flexion moment (patient normal p < 0.010, patient fast and very fast p = 0.002). Additionally, average y displacement change in centre of mass (healthy normal p = 0.011, patient slow p < 0.001, patient normal p = 0.009 and patient fast p = 0.002). There was also statistical significance for average knee stiffness (healthy slow, normal, fast and very fast were all p < 0.001, patient slow p < 0.001, patient normal p = 0.003, patient fast p = 0.007 and patient very past p < 0.001). Additionally, there was statistical significance for total mean contact loading forces lateral portion (healthy slow, normal, fast and very fast all had p < 0.001 and patient slow p = 0.019). Finally, there was statistical significance for CCI Three (patient very fast p < 0.001), and for CCI Four (healthy very fast p < 0.025).
Table 6: Shapiro-Wilk test for normality on the biomechanical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Walking category</th>
<th>Shapiro-Wilk</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>Normal</td>
</tr>
<tr>
<td>Average speed</td>
<td>Healthy</td>
<td>p = 0.035*</td>
<td>p = 0.749</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.609</td>
<td>p = 0.667</td>
</tr>
<tr>
<td>Peak knee flexion angle</td>
<td>Healthy</td>
<td>p = 0.065</td>
<td>p = 0.187</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p &lt; 0.001*</td>
<td>p = 0.099</td>
</tr>
<tr>
<td>Peak internal knee flexion moment</td>
<td>Healthy</td>
<td>p = 0.083</td>
<td>p = 0.361</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.398</td>
<td>p = 0.010*</td>
</tr>
<tr>
<td>Average max ground reaction force</td>
<td>Healthy</td>
<td>p = 0.490</td>
<td>p = 0.520</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.833</td>
<td>p = 0.736</td>
</tr>
<tr>
<td>Average y displacement change in centre of mass</td>
<td>Healthy</td>
<td>p = 0.102</td>
<td>p = 0.011*</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p &lt; 0.001*</td>
<td>p = 0.009*</td>
</tr>
<tr>
<td>Average leg stiffness</td>
<td>Healthy</td>
<td>p = 0.129</td>
<td>p = 0.988</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.289</td>
<td>p = 0.717</td>
</tr>
<tr>
<td>Average knee stiffness</td>
<td>Healthy</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p &lt; 0.001*</td>
<td>p = 0.003*</td>
</tr>
<tr>
<td>Total mean contact loading forces medial portion</td>
<td>Healthy</td>
<td>p = 0.460</td>
<td>p = 0.208</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.073</td>
<td>p = 0.077</td>
</tr>
<tr>
<td>Total mean contact loading forces lateral portion</td>
<td>Healthy</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.019*</td>
<td>p = 0.078</td>
</tr>
<tr>
<td>CCI One</td>
<td>Healthy</td>
<td>p = 0.343</td>
<td>p = 0.594</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.088</td>
<td>p = 0.495</td>
</tr>
<tr>
<td>CCI Two</td>
<td>Healthy</td>
<td>p = 0.072</td>
<td>p = 0.294</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.299</td>
<td>p = 0.711</td>
</tr>
<tr>
<td>CCI Three</td>
<td>Healthy</td>
<td>p = 0.072</td>
<td>p = 0.294</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.009</td>
<td>p = 0.019</td>
</tr>
<tr>
<td>CCI Four</td>
<td>Healthy</td>
<td>p = 0.086</td>
<td>p = 0.564</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.331</td>
<td>p = 0.545</td>
</tr>
<tr>
<td>CCI Five</td>
<td>Healthy</td>
<td>p = 0.203</td>
<td>p = 0.364</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>p = 0.684</td>
<td>p = 0.758</td>
</tr>
</tbody>
</table>

(Significance) | * Statistically significant

Table 7 demonstrates the statistical analysis through the use of an ANOVA on the healthy and patient between-group interaction. Any non-parametric data utilised the ART method before being examined with the same ANOVA and is marked accordingly. The green areas show values of statistical significance whilst the red areas show where statistical significance was not satisfied. The colour coding is present due to the number of results reported in this table.

Speed as a main effect provided some significant results (test of within-subject effects). These were average speed (p < 0.001), peak knee flexion angle (p < 0.001), and peak internal knee flexion moment (p = 0.041). Also average max group reaction force (p = 0.042), average y displacement change in centre of mass (p < 0.001) and average leg...
stiffness (p=0.001). Additionally, total mean contact loading forces medial portion (p=0.001), and lateral portion (p<0.001).

The group effect (test of between-subject effects) also provided some significant results. The peak knee flexion angle (p=0.010), peak internal knee flexion moment (p=0.005), total mean contact loading forces medial portion (p=0.001), and lateral portion (p=0.019) were all statistically significant. Additionally, CCI One (p=0.019), CCI Two (p=0.001) and CCI Five (p=0.010) were also all statistically significant.

For the most central analyses, tests of within-subject effects for speed and group, only peak internal knee flexion moment (p=0.003) and CCI Four (p=0.039) were statistically significant which requires more investigation in this results section. This means for internal knee flexion moment and CCI Four, the groups differed in how they responded to the different speeds. The internal knee flexion moment was lower for patients, whilst the CCI Four was initially higher for slow and normal walking, and then lower than for healthy individuals for fast and very fast walking. Average knee stiffness shows that there is no statistical significance (including no sphericity) across all analyses.
Table 7- Healthy and patient between group interaction repeated measures ANOVA

<table>
<thead>
<tr>
<th>Healthy and patient between-group interaction</th>
<th>Mauchly’s test of sphericity</th>
<th>Tests of Within-Subject Effects</th>
<th>Tests of Between-Subject Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig.</td>
<td>Sphericity</td>
<td>Speed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>assumed</td>
<td>Greenhouse-Geisser</td>
</tr>
<tr>
<td>Average speed†</td>
<td>p = 0.304</td>
<td>p &lt; 0.001</td>
<td>p = 0.935</td>
</tr>
<tr>
<td>Peak knee flexion angle‡</td>
<td>p = 0.304</td>
<td>p &lt; 0.001</td>
<td>p = 0.041</td>
</tr>
<tr>
<td>Peak internal knee flexion moment‡</td>
<td>p = 0.301</td>
<td>p &lt; 0.001</td>
<td>p = 0.042</td>
</tr>
<tr>
<td>Average max ground reaction force</td>
<td>p = 0.001</td>
<td>-</td>
<td>p = 0.042</td>
</tr>
<tr>
<td>Average y displacement change in centre of mass‡</td>
<td>p = 0.036</td>
<td>p &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Average leg stiffness</td>
<td>p = 0.001</td>
<td>-</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Average knee stiffness‡</td>
<td>p &lt; 0.001</td>
<td>p = 0.097</td>
<td>-</td>
</tr>
<tr>
<td>Total mean contact loading forces medial portion</td>
<td>p = 0.001</td>
<td>-</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Total mean contact loading forces lateral portion‡</td>
<td>p = 0.001</td>
<td>p &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>CCI One</td>
<td>p = 0.296</td>
<td>p = 0.307</td>
<td>-</td>
</tr>
<tr>
<td>CCI Two</td>
<td>p = 0.182</td>
<td>p = 0.194</td>
<td>-</td>
</tr>
<tr>
<td>CCI Three‡</td>
<td>p = 0.206</td>
<td>p = 0.255</td>
<td>-</td>
</tr>
<tr>
<td>CCI Four‡</td>
<td>p = 0.358</td>
<td>p = 0.435</td>
<td>-</td>
</tr>
<tr>
<td>CCI Five</td>
<td>p = 0.207</td>
<td>p = 0.195</td>
<td>-</td>
</tr>
<tr>
<td>(Significance)</td>
<td>Sig &gt; 0.1</td>
<td>Sig &lt; 0.05</td>
<td>Sig &lt; 0.05</td>
</tr>
</tbody>
</table>

† Data which used the ART method

Table 8 establishes the common parameters of interest for both healthy and patient groups. Any non-parametric data utilised the ART method before being examined with the same ANOVA. Using an ANOVA, there is statistical significance between the 4 speeds for healthy and patient individuals (both p < 0.001) and the peak knee flexion angles for healthy and patient individuals (both p < 0.001). There is also statistical significance between the average maximum GRF for healthy individuals (p = 0.006) and the average y displacement change in the CoM for healthy (p < 0.001) and patient individuals (p = 0.003).
Table 8- Mean, standard deviations and significance levels are provided for key knee biomechanical parameters (for the first half of the stance phase only)

<table>
<thead>
<tr>
<th>Group</th>
<th>Factor</th>
<th>Speed 1</th>
<th>Speed 2</th>
<th>Speed 3</th>
<th>Speed 4</th>
<th>Individual group interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.42 (±0.08)</td>
<td>0.83 (±0.29)</td>
<td>1.44 (±0.34)</td>
<td>1.93 (±0.30)</td>
<td>p=0.114* p&lt;0.001*</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Average speed † (for full walk)</td>
<td>0.70 (±0.39)</td>
<td>1.04 (±0.40)</td>
<td>1.56 (±0.42)</td>
<td>2.09 (±0.41)</td>
<td>p=0.017 p&lt;0.001*</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>22.09 (±9.32)</td>
<td>24.22 (±8.02)</td>
<td>28.77 (±8.11)</td>
<td>30.84 (±7.35)</td>
<td>p=0.278* p&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Peak knee flexion angle ‡ (deg)</td>
<td>11.04 (±8.80)</td>
<td>13.09 (±7.75)</td>
<td>18.63 (±7.87)</td>
<td>22.34 (±8.98)</td>
<td>p=0.938* p&lt;0.001*</td>
</tr>
<tr>
<td>PATIENT</td>
<td></td>
<td>0.04 (±0.03)</td>
<td>0.03 (±0.09)</td>
<td>-0.08 (±0.24)</td>
<td>-0.22 (±0.35)</td>
<td>p=0.083 p&lt;0.111</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.06 (±0.03)</td>
<td>0.11 (±0.05)</td>
<td>0.11 (±0.17)</td>
<td>0.07 (±0.33)</td>
<td>p=0.343* p&lt;0.001*</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Average max ground reaction force ‡ (GRF) (kN)</td>
<td>0.84 (±0.19)</td>
<td>0.86 (±0.20)</td>
<td>0.99 (±0.29)</td>
<td>1.00 (±0.46)</td>
<td>p=0.001 p&lt;0.363</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.86 (±0.14)</td>
<td>0.88 (±0.15)</td>
<td>0.99 (±0.20)</td>
<td>1.099 (±0.21)</td>
<td>p=0.001 p&lt;0.006*</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Average y displacement change in centre of mass ‡ (CoM) (m)</td>
<td>0.03 (±0.02)</td>
<td>0.04 (±0.02)</td>
<td>0.05 (±0.03)</td>
<td>0.05 (±0.01)</td>
<td>p=0.161* p&lt;0.003*</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.03 (±0.01)</td>
<td>0.04 (±0.01)</td>
<td>0.05 (±0.01)</td>
<td>0.06 (±0.01)</td>
<td>p=0.343* p&lt;0.001*</td>
</tr>
</tbody>
</table>

* Statistically significant
‡ For all of the gait cycle
† Data which used the ART method

Table 9 displays the stiffness and loading parameters for both the healthy and patient participants. Any non-parametric data utilised the ART method before being examined with the same ANOVA. Analysing the individual group interaction with an ANOVA, there was an individual group statistical significance for average leg stiffness for healthy individuals (p= 0.007) and for the average knee stiffness for patients (p= 0.036). There was also statistical significance for medial total mean contact loading forces for healthy individuals (p= 0.008) and patients (p= 0.002), and for the total lateral mean contact loading forces for healthy individuals (p= 0.008) and patients (p= 0.003).
**Table 9- Mean leg and knee (or joint) stiffness with medial and lateral total mean forces (for the first half of the stance phase only)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Factor</th>
<th>Speed 1</th>
<th>Speed 2</th>
<th>Speed 3</th>
<th>Speed 4</th>
<th>Individual group interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENT</td>
<td>Average leg stiffness ₪</td>
<td>0.40 (±0.17)</td>
<td>0.36 (±0.15)</td>
<td>0.28 (±0.10)</td>
<td>0.30 (±0.10)</td>
<td>p= 0.148*</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.38 (±0.14)</td>
<td>0.29 (±0.07)</td>
<td>0.25 (±0.03)</td>
<td>0.22 (±0.04)</td>
<td>p= 0.001</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Average knee stiffness ‡</td>
<td>0.23 (±0.50)</td>
<td>0.12 (±1.52)</td>
<td>-0.04 (±0.05)</td>
<td>-0.06 (±0.06)</td>
<td>p= 0.001</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.10 (±0.45)</td>
<td>-0.12 (±0.51)</td>
<td>-0.17 (±0.54)</td>
<td>-0.33 (±0.9)</td>
<td>p= 0.002</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Total mean contact loading forces medial portion (N/BW)</td>
<td>17.97 (±7.85)</td>
<td>20.40 (±7.79)</td>
<td>25.54 (±8.30)</td>
<td>27.34 (±8.19)</td>
<td>p= 0.003</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>29.87 (±7.95)</td>
<td>33.61 (±5.71)</td>
<td>40.14 (±8.92)</td>
<td>44.88 (±8.78)</td>
<td>p= 0.001</td>
</tr>
<tr>
<td>PATIENT</td>
<td>Total mean contact loading forces lateral portion (N/BW)</td>
<td>21.55 (±10.33)</td>
<td>23.16 (±10.78)</td>
<td>25.32 (±13.25)</td>
<td>30.32 (±14.32)</td>
<td>p= 0.033</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>11.57 (±8.69)</td>
<td>13.25 (±10.92)</td>
<td>18.22 (±23.97)</td>
<td>20.90 (±23.11)</td>
<td>p= 0.005</td>
</tr>
</tbody>
</table>

*Statistically significant

% For all of the gait cycle

镲 Data which used the ART method

When examining the results, the first peak for medial and lateral average forces over the stance phase was more likely to be higher than the second peak for the patient volunteers (the opposite was true for healthy volunteers). The second peak being higher is unusual and appears to have been associated with the GRF information; the GRFs start to unload rapidly at the end of the step for healthy individuals, and more so than for the patient participants. There is also precedence for the second peak being higher than the first peak; for the medial compartment with the same model for healthy individuals (Van Rossom et al. 2019) and in terms of the range of the second peak being higher than the range of the first peak for both medial and lateral compartments (Miller et al. 2015). Total mean contact forces were collected for the medial and lateral potions and not instant peak contact forces as these are more representative of the joint surfaces for the full stance loading portion as discussed in section 4.2.4.1. Figure 29 demonstrates the total contact forces for the medial and lateral portions of the tibiofemoral joint for the first half of stance, derived as the sum of the values from the first half of stance (not to the peak force value). This figure demonstrates that medial forces appear to be
generally higher than lateral forces for healthy individuals and the opposite is true for patient individuals.

Figure 29- Total mean medial and lateral contact forces per walk for the first half of stance for healthy and patient subjects (medial p= 0.008 and p= 0.002, and lateral p= 0.008 and p= 0.003 respectively)

Figure 30 shows the relationship between increasing speed and the results for the healthy and patient groups in terms of loading on the medial and lateral compartments of the tibiofemoral joint with a second-order line of best fit. The adjusted $R^2$ suggests an
excellent fit for the line for medial loading for healthy individuals, however, the other
lines are of a less predictable fit (Kissell and Poserina 2017). The measured speed rather
than the speed category is shown to understand the influence of different perceptions
of a speed category; one individual may translate ‘normal speed’ as very fast compared
to another. Hence separating the data by actual speed may allow for clearer
biomechanical understanding. A second-order line of best fit was chosen when the data
was not linear in behaviour to aid in establishing trends. Statistically, and as shown in
Table 7 (healthy and patient between group interaction repeated measures ANOVA)
there is a significant difference between the medial and lateral total mean contact
forces and the speed (p= 0.001 and p= 0.011 respectively), but only statistical
significance for the medial portion for between-subject effects (p= 0.001). As can be
seen, the patient participants consistently display lower medial forces but higher lateral
forces at all speeds compared to their uninjured counterparts, an opposite result to
what was expected. Furthermore, all lines of best fit for both groups suggest a close-to-
linear relationship between speed and loading forces, indicating that as speed increases,
so does the loading (on either compartment). However, this is not true for the medial
compartment of the patient group, as the line of best-fit curves significantly away from
the healthy line of best-fit when speeds are approximately over 1.5m/s. This suggests
that for patients at faster speeds, the medial compartment is not as loaded as would be
expected, and more crucially, this change in loading does not seem to be shifted into the
lateral compartment. There is a significant change in the peak internal flexion moments
for these faster speeds for patient individuals, and interestingly the peak internal flexion
value is a negative, suggesting an extension moment at these faster speeds. There is the
possibility that the moment changes in the sagittal plane, and probably in the frontal
plane too though not examined in this research study, are changing, causing this change
in the loading pattern.

Additionally, as a note for all graphs comparing healthy and patient data in this chapter
and when comparing to measured speed, it can be seen that the range on the x-axis is
far less for the patients compared to healthy individuals meaning that the lines of best
fit are offset, suggesting that patients produced less range of speeds than their healthy
counterparts. However, there are only a few data points that are around or above
2.5m/s from the healthy group, suggesting that this may be an isolated finding of this
research. Therefore, more analysis into the differences between the medial forces for
both healthy and patient individuals is examined in the discussion with more focus on
other possible biomechanical and physiological factors.
Figure 30 - Healthy and patient medial (adjusted $R^2 = 0.51$ and 0.08 respectively) and lateral loading (adjusted $R^2 = 0.01$ and 0.10 respectively), with a second order line of best fit

Now that stiffness between groups and for speed categories and measured speeds have been examined, the leg and knee stiffness can be investigated.

Figure 31 shows the leg stiffness for the patient group with the average displayed as a black cross. As can be observed from the general decrease (apart from the slight increase of the average line for the very fast walk for patients), the leg stiffness generally decreases as the speed category increases. However, it is unclear how the measured speed may have affected the leg stiffness results, so this is examined further in Figure 34.
Figure 31- Leg stiffness for the healthy and patient groups ($p = 0.007$ and $p = 0.068$ respectively)

Figure 32 shows the knee stiffness for the patient group with the average displayed as a black cross. Due to extremely large error bars changing the scaling on the y-axis significantly, it is difficult to examine the knee stiffness results visually and how they are affected by an increase in the speed category. Therefore, Figure 33 is derived from Figure 32 and shows a sub-section of the graph, to be able to examine values with quite similar ranges. Figure 33 demonstrates the same as for the healthy group, that as the speed category increases, the knee stiffness decreases slightly.
There are very large error bars for this figure; a very small value for the change in angle in the initial calculations causes a larger knee step stiffness and in turn, larger error bars. This is because, for some of the modelled angle results, some participants had no real change in angle from IC to halfway through the stance phase, which is the approximate area considered when deriving the knee stiffness values, so much so that the recorded knee angle for this period were either near zero or slightly decreased from a positive angle number during the period.

*Figure 32*- Knee stiffness for the healthy and patient groups (p= 0.237 and p= 0.036 respectively)
As mentioned above, it is therefore unknown how the actual measured speed could have affected these stiffness results. Therefore, Figure 34 displays leg stiffness with a second-order line of best fit for the measured speed. As can be seen, the data still behaves in the same way as it did when plotted through the speed category; as the speed increases, the leg stiffness decreases to a point (approximately 2m/s and 1.75m/s respectively), when the leg stiffness then increases slightly with an increasing speed. The adjusted R² suggests that the line of best fit for healthy individuals is of an excellent fit, whilst the line of best fit for patient individuals is of a good fit (Kissell and Poserina...
This graph is particularly featured for a comparison to Figure 35, as discussed below.

Figure 34 - Leg stiffness accounting for measured speed for healthy and patient participants, second order line of best fit (adjusted $R^2 = 0.67$ and 0.20 respectively)

Once again, accounting for measured speed, Figure 35 shows knee stiffness with a second line of best fit for the patient participants. The adjusted $R^2$ suggests that both lines of best fit are of a less predictable fit (Kissell and Poserina 2017). Commonly in this research, a second order line of best fit has been featured due to lack of linearity in the data points. However, the line of best fit behaves very linearly, suggesting that knee
stiffness decreases as the actual speed increases and Figure 35 demonstrates a similar behaviour to Figure 33 (the knee stiffness with the speed category).

This means that leg stiffness and knee stiffness for a patient population behave similarly to that for the healthy population, with the former being quadratic and the latter being linear in nature. However, as mentioned earlier, there is a concern as to how the knee stiffness result may have been influenced by unusual knee angles at IC. Due to concern as to whether this is from individual style or a modelling issue, leg stiffness is a measure that can be followed with more certainty for the rest of this study.

![Figure 35](image)

*Figure 35*- Knee stiffness accounting for measured speed for healthy and patient participants, second order line of best fit (adjusted R² = 0.05 and 0.05 respectively)
It is now of interest to directly plot the healthy and patient stiffness data together so that clearer trends can be established. Knee stiffness seems to maintain extremely similar values no matter what the speed of the walk, suggesting in a way that these knee stiffness graphs are just displaying very small fluctuations of a constant parameter. If knee stiffness is a constant parameter, it is pertinent for this chapter to examine leg stiffness more.

Figure 36 shows the healthy and patient leg stiffness results according to produced speed. The adjusted $R^2$ suggests that the healthy line of best fit is of an excellent fit whilst the patient line of best fit is of a good fit (Kissell and Poserina 2017). As mentioned previously, both sets of data behave like inverse parabolas; as measured speed increases, leg stiffness decreases to a point. For the faster speeds, leg stiffness then increases slightly. Interestingly, the speed at which this ‘turn’ occurs is lower for patients (approximately 1.75m/s) than for healthy participants (approximately 2m/s). Furthermore, the leg stiffness is similar for both healthy and patient groups for the slowest speeds until about 0.8 m/s, suggesting that for the lowest speeds, leg stiffness for a patient is similar to the leg stiffness they had before their injury. For all speeds between approximately 0.8m/s and 1.75m/s, the leg stiffness is almost linear for both groups, however, patients are consistently stiffer than their healthy counterparts in this speed range.

*Figure 36- Healthy and patient leg stiffness accounting for measured speed, with a second order line of best fit (adjusted $R^2= 0.67$ and 0.20 respectively)*
Now that loading and stiffness have been examined, the analysis of the co-contraction indices results can occur.

Figure 37 and Figure 38 demonstrate the five calculated co-contraction indices for the four speed categories for the healthy and patient groups; Figure 37 represents the first three, whilst Figure 38 represents the last two indices. Figure 37 shows large whiskers on the boxplots suggesting a large range in the data and all the medians suggest that CCIs act parabolic in nature; all increase to a point and then decrease at the fastest speeds and are therefore concave, apart from CCI Three for patients that acts in a convex nature. Also CCI One for healthy subjects increases linearly.

![Figure 37](image)

*Figure 37- Co-contraction indices for CCI One, CCI Two and CCI Three (paired indices) for the healthy and patient groups for the four walking categories (healthy p= 0.360, p= 0.676, p= 0.613 and patient p= 0.486, p= 0.211, p= 0.346)*

In Figure 38, CCI Five for patient subjects shows an extremely large range of data. The direction of median CCI results as the speed increases is not as clear for the grouped CCIs.
As has been done before, it is now of interest to examine the CCI results for the measured speed to see if there are any differences and to also explore the relationship between the CCI measures for both the healthy and patient groups. Figure 39 demonstrates the CCI results comparing the healthy and patient groups with a second-order line of best fit. The spread in the data points could show why no linear statistical significance such as for the ANOVA mentioned above could be found. Figure 39 shows that for the paired measurements, the patients are consistently more contracted than their healthy counterparts. The adjusted $R^2$ suggests that there is a less predictable fit for all lines of best fit, apart from healthy individuals in CCI One, where a good fit is established (Kissell and Poserina 2017). Interestingly for these first 3 CCI measurements, the lines of best fit visually act slightly similar for the two groups. This is true also for the grouped measurement CCI Five but interestingly not true for the grouped CCI measurement, CCI Four. Whilst the patients seem to be once again consistently higher for both grouped calculations, that is not true once the patient participants walk over 1m/s in the CCI Four measurement; the CCI linearly decreases across all measured speeds for the patient group. For CCI Four, this change in how the line of best-fit acts and the fact that the CCI is lower for the patients compared to healthy individuals over 1m/s when all other CCI measurements do not suggest that, is of particular interest.
From the direct findings from the last figure mentioned above, the three muscles that contribute to CCI Four can be explored in more depth. Figure 40 demonstrates the maximum percentage activation (a value between 0 and 1) for the tibialis anterior, gastrocnemius lateral head and soleus and provides a second-order line of best fit. The adjusted $R^2$ values suggest a less predictable fit for all lines of best fit (Kissell and Poserina 2017). It is worth noting each muscle was normalised to its maximum from the EMG data collected between the very start and end of the gait test, and each maximum value was derived across the whole of the stance phase. As can be seen, patients are consistently more activated for all three muscles compared to their healthy counterparts. Furthermore, the tibialis anterior and gastrocnemius lateral head seem to
demonstrate similar behaviours between groups. However, the soleus demonstrates different behaviour between the two groups at the same speed points, suggesting that an uninjured individual maintains similar maximum activation of the soleus no matter what walking speed is being performed. However, patients increase the maximum activation of the soleus as the speed increases.

Figure 40- Healthy and patient maximum contraction percentages for tibialis anterior, gastrocnemius lateral head and soleus, with a second order line of best fit (adjusted $R^2= 0.07/0.16$, $0.10/0.01$, and $0/0.12$ respectively)

* Adjusted $R^2$ produced a negative number which was adjusted to zero

Table 10 shows the mean co-contraction indices between healthy and patient groups, and the corresponding statistics, to complement the figures above. Any non-parametric data utilised the ART method before being examined with the same ANOVA. There was no statistical significance for the healthy or patient group CCI results across the four speeds using an ANOVA. Table 3 details the muscles used in each CCI measurement.
<table>
<thead>
<tr>
<th>Group</th>
<th>Factor</th>
<th>Speed 1</th>
<th>Speed 2</th>
<th>Speed 3</th>
<th>Speed 4</th>
<th>Mauchly’s Test of Sphericity</th>
<th>ANOVA, Sphericity Assumed, 9 subjects</th>
<th>ANOVA, Greenhouse-Geisser, 9 subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENT</td>
<td>CCI One</td>
<td>0.72 (±0.31)</td>
<td>0.72 (±0.27)</td>
<td>0.83 (±0.35)</td>
<td>0.75 (±0.45)</td>
<td>p= 0.177*</td>
<td>p= 0.486</td>
<td>-</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.51 (±0.26)</td>
<td>0.54 (±0.22)</td>
<td>0.56 (±0.21)</td>
<td>0.65 (±0.19)</td>
<td>p= 0.403*</td>
<td>p= 0.360</td>
<td>-</td>
</tr>
<tr>
<td>PATIENT</td>
<td>CCI Two</td>
<td>0.68 (±0.25)</td>
<td>0.85 (±0.29)</td>
<td>0.84 (±0.30)</td>
<td>0.73 (±0.43)</td>
<td>p= 0.640*</td>
<td>p= 0.211</td>
<td>-</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.39 (±0.17)</td>
<td>0.50 (±0.16)</td>
<td>0.48 (±0.19)</td>
<td>0.41 (±0.26)</td>
<td>p= 0.264*</td>
<td>p= 0.676</td>
<td>-</td>
</tr>
<tr>
<td>PATIENT</td>
<td>CCI Three</td>
<td>0.43 (±0.47)</td>
<td>0.42 (±0.40)</td>
<td>0.44 (±0.53)</td>
<td>0.40 (±0.54)</td>
<td>p= 0.374*</td>
<td>p= 0.346</td>
<td>-</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.27 (±0.18)</td>
<td>0.25 (±0.15)</td>
<td>0.29 (±0.17)</td>
<td>0.23 (±0.15)</td>
<td>p= 0.239*</td>
<td>p= 0.613</td>
<td>-</td>
</tr>
<tr>
<td>PATIENT</td>
<td>CCI Four</td>
<td>0.56 (±0.37)</td>
<td>0.56 (±0.26)</td>
<td>0.47 (±0.36)</td>
<td>0.44 (±0.28)</td>
<td>p= 0.339*</td>
<td>p= 0.750</td>
<td>-</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.41 (±0.21)</td>
<td>0.44 (±0.21)</td>
<td>0.53 (±0.24)</td>
<td>0.48 (±0.32)</td>
<td>p= 0.250*</td>
<td>p= 0.330</td>
<td>-</td>
</tr>
<tr>
<td>PATIENT</td>
<td>CCI Five</td>
<td>1.29 (±0.66)</td>
<td>1.60 (±0.44)</td>
<td>1.55 (±0.59)</td>
<td>1.33 (±0.77)</td>
<td>p= 0.483*</td>
<td>p= 0.146</td>
<td>-</td>
</tr>
<tr>
<td>HEALTHY</td>
<td></td>
<td>0.86 (±0.38)</td>
<td>0.88 (±0.22)</td>
<td>0.90 (±0.21)</td>
<td>0.86 (±0.22)</td>
<td>p= 0.025</td>
<td>-</td>
<td>p= 0.913</td>
</tr>
</tbody>
</table>

* Statistically significant
† Data which used the ART method

Now that the simple biomechanical measures such as angles and moments have been produced, and more complex measurements such as loading, stiffness and CCI information have been analysed in isolation using ANOVA to study this categorical data, the interaction between some of these more complex measures needs to be investigated. Linear regression was used to investigate the link between loading and either stiffness or CCI. Stiffness could be compared with CCI but as both are considered inputs, they are not compared in this way. As linear regression considers continuous data, the speed category or the actual speed was not considered. Instead, inputs of either stiffness or CCI and outputs of loading at different speeds were considered, with all the results being added as one column vector. This means no adjusting for actual speed was needed as it was in previous figures; if the data were to have been moved to the actual speed order there would have been no effect on the linear regression results. This means that linear regression is a robust measure to analyse two variables against each other without directly considering the effect of the speed. This combination of analysing the categorical data through ANOVA and continuous data through linear regression is formed to investigate the data from both viewpoints.
Therefore, the interaction of the two inputs (CCI or leg stiffness) and the one output (loading; whether that is medial or lateral loading) required analysing for the healthy and patient groups. Table 11 shows the linear regression results for these relationships, and section 4.2.6 expands on the meaning of each of the column titles. There were limited statistically significant results for this comparison, and all for healthy individuals only. There is statistical significance for lateral loading based on CCI Two ($F(1,34)= 4.228$, $p= 0.047$) with an $R^2$ of 0.111. Additionally, there was statistical significance for lateral loading based on CCI Five ($F(1,34)= 4.339$, $p= 0.045$) with an $R^2$ of 0.113. Finally, there was statistical significance for medial loading based on leg stiffness ($F(1,34)= 5.915$, $p= 0.020$) with an $R^2$ of 0.148. The three aforementioned $R^2$ values suggest a less predictable fit for the lines of best fit between the two variables (Kissell and Poserina 2017). No statistical significance could be established from the linear regression analyses for patient individuals.
### Table 11: Linear regression results for healthy and patient participants

<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>Regression df</th>
<th>Residual df</th>
<th>F change</th>
<th>Sig F change</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Medial loading based on CCI One</td>
<td>1</td>
<td>34</td>
<td>0.000340</td>
<td>p= 0.985</td>
<td>0.000010</td>
</tr>
<tr>
<td>Healthy Medial loading based on CCI One</td>
<td>1</td>
<td>34</td>
<td>1.190</td>
<td>p= 0.283</td>
<td>0.034</td>
</tr>
<tr>
<td>Patient Lateral loading based on CCI One</td>
<td>1</td>
<td>34</td>
<td>0.017</td>
<td>p= 0.896</td>
<td>0.001</td>
</tr>
<tr>
<td>Healthy Lateral loading based on CCI One</td>
<td>1</td>
<td>34</td>
<td>0.272</td>
<td>p= 0.605</td>
<td>0.008</td>
</tr>
<tr>
<td>Patient Medial loading based on CCI Two</td>
<td>1</td>
<td>34</td>
<td>0.009</td>
<td>p= 0.925</td>
<td>0.000267</td>
</tr>
<tr>
<td>Healthy Medial loading based on CCI Two</td>
<td>1</td>
<td>34</td>
<td>0.032</td>
<td>p= 0.859</td>
<td>0.001</td>
</tr>
<tr>
<td>Patient Lateral loading based on CCI Two</td>
<td>1</td>
<td>34</td>
<td>0.177</td>
<td>p= 0.677</td>
<td>0.005</td>
</tr>
<tr>
<td>Healthy Lateral loading based on CCI Two</td>
<td>1</td>
<td>34</td>
<td>4.228</td>
<td>p= 0.047*</td>
<td>0.111</td>
</tr>
<tr>
<td>Patient Medial loading based on CCI Three</td>
<td>1</td>
<td>34</td>
<td>0.608</td>
<td>p= 0.441</td>
<td>0.018</td>
</tr>
<tr>
<td>Healthy Medial loading based on CCI Three</td>
<td>1</td>
<td>34</td>
<td>0.060</td>
<td>p= 0.809</td>
<td>0.002</td>
</tr>
<tr>
<td>Patient Lateral loading based on CCI Three</td>
<td>1</td>
<td>34</td>
<td>2.925</td>
<td>p= 0.096</td>
<td>0.079</td>
</tr>
<tr>
<td>Healthy Lateral loading based on CCI Three</td>
<td>1</td>
<td>34</td>
<td>0.528</td>
<td>p= 0.472</td>
<td>0.015</td>
</tr>
<tr>
<td>Patient Medial loading based on CCI Four</td>
<td>1</td>
<td>34</td>
<td>0.977</td>
<td>p= 0.330</td>
<td>0.028</td>
</tr>
<tr>
<td>Healthy Medial loading based on CCI Four</td>
<td>1</td>
<td>34</td>
<td>0.154</td>
<td>p= 0.697</td>
<td>0.005</td>
</tr>
<tr>
<td>Patient Lateral loading based on CCI Four</td>
<td>1</td>
<td>34</td>
<td>1.778</td>
<td>p= 0.191</td>
<td>0.050</td>
</tr>
<tr>
<td>Healthy Lateral loading based on CCI Four</td>
<td>1</td>
<td>34</td>
<td>3.807</td>
<td>p= 0.059</td>
<td>0.101</td>
</tr>
<tr>
<td>Patient Medial loading based on CCI Five</td>
<td>1</td>
<td>34</td>
<td>0.241</td>
<td>p= 0.627</td>
<td>0.007</td>
</tr>
<tr>
<td>Healthy Medial loading based on CCI Five</td>
<td>1</td>
<td>34</td>
<td>0.177</td>
<td>p= 0.677</td>
<td>0.005</td>
</tr>
<tr>
<td>Patient Lateral loading based on CCI Five</td>
<td>1</td>
<td>34</td>
<td>0.024</td>
<td>p= 0.878</td>
<td>0.001</td>
</tr>
<tr>
<td>Healthy Lateral loading based on CCI Five</td>
<td>1</td>
<td>34</td>
<td>4.339</td>
<td>p= 0.045*</td>
<td>0.113</td>
</tr>
<tr>
<td>Patient Medial loading based on leg stiffness</td>
<td>1</td>
<td>34</td>
<td>0.935</td>
<td>p= 0.340</td>
<td>0.027</td>
</tr>
<tr>
<td>Healthy Medial loading based on leg stiffness</td>
<td>1</td>
<td>34</td>
<td>5.915</td>
<td>p= 0.020*</td>
<td>0.148</td>
</tr>
<tr>
<td>Patient Lateral loading based on leg stiffness</td>
<td>1</td>
<td>34</td>
<td>0.015</td>
<td>p= 0.904</td>
<td>0.000435</td>
</tr>
<tr>
<td>Healthy Lateral loading based on leg stiffness</td>
<td>1</td>
<td>34</td>
<td>0.703</td>
<td>p= 0.407</td>
<td>0.020</td>
</tr>
</tbody>
</table>

* Statistically significant

The lack of statistical significance of these last results may be because the two variables under consideration do not have a linear relationship. CCI results were not strong statistically in Table 10, and a link between CCI and stiffness cannot be established. Knowing that data plotted against the actual speed displays clearer trends than for the speed categories, recategorizing the speed groups according to the produced speed could suggest clearer trends when comparing one factor against another. There also could be other mechanisms at work which have not been foreseen, like how the leg stiffness decreased with speed.

In summary, this research chapter aimed to collect biomechanical measures for healthy and patient individuals including the four measures to define dynamic knee joint
stability (namely knee stiffness, leg stiffness, CCI calculations and knee joint contact loading). From there, this research chapter aimed to compare healthy and patient results to obtain an understanding of the difference in movement strategies for those that had received an ACLr. The statistical significance of the differences between the two groups was variable. When examining the graphs, very different strategies could be observed.
4.4 Discussion
4.4.1 Overview
The purpose of this study was to establish how gait and changes in walking speed affect knee function for healthy individuals and individuals with ACLr. Dynamic knee measures such as stiffness, co-contraction indices, knee contact loading and biomechanical indicators were used to differentiate between healthy and patient individuals. Statistically, the Shapiro-Wilk test, a two-tailed independent t-test, and a repeated measures ANOVA were used. All t-tests and ANOVAs mentioned in this discussion are independent and repeated measures respectively unless mentioned otherwise.
Initially, the normality of the subject characteristics of both groups was tested using the Shapiro-Wilk test, and no statistical significance was found, meaning all the variables satisfied normal distribution. When comparing the subject characteristics between groups using a t-test, no statistical significance could be established, suggesting that the groups were well-matched.
Considering the biomechanical results next, a Shapiro-Wilk test was conducted again. Where statistically significant results were found, meaning normal distribution was not found, the ART method was used to align the data so that it could be used by the same ANOVA procedure (Conover and Iman 1981; Mansouri 1998; Leys and Schumann 2010). The ANOVA compared the two groups at all four speed categories. Tests of between-subject effects for the group showed statistical significance for the peak knee flexion angle (p= 0.010), and peak internal knee flexion moment (p= 0.005). Statistical significance was also found for total mean contact loading forces for the medial portion (p= 0.001) and the lateral portion (p= 0.019). Additionally, statistical significance was found for CCI One (p= 0.019), CCI Two (p= 0.001) and CCI Five (p= 0.010).
Considering the ANOVA within-subject effects for speed, there was statistical significance for the average speed (p< 0.001) and peak knee flexion angle (p< 0.001). There was also statistical significance for peak internal knee flexion moment (p= 0.041), average maximum ground reaction force (p= 0.042) and average y displacement change in the centre of mass (p< 0.001). There was also statistical significance for average leg stiffness (p= 0.001), total mean contact loading forces medial portion (p= 0.001) and lateral portion (p< 0.001).
The ANOVA within-subject effects for the speed and the group found statistical significance for peak internal knee flexion moment (p= 0.003) and CCI Four (p= 0.039).
A small amount of statistical significance was found for the linear regression results for the healthy group, lateral loading based on CCI Two (p= 0.047), lateral loading based on CCI Five (p= 0.045), and medial loading based on leg stiffness (p= 0.020). No statistical significance was found in the linear regression results for the patient group.
Analyses using ANOVA consider linear behaviours, however, it is clear from some of the graphs in the results section, that some quite parabolic behaviours are occurring.

4.4.2 Hypotheses
This hypotheses section discusses the statistical significance of the variables and whether the hypotheses are accepted or rejected. More discussion on the findings from these results is found in the subsequent discussion sections.

Considering basic biomechanical variables at increasing walking speeds between healthy and patient participants, the null hypothesis can only be rejected for the peak internal knee flexion moment, where the within-subject effects for group and speed were considered (p= 0.003). Therefore there is a significant difference between healthy and patient individuals for the internal flexion moment at increasing speeds.

The null hypothesis cannot be rejected for the average speed, the peak knee flexion angle, the average maximum ground reaction force or the average y displacement change in the centre of mass as no statistical significance for within-subject effects for speed and group was found. However, these basic biomechanical variables did produce some statistical significance for either between-subject effects for group or within-subject effects for speed and these are seen in Table 7.

Considering only the co-contraction at increasing speeds around the knee joint, the null hypothesis can only be rejected for CCI Four; an ANOVA between the healthy and patient group interaction demonstrated that CCI Four had statistical significance for within-subject effects for group and speed (p= 0.039). Visually, the co-contraction for CCI Four lowered more for the patient group than for the healthy group in Figure 39, which was an unexpected result. For CCI One, Two, Three, and Five no statistical significance with an ANOVA for group and speed could be found.

Additionally, the null hypothesis cannot be rejected for increasing speeds with knee or leg stiffness either. Considering knee stiffness, no statistical significance for within-subject effects (for group with speed) could be found between the healthy and patient groups. Examining each group individually in Table 9, there is statistical significance for patients only (p= 0.036). In this research, this is the only statistically significant result found between changing speeds and knee stiffness. Considering leg stiffness and increasing speeds, the null hypothesis is not rejected. There are no within-subject effects with an ANOVA for speed and group between the healthy and patient individuals nor for the group between-subject effects. However, examining each group separately in Table 9, there was statistical significance for the healthy individuals only (p= 0.007), which is also represented visually as a decrease in leg stiffness (to a point) as speed increases as seen in Figure 36. Interestingly for leg stiffness, there was statistical
significance with the ANOVA examining within-subject effects considering speed only 
(p= 0.001). This suggests a more complex behaviour for leg stiffness, more related to 
changes in speed than differences between healthy and patient individuals.

Considering knee joint loading and increasing speeds, the null hypothesis cannot be 
rejected. For the most central statistical test, the within-subject effects for speed and 
group there was no statistical significance. Statistical significance was however found for 
the medial and lateral portions for within-subject effects for speed only (p= 0.001 and p< 
0.001 respectively) and for the medial and lateral portions for between-subject effects 
for group (p= 0.001 and p= 0.019 respectively). Therefore medial and lateral loading is 
significantly lower and higher respectively for patients compared to healthy individuals, 
not considering changes in speed. Significance was found in the individual group 
analyses for the healthy group (both p= 0.008) and the patient group in both the medial 
and lateral portions (p= 0.002 and p= 0.003 respectively). This was supported visually by 

knee contact loading for both compartments increasing to a point as the measured 
speed increases as seen in Figure 30.

4.4.3 Biomechanical Discussion

As discussed in the literature in section 2.1.3.1 and as seen in Figure 2, it was proposed 
that there was a four-part relationship to represent the dynamic stability of a joint. The 
leg stiffness, co-contraction, joint stiffness and joint loading can unite to create a full 
picture of how the knee joint maintains its stability during activity. The inputs of a 
motion could be identified as the leg stiffness, co-contraction and joint stiffness, 
whereas the outcome or output was the impact on the joint loading.

However, during the analysis of the results, there was some concern with the knee 
stiffness results. Occasionally, there were unusual knee angles at IC, with the knee angle 
either being near zero or decreasing very early during the first half of the stance phase. 
This meant that the change in angle could often be a negative value, in turn meaning 
that the knee stiffness value for an individual in a certain speed category was sometimes 
a negative, however finding the mean for a walking category where the individual knee 
stiffnesses were positive or negative was still reasonable. It was unknown when these 
results were collected why this negative change in an angle was occurring as it did not 
appear to be demonstrated in the literature around calculating knee stiffness, though it 
was more likely an individual’s style than a model issue. Additionally, the leg stiffness 
gained statistical significance in Table 7 whilst the knee stiffness did not, and the knee 
stiffness has been the least statistically significant measure in this research. These 
unusual knee stiffness results appear to be from an individual’s gait style; the protocol 
was adhered to in the same way by the author. Furthermore, a few articles in previous
literature have demonstrated this lack of the smaller first peak in the flexion angle and lack of range of variation for the knee in the first half of stance, and this is not just a slow speed-related issue (Morais Filho et al. 2010; Fukuchi et al. 2019b; Ong et al. 2019; Robert-Lachaine et al. 2020). Additionally, there is recent precedence for knee stiffness being very similar at different increasing speeds as found in this study and also not achieving statistical significance, suggesting an interesting thought that knee stiffness does not change with different speeds but is regulated to keep constant through the changing of the variables that comprise the knee stiffness calculation (Akl et al. 2020). Knee stiffness appeared quite similar across changing speeds, suggesting some modulation was occurring to maintain knee stiffness results. However, leg stiffness did appear to change graphically at changing speeds, for both groups too, and at different rates too, as can be observed by different ‘turning points’ in the leg stiffness graph plotted against actual speeds (Figure 36). Figure 41 shows the statistical significance for both the healthy and patient data respectively, and considers the four-part relationship between leg stiffness, knee stiffness, co-contraction and joint loading to form dynamic joint stability. As can be seen, the most statistical significance for the healthy group was for medial loading and leg stiffness, both independently and interlinked. There was however some statistical significance for lateral loading, between CCI Two and lateral loading, and CCI Five and lateral loading for the healthy group. There was less statistical significance for the patient group, for knee stiffness, and the lateral and medial load. Whilst correlation quantifies the strength of the relationship, linear regression establishes the relationship as an equation (Bewick et al. 2003) and was deemed more pertinent to this research.
Therefore, analysing the relationships between loading (both compartments), leg stiffness and co-contraction in general, there appears to be a relationship for healthy individuals. As speed increases, leg stiffness decreases and is linked to an increase in the medial loading. As lateral loading increases, it appears to be linked to CCI Two and Five, though the outcome of these CCIs is not known. For patients, the link between the dynamic knee stability factors is not known.

For the highest speeds, leg stiffness is slightly higher, and resultant loading is high. These parabolic results for leg stiffness in Figure 34 appear to change when speeds go from fast to very fast. While these speeds are not fast enough to be classed as running speed, this change could be because the participants started to demonstrate almost a slow jog rather than a fast walk, which is possible at a similar speed to each other (Keller et al. 1996).

When understanding why more statistical significance was gained for healthy individuals, two specific questions from Figure 41 arise; (1) why the medial loading increases whilst the leg stiffness decreases, and (2) why are CCI Two and CCI Five statistically significant when linked to lateral loading.

Considering the first query, and validity of the methods, healthy individuals analysed for this study are representative of a wider population, with normality of all their subject characteristics. Additionally, some basic and popularly analysed biomechanical results displayed statistical significance; the average speed in each speed category (p< 0.001) and the peak knee flexion angles (p< 0.001). Also, the independent measures that come together to form leg stiffness were also statistically significant, the average maximum GRF (p= 0.006) and the average y displacement change of CoM (p< 0.001). Examining
these two factors shows that whilst both increase as speed increases, the average y displacement must increase more as it is the denominator of the sum meaning that the resultant leg stiffness decreases as speed increases. Whilst it is reasonable to assume that the GRFs increase as the speed increases (Keller et al. 1996), there is also legitimacy to the average y displacement of CoM increasing as walking speed increases (Orendurff et al. 2004). While medial loading is being linked to leg stiffness here, knee stiffness has not been linked to medial loading due to knee stiffness providing almost constant results at different speeds.

As leg stiffness considers the leg being analysed under maximum compression, the point of interest is between IC and midstance (Bishop et al. 2006). However, the other foot is in contact with the ground at the start of this period, hence the body is in a ‘double support’ phase. Some leg stiffness analyses have separated the double and single support sections for analyses, defining each leg with a spring (Geyer et al. 2006), or a spring and damper (Kim and Park 2011). Those that separated the ‘leading’ leg during single support did indeed establish that leg stiffness increases for increases in speeds (Kim and Park 2011). As the leg stiffness calculation does not analyse the effect of this double support phase from the other leg, a simplistic way to check for any effects could be to plot the GRF against the vertical CoM for both landing and push-off and if both lines corroborate, a linear spring behaviour is occurring (Brughelli and Cronin 2008). For those that analysed leg stiffness in the same way as this research, leg stiffness decreased for an increase in speed (Akl et al. 2020) as was found in this research. It was also argued that whilst the leg stiffness decreases, this is because the denominator is increasing in the leg stiffness summation (Akl et al. 2020). As long as both the GRF and vertical displacement of CoM are increasing with increasing speeds, which is true for this study, then leg stiffness in fundamental components is in fact, increasing (Akl et al. 2020).

Therefore, it can now be understood why the medial load is increasing with an increase in speed whilst the leg stiffness is decreasing; because the latter is actually increasing in fundamental terms, which is an important point to learn in this research. Interestingly, it was also suggested that leg stiffness is not linked to the lower limb joint stiffnesses during initial loading (eccentric contraction) and that knee stiffness works independently, all of which could support the other findings in this research (Akl et al. 2020).

Considering the second query as to why specifically CCI Two and CCI Five had significance with the lateral loading (through linear regression), the muscles comprising each CCI measurement should initially be examined. If CCI Two and CCI Five are compared to find the muscles in common, both the gastrocnemius lateral head and
vastus lateralis are in both measurements (Souissi et al. 2017; Mohr et al. 2018). Which muscle is of interest then needs to be established. Gastrocnemius lateral head is in CCI Two, CCI Four and CCI Five (Souissi et al. 2017; Mohr et al. 2018), whilst vastus lateralis is in CCI One, CCI Two, CCI Three and CCI Five (Richards and Higginson 2010; Souissi et al. 2017; Jones et al. 2018; Mohr et al. 2018). If just one of these muscles was significant, the other CCI measurements covered by a specific muscle would have been significant. As both muscles are only covered by CCI Two and CCI Five, it is this combination of these two muscles which have produced the significance and needs examination. Analysing previous literature about these two muscles during gait suggests that there should be more of a medial activation effect than a lateral one as seen in these results (Heiden et al. 2009). Interestingly, activation becomes more lateral in patients with OA who are trying to reduce their eKAM (Heiden et al. 2009).

Gait on a treadmill could change the step width, and if increased, would increase the eKAM and could explain the increased lateral activation. It is therefore proposed that the next chapter will investigate if step width changes on a treadmill, and if an eKAM change also occurs. If lateral gastrocnemius and vastus lateralis are indeed more activated due to offsetting an increase in eKAM (Heiden et al. 2009), then this could explain the link to the lateral loading.

4.4.4 Gait Speed Implications
The speed categories for the patients had a minimum of 34% speed-on-speed increase, the same minimum as for the healthy participants, and tests of within-subject effects (in the overall ANOVA) showed that for speed alone, statistical significance was found (p< 0.001). Patients were on average slower than the healthy average speed (though within-subject effects for speed and group did not signify that this was statistically different).

Upon visually examining the effect of speed on each group, as speed increases, total mean medial forces increase linearly for healthy people (p= 0.008), whilst they initially increase then decrease parabolically for patients (p= 0.002), suggesting a more complex strategy for patient participants. There were between-subject effects between the groups (p= 0.001) or within-subject effects for the different speeds (p= 0.001). However, total mean medial forces do not establish statistical significance for within-subject effects for speed and group. There is therefore not enough difference between the groups, and at the different speeds, to distinguish them apart. It can however be agreed that there is a statistically lower loading of the medial portion for the patients compared to healthy individuals (p= 0.001), which is explored more below in section 4.4.7. Lateral forces increased linearly for both healthy (p= 0.008) and patients (p= 0.003), though patients always show higher total mean forces at the same speed categories than
healthy individuals (p = 0.019). There was significance for the between-subject effects of group (p = 0.019), and for within-subject effects for speed (p < 0.001), but there was no significance for within-subject effects for speed and group. This suggests that comparable to the medial loading, insufficient difference in lateral loading was found between groups at different speeds.

Leg stiffness visually decreases parabolically for both healthy (p = 0.007) and patient groups (p = 0.068 therefore no statistical significance) as speed increases. There is statistical significance of within-subject effects for speed (p = 0.001) but not for speed and group. There was also no statistical significance for between-subject effects for group. Leg stiffness appears more statistically linked to changes in speeds than groups. Finally, there is a lot of variation between different CCI calculations for the healthy and patient groups, however, the line of best fit for the healthy and patient groups seem to act visually similarly between the two groups for each CCI measurement, apart from CCI Four. Whilst the CCI results demonstrate some interesting details, they should be interpreted with extreme caution due to the lack of statistical significance throughout. The analysis of healthy or patient groups alone for the different CCI measurements over the four speed categories did not show any statistical significance. Additionally, tests of between-subject effects for the groups showed significance for CCI One (p = 0.019), CCI Two (p = 0.001) and CCI Five (p = 0.010). The most central test, the test of within-subject effects for speed and group, showed significance for CCI Four alone (p = 0.039). It could be deduced that the similarities between the two groups for the CCI measures suggest that the patient group does not seem to have sustained an effect from their ACLr into their activation patterns yet. Though CCI Four is of particular interest due to its highly different nature with the lines of best fit for each group. This is investigated further in the co-contraction section 4.4.6.

4.4.5 The Role of Stiffness

It has been found that more statistical significance was established for leg stiffness than knee stiffness. This is probably due to the different angles displayed at IC in the modelled results of some individuals influencing the knee stiffness calculation as mentioned in section 4.4.3. This could also possibly suggest that individuals maintain similar knee stiffness values at different speeds, therefore knee stiffness is not speed-dependent, which in itself is a finding. This lack of variation could explain the lack of statistical significance. Interestingly, there was statistical significance for average knee stiffness in patients (p = 0.036), which could suggest that for patients, this regulation of knee stiffness is interrupted, though statistical significance was not found when comparing the groups with between-subject effects. Due to more statistical significance
with the leg stiffness measure, it does suggest that leg stiffness can tell us more about limb control as a whole. Leg stiffness is lower at what could be defined as ‘normal’ speeds; when a person is asked to walk at a slow or very fast speed the leg stiffens. However, more data would be required from individuals walking at much faster speeds (without breaking into a run) to establish if this second-order deduction is indeed correct.

The term stiffness is subject to much debate and is often used to classify very different circumstances (Butler et al. 2003). In this study, leg stiffness is calculated within the first half of stance and assumes vertical leg stiffness, so does not consider any additional angular input; please see section 2.1.3.2 for more discussion and explanation as to the additional input from an angular component. This calculation is considered part of dynamic movement and has both passive and active elements to analyse under one calculation and therefore needed simplifying. It is also important to note that this study does not consider the leg stiffness in any other situation than a compression of the spring phase, as the second phase of landing would be considered a separate stiffness value (Latash and Zatsiorsky 2016). As leg stiffness is taken at one point in time and does not represent the human system in equilibrium, the term “quasi-stiffness” could be a more appropriate term (Latash and Zatsiorsky 1993). Crucially quasi-stiffness does not fully consider elastic forces and the associated accumulation of elastic energy (Latash and Zatsiorsky 1993). This could fundamentally explain why it is so difficult to link leg stiffness results with the co-contraction indices in this research as they are deduced in very fundamentally different ways. Quasi-stiffness only considers the linear portion of the moment-angle graph from heel strike to peak knee flexion angle which is approximately halfway through the stance phase. However, the CCI calculations for this research were derived between the initial heel strike, with an inbuilt EMD of 40ms subtracted off the heel strike time which is derived in Chapter 6, and the maximum moment time, without an EMD removed off the end of the sampled time. There are therefore subtle differences between the two sampled time periods. If quasi-stiffness does not fully consider elastic forces and elastic potential energy (primarily from the muscles), then the two factors cannot be compared; stiffness is an external mechanical representation, whilst CCI is an internal elastic-based representation. That is not to say, however, that both have produced interesting results that should be investigated further.

It could also be difficult to link leg stiffness to the CCI results because of large amounts of noise on the sampled voltages from equipment issues, and in turn, poor statistical significance of the CCI results. It is worth noting that as discussed in section 4.4.3, both
components to calculate leg stiffness do increase as the speed increases, suggesting that the leg stiffness is actually increasing, until faster walking speeds at least (Akl et al. 2020). Additionally, the lack of statistical significance between knee stiffness and knee contact joint loading has been found in previous research (Gustafson et al. 2019), further suggesting that it may be more beneficial to investigate the relation between leg stiffness and knee contact joint loading in the future instead.

Leg stiffness starts from extremely similar values for slow speeds for both healthy and patient participants, before both decreasing and returning to higher values for higher speeds. Whilst the patients seem to produce higher stiffness results for middle-range walking speeds compared to healthy individuals, GRFs and average y displacement change in the CoM are both extremely similar for the two groups. However statistical significance was only found within the healthy group for these two variables p= 0.006 and p< 0.001 respectively, and for the patient group average y displacement change in the CoM p= 0.003. This suggests caution is required once again, and no statistical significance was found for within-subject effects of speed and group for these two variables. It is possible that in a future study, a similar speed versus leg stiffness graph could be produced with the average y displacement change being lower for patients (as it is currently), which would suggest a stiffer spring behaviour. This can occur through patients being ‘less bouncy’ in gait, and travelling through less range of motion, such as found in this research that the peak knee flexion angle is more across the four speeds (p< 0.001). Whilst the lower values for peak internal knee flexion moments at angle time were not statistically significant for just patients, this measure was statistically significant for between-subject effects for group (p= 0.005), and within-subject effects for speed (p= 0.041), and for group with speed (p= 0.003). This suggests that the lower values for the peak internal knee flexion moments for the patients indicate that the knee is not ‘encouraged’ to straighten, possibly remaining more flexed throughout the gait cycle. This would, in an exaggerated concept, mean the patient individuals were slightly more crouched at the knee, leading to less of a change in the overall CoM.

4.4.6 Co-Contraction
As discussed in section 2.1.3.3, if the CCI measures were ranked from those that are activated the longest to the least of the stance phase of gait, the following order is found; CCI Five, CCI Four, CCI Two, CCI One and CCI Three. This however, studied the coverage of the stance phase activations and not the actual ‘normal’ activation amount. Additionally, if purely only considering the loading portion of the stance phase of the gait cycle, muscles that were activated across different portions of the stance phase are not preferable. This is because it makes it more difficult to isolate which section of
stance the muscle is contributing to; therefore, in this research, the vastus lateralis, gastrocnemius lateral head and the soleus do cross and are not as preferable. Unfortunately, each CCI calculation in this study features at least one of these less preferable muscles for the initial loading, so it is difficult to distinguish which calculation is more favourable.

No statistical significance was found for any of these 5 CCI measures for either the patient or healthy groups in Table 10, remembering that the co-contraction indices were measured using the linear analysis of an ANOVA, though they could display a more quadratic behaviour. However, for between-subject effects (for the group differences only and not considering the effect of the speed categories) there was significance for CCI One (p= 0.019), CCI Two (p= 0.001) and CCI Five (p= 0.010).

Considering within-subject effects (speed and group interaction) there was only statistical significance for CCI Four (p= 0.039). This means that something particularly different and important between the groups occurs in CCI Four, but it potentially has to be something that is not covered by the other calculations (as there would have been statistical evidence). Examining the muscle pairings and groups, it can be seen that the soleus is the only muscle not featured in the other calculations but is featured in CCI Four. As can be seen in Figure 40, there is a very different activation behaviour for the soleus in a patient group. It appears that CCI Four decreases in patients due to the increased activation of soleus for patients (an inverse relationship due to the nature of the CCI calculation), though this statistical variation was not established with CCI Four of the patient group in Table 10.

The change in the role of the soleus during gait between a healthy knee and post-ACLr needs to be examined. The ACL is known to prevent anterior tibial translation, where tibial translation can cause pathological gait (Dhillon et al. 2011). If the ACL has been damaged and reconstructed but not to a successful level of function, it is reasonable that the musculature of the lower limbs would want to prevent this anterior tibial translation. It has been found that the soleus in particular acts as an ACL agonist to resist this anterior tibial translation (Elias et al. 2003). It could be questioned how this happens as the soleus does not cross the knee joint. It occurs by causing a rotation of the tibia at the ankle and a shift of the tibia posteriorly at the knee (Elias et al. 2003). Previous research has shown this is true in vitro (Elias et al. 2003), for single-leg landing (Mokhtarzadeh et al. 2013) and sidestep cutting (Maniar et al. 2018). This could also be relevant to less extreme dynamic movements like gait too. This theory is supported by this research, where the soleus is more activated in individuals with ACLr due to increases in instability compared to intact ACL individuals at different speeds.
4.4.7 The Result on Loading
Contact force was chosen over contact pressure due to the more common use of this measurement by previous studies, and so that results could be compared to check the model was implemented correctly (Meireles et al. 2017; van Rossum et al. 2018; Van Rossom et al. 2019). Peak forces were not used in the analyses, as the peak is a point in time which may occur for a long or short period, and there is precedent for similar peak forces occurring when very different strategies occur (Dabbs et al. 2015; Rasnick et al. 2016). Therefore, the area under the forces graph from IC to the first half of the stance phase was integrated, as discussed in section 4.2.4.1.

With both groups, there was a total increase in loading of the tibiofemoral joint with an increase in speed (healthy medial p= 0.008 and lateral p= 0.008, patient medial p= 0.002 and lateral p= 0.003), which is supported by previous literature (Lerner et al. 2014). It cannot be deduced whether this is from the GRFs in Table 8 or the muscle forces via the CCI measures in Table 10. Interestingly, the peak internal knee flexion moment in this research acts differently at increasing speed categories between healthy and patient groups (p= 0.005). However statistical significance was not established for internal flexion moment for each group individually, suggesting that the flexion moment is not a good substitute measurement of tibiofemoral joint loading, even though net muscle moments have been linked to joint loading historically (Lerner et al. 2014).

Another interesting result for loading for patients was that both medial and lateral forces at the tibiofemoral joint had a higher first peak when compared to the second, with the reverse happening for the healthy participants. This could be explained due to the increased lack of stability of patient individuals during IC when the knee is required to be stable and ready for the increase in loading that it receives, causing increased muscle activation and in turn from this, even more loading to the knee joint. As the CCI results only consider approximately the first half of stance, the percentage muscle activation for three of the muscles investigated in this research, tibialis anterior, gastrocnemius lateral head and soleus can be seen to all be more activated for patients, supporting this theory (Figure 40).

As mentioned previously in section 4.4.2, medial loading behaves differently between groups, whilst lateral is extremely similar just offset in its values, with patients having higher lateral loading. However, medial and lateral loading results appear to produce the same amount of statistically significant results.

It is worth considering if these results were produced incorrectly because of a methodological issue. Markers and EMG on the subject were placed by the same person who had training in anatomy and muscle palpation. Instructions were given to the
participant, and data collection and processing were all done by one person (the author). The model was run in the same way for each participant with the usage of the same scripts in Matlab to ensure no small ‘typo’ errors occurred. The methods and the model were as rigorous and methodical as they could be to ensure the consistency of the results.

Therefore, with a focus on these loading results, it can be proposed that as speed increases, medial compartment loading increases to a point for patients, and then decreases slightly for faster speeds. This could be further confirmed by gathering data from patients walking at far higher speeds, somewhat a juxtaposition in itself as it is known those post-ACLr generally walk slower (Garcia et al. 2023). It can be seen that there are similar GRFs for both groups (Table 8), which directly impacts the knee loading results as the GRF is partially used to compute the contact forces in the model (Lenhart et al. 2015a). Two questions when comparing the patient group to the healthy group is where do these expected higher medial forces at higher speeds go for the patient group and why? It could be suggested that there is a shift to more lateral loading for patients. So shifting of forces more laterally by a patient individual could be a secondary outcome from these issues, which can be supported by the knowledge from this research that there are graphically higher lateral loads for patients. The lateral loading results did produce statistically significant results between the healthy and patient groups (p=0.019). However, it is also still possible as mentioned in section 4.3 that the changes in the frontal or sagittal moments at higher speeds for patient individuals could also be behind these changes.

Therefore, Figure 42 shows the summary of statistically significant healthy and patient results obtained to define dynamic knee stability and is placed here to aid in identifying the medial, and in turn lateral, loading queries discussed above.
At this point, any theories on change in loading for patients at higher speeds is a proposal alone due to the lack of statistical significance in some of the parameters now being explored. There are three solutions to this decrease in loading at higher speeds for patients. The first is that the forces shift from the medial to lateral components of the tibiofemoral joint at higher speeds. The second is that the muscles (and other soft tissues) around the knee absorb the extra medial forces through elastic dampener tissue behaviour. The third is theoretically, not enough data has been collected for patients at higher speeds, and with more data points, the parabolic behaviour turns into linear behaviour (the reverse could also be possible). A combination of all three solutions is also possible.

For point one, patients can load their knees more laterally to avoid further wear and damage to the medial portion of the tibiofemoral joint. Statistical significance was found for between-subject effects for group for the lateral contact loading ($p= 0.019$).

However, another plane could also be affecting the results. In Chapter 5 where there was a discussion on step width changes and knee rotation, the peak contact forces for the over ground condition had lower medial but higher lateral peak contact forces compared to the treadmill condition (this pattern of having higher lateral but lower medial forces is similar to patients in this research study). The over ground condition also had higher peak internal rotation angles compared to the treadmill condition. As established in Chapter 5, the soleus acts as an ACL agonist to ensure that the tibia remains in a more posterior position and contracts eccentrically in early stance to aid...
plantarflexion. Soleus also contracts to decelerate tibial internal rotation (Mulligan 2012). As known from this study and discussed in section 4.4.6, soleus is more activated for the patient group. This could be because soleus is attempting to reduce more tibial internal rotation for the patient group, in effect producing a similar behaviour seen in Chapter 5 for the over ground condition. If the patient group is therefore less internally rotated due to the efforts of the soleus to contract more to deaccelerate the rotation, the tibia is less anterior for the patient group than for the healthy group, affecting the loading pattern on the medial and lateral compartments. It is known that a more varus knee, produced by more internal tibial rotation, causes more medial loading (Van Rossom et al. 2019), which supports the findings of higher medial loading for healthy compared to patient individuals in this research study. Additionally, there is a suggestion that when one compartment decreases in load, the other increases (Van Rossom et al. 2019), though this in itself does not explain why at faster walking speeds, the medial compartment offloads in a non-linear pattern whilst the lateral compartment increases its load linearly.

For point two, it is also reasonable that some forces can be absorbed into the knee tissues as material dampening, not mechanical dampening; it has been previously established that material dampening is required to dissipate increases in energy from larger amounts of momentum (Lin and Rymer 2000). Increased dampening relating to higher momentum can question why there are changes, particularly at these higher speeds. Additionally, it would be reasonable to consider the forces must be absorbed through activated muscles and not passive ones, as the CCI values are higher for patients, although between-subject effects for group did not always gain statistical significance. However, if the muscles are more activated to aid the stability of the joint, they may be receiving this dampening effect either passively or actively. The muscles could become more activated at higher speeds as a response to this increased load, with the muscles possibly acting isometrically to also ‘stiffen’ the leg spring as a whole. If this is the case, the CCI is somewhat an output as well as simultaneously being an input.

Finally, for point three, there can still be a lack of power to the study which can affect the line of best fit of the data, meaning that with more data points, both patient and healthy data could either both be linear or both be quadratic for both compartments. The healthy and patient groups act very differently, with patients having less medial and more lateral loading compared to their healthy counterparts. Additionally, patients appear to increase in leg stiffness with increasing speed, but their overall leg stiffness value is less than for healthy participants (meaning patients are stiffer). Additionally, patients appear to be more co-contracted across all five CCI measurements apart from a
deviation in CCI Four, though this is due to a higher relative contribution of the soleus for patient individuals.

4.4.8 Knee Injury and Loading Changes
The results in this chapter suggest that even early after injury, different patterns of stiffness, speed, and CCI are used to manage gait and loading. However, the most central statistical test used in this chapter, a repeated measures ANOVA within-subject effects for speed and group, did not yield many statistically significant results. There is the possibility that the different walking speeds were not challenging enough, or that the study was underpowered. This could mean the results of this research are more subtle.

This research study suggests that whilst co-contraction patterns may not be extensively statistically significantly different (apart from soleus), they are higher for patients compared to healthy individuals (CCI One p= 0.019, CCI Two p= 0.001 and CCI Five p= 0.010). Additionally, there is the interpretation that leg stiffness is actually increasing for patients at mid-range speeds as the individual components of leg stiffness increase. If Figure 36 is then inverted around the y-axis, the average leg stiffness is lower for patients than for healthy participants. There is the possibility to suggest (though not significant) that there is an early coping mechanism to avoid destructive loading on the compartments of the knee.

4.4.9 Limitations
Those who increase just stride length when increasing in speed, or a combination of increased stride length and increased cadence, tend to display large increases in all joint moments (Ardestani et al. 2016). Those who just increase their cadence to increase speed do not tend to experience such significant increases in their joint moments (Ardestani et al. 2016). This is an important note, though is difficult to analyse in this study, due to the sample of a fixed number of steps per speed category.

Examination of the speeds produced by each individual in each walking category shows that there was not always consistency by an individual. Participant 25 from the healthy group had a slower ‘very fast’ speed than their ‘fast’ speed, and Participant 06 from the healthy group had a similar speed for ‘slow’ and ‘normal’ speeds. These issues may be from fatigue, lack of understanding (despite a full description and time given to each participant to experience the treadmill) or simply wanting to complete the task quicker.

If this study were to be expanded upon in the future, the speed categories should be more defined for the volunteers so that they perform each walk category within a certain tolerance. Verbal prompting could be used if they go too slow or fast, to reduce
the effect of the speed category shifting between individuals, and even within an individual’s performance. Clearly though, this is also problematic as giving participants their own self-paced choice of speed aids the participant to produce more natural biomechanical data. Furthermore, treating speed as a discrete measure and a continuous measure simultaneously can be confusing. The speed category made a discrete measure which aided the statistical analyses, however many of the graphs utilised speed in a continuous form.

Two patient participants had two-sided injuries and though they did not appear to struggle, or comment on struggling, it is worth discussing. Whilst it may have been more reasonable to investigate only those with one-sided injury, there were not the numbers required from volunteers to remove these participants from the analysis. It is a common issue when one leg gains an injury that the other side overcompensates and can become injured in time (Garcia et al. 2023). For this reason, it was important to keep these individuals within the analysis; it is more representative of what occurs in real life. Interestingly, one study with participants who were ACLd and then had a reconstruction, have self-reported dramatic improvements in control of the knee, though they did not produce such improvements in measured tasks (Smale et al. 2019). Hence it may be more important to consider the psychological perceptions of those who have received ACLr in the future (Du et al. 2022).

Statistically, the sample size of 18 participants did not reach the recommended 19 participants required for this research, with the lack of patient participation as the main cause. This research study was underpowered and due to the unpredictable nature of research, could not have been anticipated or resolved. More statistically significant relationships could have been established with a larger sample size. A post hoc power analysis could have been performed at this point, which is often performed when there are some non-significant results, however performing a power analysis would have been analytically misleading (Zhang et al. 2019). However, some statistical significance was found in this study, and therefore those results successfully overcame a Type II error (Peterson and Foley 2021).

During analysis of the data, typically a second-order line of best fit was assigned to the data, due to the lack of appearance of a linear data alignment. This meant that one ‘turn’ in the line of best fit would be present. This discussion has mentioned parabolic behaviour, and the second-order line of best fit could have accidentally caused this to appear. However, gait by its very nature is a parabolic activity, and the results over a range of speeds could also give rise to this behaviour, especially when gait approaches jogging or running.
The musculoskeletal model used in this research cannot be readily adapted to model injured patients to represent the possibly still present changes of a less functional ACL. Therefore, further investigations are required to analyse the results of the model to understand its muscle forces validity. As the muscle forces are produced from the balance between the external moments of the knee and the calculated internal muscle moments, the musculoskeletal model does not give the option to specify the level of co-contraction in an individual. It is therefore imperative to understand what extent the contact forces that result from the muscle force distribution patterns mirror the observed CCI values. This can be done by comparing the collected EMG of the healthy and patient groups to the modelled muscle activation results and will be investigated in more depth in Chapter 6.

Some medial and lateral force plots for individuals displayed some lateral offloading. It would be insightful to investigate this during Chapter 5 along with whether there is a change in the eKAM that could be present if step width was wider than expected. This could create a longer moment arm between the knee and the vertical line to the CoM and could explain why the CCI results suggested more lateral activation than medial side activation.

4.5 Conclusions

Patients approximately 6 years post-ACLr surgery display different gait strategies for different speeds compared to their non-injured counterparts.

A post-ACLr group moderate their internal flexion moments in response to increasing walking speeds differently to healthy individuals (p= 0.003), with CCI Four and the contribution from the soleus acting in a statistically significantly separate way (p= 0.039). Additionally, patients walk with lower internal flexion moments compared to healthy individuals (p=0.005). The resultant tibiofemoral contact load is less on the medial portion (p= 0.002) compared to uninjured individuals (p= 0.008) suggesting a shift in strategy with an increase in the lateral portion for individuals with ACLr (p= 0.003) compared to healthy individuals (p= 0.008).

Caution should be used when analysing speed in discrete categories rather than absolute values and participants should be monitored closely to ensure that they perform within a certain tolerance of speed.

Further work is required to understand the link CCI has to play in terms of an input alongside leg stiffness and as an output alongside medial compartment loading and how different categories of walking style, along with speed, could aid more personalised rehabilitation programmes.
Further work is also required to investigate the relationship between collected EMG and modelled muscle activations for both the healthy and patient groups which are investigated in Chapter 6.

Investigation is also required to analyse frontal kinematics, specifically considering treadmill and over ground kinematics, to understand their effect on external knee adduction moments (eKAM) which could explain why the soleus contributed to statistical significance in this study, which is examined next in Chapter 5.
5. The Gait Differences for Healthy Volunteers between Walking on a Treadmill-based Gait-Analysis System and an Over ground-based System to Establish the Effects on the Varus Valgus Moment of the Knee

5.1 Introduction and Additional Literature Review
This literature review investigates additional literature to that of Chapter 2 as a direct result of the queries generated at the end of Chapter 4. This additional literature review has been subdivided into three categories: knee background, biomechanical implications, and modelling and loading. The purpose of this study was to identify the frontal biomechanical changes in gait between treadmill and over-ground walking.

5.1.1 Knee Background
5.1.1.1 Step Width
Many studies have sought to compare the biomechanical differences between treadmill and over-ground gait (Bello et al. 2013; Rozumalski et al. 2015; Hollman et al. 2016; Srivastava et al. 2016; Yang and King 2016). Treadmill motion analysis systems are a preferred method of data collection for data-rich datasets in less time and space than conventional over ground data analysis systems. Previous research on step width on single belt (Rosenblatt and Grabiner 2010) and dual-belt treadmill-based gait (Zeni and Higginson 2010; Altman et al. 2012; Oude Lansink et al. 2017) has suggested an increased step width in these situations when compared to other walking environments, though some suggest an enforced treadmill familiarisation period can significantly reduce the difference in step width (Zeni and Higginson 2010; Altman et al. 2012; Oude Lansink et al. 2017), although this has been contested (Rosenblatt and Grabiner 2010; Rutherford et al. 2017a). Increasing the age of those using the treadmill is also likely to increase the step width (Hurt et al. 2010), as well as visual field disturbance (Saucedo and Yang 2017). Additionally, fitness ability has its part to play in kinematic variability, though the variability reduces after as little as 30 minutes of exercise on a treadmill (Da Rocha et al. 2017). Some suggest that treadmill gait produces less gait variability than over ground variability, meaning different styles are produced (Hollman et al. 2016). Increased step width can cause changes in full body kinematics, such as lateral CoM displacement, increased lateral trunk lean and increased eKAMs (Anderson et al. 2018), changes in mediolateral gait stability (Stimpson et al. 2017) and even changes in the control of foot placement (Perry and Srinivasan 2017).

As a change in the step width will cause changes in biomechanical gait results, it is reasonable to deduce that this will be an important factor for those with an ACLr and how step width may link to mal-alignment in the frontal plane. Therefore, investigating
step width changes in the most common ‘normal’ environment, over ground walking needs to be compared to treadmill walking, a commonly used environment to gather biomechanical data. It can then be ascertained as to the size of the effect on the frontal plane biomechanics kinematics and kinetics and also including knee contact loading for healthy individuals. This is to ensure that any changes for individuals with ACLr are from movement changes relating to the injury and ACLr and not from issues caused by the data collection setting.

5.1.1.2 OA
Increased step width can lead to increased eKAM (Anderson et al. 2018), which in turn can cause an increase in the force passing through the medial compartment (Teoh et al. 2013). Incorrect knee frontal angles can place stress on other tissues in the knee, such as the articular cartilage, ligaments, menisci, and subchondral bone (Sharma 2007). With the added issue of repeated exposure, OA could develop and worsen (Sharma 2007). For obese subjects, increased eKAM increases the likelihood of developing obesity-related knee OA (Yocum et al. 2018). Being overweight or obese is a regular correlation with future knee problems (Blagojevic et al. 2010; Adouni and Shirazi-Adl 2014). Those with pre-existing lower limb injuries are at an increased risk of early-onset OA (Long et al. 2017).

5.1.2 Biomechanical Implications
5.1.2.1 External Knee Adduction Moments
This section addresses wider step width and changes in eKAMs. Higher eKAMs have been found in individuals with ACLr previously (Patterson et al. 2014; Kumar et al. 2019), though lower eKAMs have occasionally been found (Patterson et al. 2014; Khandha et al. 2016). Higher eKAMs are associated with the progression of knee OA (Gerbrands et al. 2014). As this research study uses healthy individuals, this section is used to characterise those with injuries and possible OA to complement the research as a whole.

A wider step width is well noted in osteoarthritic patients (Rutherford et al. 2017a; Wiik et al. 2017; Anderson et al. 2018), and subjects with OA also display a larger stance eKAM magnitude (Landry et al. 2007; Rutherford et al. 2017b; Anderson et al. 2018), higher mid-stance (defined as the period between the $1^{st}$ and $2^{nd}$ adduction peaks) portion of eKAM (Astephen et al. 2008), or a greater peak eKAM during stance (Bennett et al. 2017), and eKAM has been the subject of over ground and treadmill investigations (Richards et al. 2018; Fantini Pagani et al. 2019). However, it has also been found that healthy young subjects sometimes have a similar overall peak eKAM to those with OA, with it theorised that this is managed through the presence of healthy, and not
managed in the latter with diseased, cartilage (Andriacchi et al. 2004; Rutherford et al. 2017b). Higher levels of knee eKAM can be of particular detriment as they allow for the subject’s forces to increasingly pass through the medial compartment of the knee, and less so through the lateral compartment, particularly when there is no pretension of the lateral compartment (Schipplein and Andriacchi 1991).

Interestingly, those with OA can present with an increased lateral truck lean, to shift the CoM further over the stance leg, to reduce the eKAM (Anderson et al. 2018). Additionally, when this lateral trunk shift occurs, step width can increase to ensure the base of support of the individual (Anderson et al. 2018). Larger eKAMs are also associated with knee OA progression (Fregly et al. 2008), with OA progression more likely for individuals with ACLr (Vaishya et al. 2019). The eKAM has been used as a ‘surrogate’ outcome measure for the progression of medial compartment OA and loading (Hunt et al. 2008; Yocum et al. 2018). As an additional note, it has been thought historically that to increase the stability of the joint when there are higher adduction moments, more muscle force could be present (Schipplein and Andriacchi 1991), which could occur when the step width is wider.

5.1.2.2 Kinematics in Other Joints
Figure 43 on the left shows the knee in slight varus, known as ‘bow-legged’, as a healthy knee normally displays an external adduction moment during the whole of the stance phase, though there is a very minor abduction moment at the point of IC (Andrews et al. 1996). The figure on the right shows the assumption in this research of what occurs with a wider step width on the external moments on the hip, knee and ankle joints. This figure is considered more from a ‘z’ configuration, where an increase in the moment on one side of the frontal axis will lead to a decrease in the moment on the other side of the frontal axis. Hence while it could be proposed that a wider step width would decrease the external hip adduction moment and the external ankle adduction moment, this is the same as increasing the external hip abduction moment and increasing the external ankle abduction moment. From this figure, it is worthwhile to establish further information on the external abduction moments at the hip and knee joints.
In one comprehensive systematic review, it is not well established what effect modifying the treadmill gait to reduce the eKAM from increased step width for those with medial OA would have on the hip and ankle in the frontal plane (Bowd et al. 2019). Whilst this is not for individuals with ACLr, there appears to be more literature between eKAM, other joints and OA individuals (Bowd et al. 2019), though there is some recent literature of relevance for ACLr (Lyle et al. 2022) or healthy individuals (Stief et al. 2021). There is a robust link to discuss kinematic changes for OA individuals due to the association between ACLr and OA development as discussed in section 2.1.2.1 (Vaishya et al. 2019).

It is not of benefit to reduce the joint loading in one joint to then potentially increase it in another. Some studies have suggested that hip moments do not increase when adjusting gait to reduce eKAM through taught modifications and live feedback (again for a population with medial OA), but instead, there are some increases in the knee flexion and ankle adduction moments (Richards et al. 2018), whilst others suggest that people with knee OA tend to have decreased peak hip adduction moments and many more hip and knee modifications in the sagittal, as opposed to frontal, plane (Astephen et al. 2008). The hypotheses are therefore focused on the frontal hip, knee and ankle moments.

5.1.3 Modelling and Loading

5.1.3.1 Knee Joint Loading

The knee joint is maximally loaded when the leg in question is in contact with the ground. In this investigation under the pattern of walking gait, it is also called the stance phase of gait. The knee joint is generally continually loaded throughout the gait cycle due to the pattern of muscle contractions but is more loaded when it has to act in stability and dampener capacities during IC with the ground. The mass of the subject is transmitted through the knee joint of the stance knee, producing a force on the ground and collected by the force plates under the subject. The force passes through the two compartments of the knee, the medial and lateral compartments. In this work, the knee
joint contact forces also have a contribution from the muscle force component deduced through the musculoskeletal model, and the knee joint contact forces are not purely deduced from the GRFs alone. The stance phase of gait can be further divided into 2 sections, that of the early or 1st half of stance, and that of the late or 2nd half of stance. This research is primarily interested in the first half of the stance phase, due to its link with the ‘loading response’ of the joints during the stance phase, a commonly used practice. The loading response can be defined as the time period between the heel strike and mid-stance (Roberts et al. 2017). As the loading response requires the combination of shock absorbance, and coordination and stability of the limb, it is considered one of the most demanding periods during gait (Perry and Davids 1992; Astephen and Deluzio 2005) and therefore is a period of great interest. Assuming that a wider step width leads to a larger eKAM, it would be beneficial to use hypotheses to link this to joint loading. Alongside a larger eKAM, it would be reasonable to assume that the medial compartment would be receiving a greater proportion of the contact forces, as theorised though not found by others previously (Saxby et al. 2016a).

5.1.3.2 Modelling Loading
As mentioned in Chapter 2, biomechanical principles such as eKAMs and joint contact forces can be produced by musculoskeletal modelling to establish reasonable estimations of what occurs externally and internally in a subject during gait. This chapter will model participants in the same model as discussed in Chapter 2 to explore the effect of step width differences during over ground and treadmill walking.
5.2 Methods and Data Processing
5.2.1 Introduction
The following sections define the study design, data collection, data processing workflow, ethics, data and safety and statistics sections of the methodology.

5.2.2 Study Design
This study was a quantitative, cross-sectional, observational, analytical study (Centre for Evidence-Based Medicine 2021). All data was collected in the RCCK laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University, Cardiff, Wales in November 2017. Ethical approval was granted by the Arthritis Research UK Centre (ARUK) for Biomechanics and Bioengineering at Cardiff University in October 2017 under the title “Step width variances between treadmill and over ground walking”. Recruitment was from healthy individuals from Cardiff University staff and student cohort, as well as contacts with links to Cardiff University, as outlined in the ARUK protocols at the time. All participants were provided with written informed consent and data was stored, and participant safety was conducted, in line with approved documentation and ARUK protocols.

5.2.2.1 Sample Size
Whilst a sample size of 27 is required for a full research study with 3 repetitions, which is discussed in section 5.2.6, it is important to note that this study was similar to a feasibility study. It was deduced that a sample size of at least 5 was required, where this small sample number being statistically justifiable in previous studies (de Winter 2013). 9 healthy participants were recruited: 5 females, and 4 males, average age 39 years (standard deviation 10 years). In the interest of matching females and males, a smaller subgroup of 3 females, and 3 males, average age still 39 years (standard deviation of 12 years) were analysed in terms of marker data and force plate data on the GRAIL (Motekforce Link. 2017).

5.2.2.2 Methods of Assessment
All subjects were evaluated using one over ground and one treadmill-based motion analysis system. Each subject was asked to walk across an over ground system three separate times at their own self-paced speed as shown in Figure 44. The two force plates were in an in-line format, with the right foot making IC with the first force plate, and the left foot making contact with the second force plate. This was to remove the effect of the subject concentrating on frontal plane foot placement. Each gait cycle speed was determined from the point the right heel marker passed over the start of the
force plate setup to when it passed over the end of the force plate setup, with the total force plate distance being 1.21m.

The derived three over ground speeds were averaged with this value being entered into the D-Flow software which is part of the GRAIL (Motekforce Link. 2017) to control the speed of the treadmill. The subject was then attached to the safety harness and slowly increased in speed until a steady-state was achieved and maintained for one minute. Nine consecutive steps were sampled from the middle of this steady-state data. Nine steps are more than the over ground three, as the same protocol was followed as for the initial healthy study, assuming no crossing of treadmill belts occurred.

![Image](image.png)

*Figure 44 - Over ground walking of a participant as shown in Vicon*

5.2.3 Data Collection
The Equipment, Vicon and D-Flow and OpenSim, SIMM and The University of Wisconsin-Madison Model sections are discussed in Chapter 3.

5.2.3.1 Study Protocol
Each subject was prepared with 57 passive reflective markers as used in the other research protocols; however, EMG data was not collected for this research. Each subject used the over ground system and then the GRAIL system. A simple T-pose calibration was taken before the GRAIL data was collected from each subject, as it was a satisfactory position for calibration. Section 5.2.2.2 discusses how the sessions were conducted.

It is now beneficial to understand the workflow that the data goes through to produce the outcomes, which is discussed below.
5.2.4 Data Processing Workflow

5.2.4.1 Calculations
Step width was obtained from the marker raw results in the .c3d file, with the angles, moments and force distributions calculated from the results from the modelling process. Previous studies have defined step width as the difference at heel strike, often referred to as IC, from a virtually derived position between the caput of the 5th metatarsal, the tip of the first toe and the heel marker (Oude Lansink et al. 2017). Others have defined step width as the difference between the contralateral heel marker position at the time when ipsilateral sagittal velocity moves from posterior to anterior (Stimpson et al. 2017). For simplicity, the step width for this study was measured as the point from the IC of the right heel marker to the IC of the left heel marker, considering the distance in the mediolateral plane only.

5.2.4.2 Matlab and Data Analysis
Figure 45 shows the data processing and analysis workflow for the step width study. Please see Appendix C for more details of these steps and examples of codes and outputs.

Step width can be collected as this is the main variable of interest. Angles and moments for the whole of the stance phase for the hip, knee and ankle are popular measures that can be compared to other research studies and can be used to explain step width results. Also, adduction/abduction in the frontal plane and flexion/extension in the sagittal plane are also of interest. As this research is interested in particularly the loading response phase of the gait cycle, this research examines the first half of the gait cycle specifically. The maximum angles and moments can also be explored to establish the most extreme situation an individual could produce and is another popular comparison to use. Additionally, the maximum contact forces in the medial and lateral compartments of the tibiofemoral joint can be explored to establish how the step width might impact on angles and moments and loading as an output. The maximum contact forces are investigated in this research, rather than a total mean as produced in Chapter 4, as this chapter is investigating a specific snapshot of movement, the worst-case scenario.
**Figure 45- Data Processing and Analysis Workflow for Step Width Study, where green represents stages different to Chapter 4**

**Step 1**
- **Processing and Validation- First script**
  - Event the data (IC to TO)
  - Note or remove treadmill belt crossing
  - Note unusual GRFs
  - Possible step events modification
  - Possible step removal
  - Possible removal of bad frames

**Step 2**
- Matlab
  - Loads .c3d file
  -Convert markers and force plates to .forces, .mot and .trc

**Step 3**
- Matlab
  - Scale cmd
  - Inverse Kinematics cmd
  - Muscle Force Distribution cmd
  - Inverse Dynamics cmd
  - Contacts cmd

**Step 4**
- Validation
  - Identify terminated files
  - Identify short files
  - Re-run when required

**Step 5**
- Processing and graphs- Second script
  - Obtains and plots angle, moment and forces figures

**Step 6**
- Validation
  - Identify unusual angle or moment plots
  - Remove unusual steps through rerunning from Step 5

**Step 7**
- Processing- Third script
  - Calculate step widths for an individual

**Step 8**
- Processing and graphs- Fourth script
  - Plots knee angles and moments in both scenarios

**Step 9**
- Processing and graphs- Fifth script
  - Plots medial and lateral forces in both scenarios

**Step 10**
- Processing and graphs- Sixth script
  - Plots hip angles and moments in both scenarios

**Step 11**
- Processing and graphs- Seventh script
  - Plots ankle angles and moments in both scenarios

**Step 12**
- Graphs- Eighth script
  - Plots step widths for all individuals
  - Plots all joint moments for all individuals (not saved in script)
5.2.5 Ethics, Data and Safety
Recruitment for data collection on healthy individuals was conducted in line with Cardiff University's Ethical procedures. Ethics information for this study is available in section 5.2.2.

The subjects were required to provide their age, gender and mass. This information was stored in lockable filing cabinets located within the RCCK laboratory, which itself is only accessible to staff and PhD students. Data collected electronically was coded simply with each subject being assigned a number in ascending order.

All subjects were required to wear a harness attaching them to the ceiling to prevent injury in the case of a fall whilst the treadmill was moving.

5.2.6 Statistics
The sample size was derived with the sample calculation used in section 4.2.6, with p₀ of 0.6, p₁ of 0.8 (Shoukri et al. 2004), an alpha of 0.05, and a beta of 0.20 (Walter et al. 1998). The number of repetitions in this study was 3, which hence derives a sample size of 26.1 or rounded up to 27 (Walter et al. 1998).

The software package SPSS was used to calculate the normality of all the data through the Shapiro-Wilk Test (Ghasemi and Zahediasl 2012).

For analysis of the biomechanical characteristics, some checking assumptions were employed to ensure the correct statistical test was employed. Firstly, a t-test was selected over an ANOVA as the group means of two factors (over ground and treadmill) were being compared, if it were more, an ANOVA would have been used (Mishra et al. 2019). Furthermore, the same group was compared under two separate scenarios, therefore it was a paired t-test (Thielen 2021). As a small sample was being analysed, it was important to identify whether a parametric or non-parametric distribution was occurring, as parametric would lead to a t-test, and non-parametric would lead to a Wilcoxon test (Thielen 2021). The assumption of normality and assumption of equal variance is required for the sample to be considered parametric (Nahm 2016). To satisfy the query of parametric distribution, and also the concern of analysing a small sample, it was understood that even for an extremely small same size of less than or equal to 5, a paired t-test can be used, assuming that the effect size is large (de Winter 2013). Statistical significance was assumed to be p< 0.05.

5.2.7 Research Hypotheses
Hypothesis 2 H₁: There is a change in step width for healthy individuals between two different data collection systems and on the resultant kinematics and kinetics (frontal
angles and moments for the hip, knee and ankle, and medial and lateral contact loading).

With the null hypothesis (H₀) of:

Hypothesis 2 H₀: There is no change in step width for healthy individuals between two different data collection systems and on the resultant kinematics and kinetics (frontal angles and moments for the hip, knee and ankle, and medial and lateral contact loading).
5.3 Results
Table 12 represents the initial group sample subject characteristics, while Table 13 represents the subject characteristics for this subgroup of this study. A Shapiro-Wilk test was taken to establish if the data (age, modelled mass and height) were normally distributed (Ghasemi and Zahediasl 2012). The subjects used in this research were different to those used in Chapter 4. Unfortunately, one of the sessions was collected at the incorrect frame rate for a male volunteer, so in the interest of matching females and males, only 6 participants were analysed further. The Shapiro-Wilk test returned significance values above 0.05, which suggests all three variables are normally distributed. All moments are discussed as external moments.

<table>
<thead>
<tr>
<th>Table 12- Subject characteristics for the initial group sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± S.D.</strong></td>
</tr>
<tr>
<td>Sample Size</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 13- Subject characteristics for the subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± S.D.</strong></td>
</tr>
<tr>
<td><strong>Shapiro-Wilk</strong></td>
</tr>
<tr>
<td>Sample Size</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Modelled mass (kg)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
</tbody>
</table>

* Statistically significant, therefore a normal distribution was not demonstrated.

Figure 46 demonstrates the median step width for each subject, during each condition, with the standard deviation as whiskers. As can be seen, all subjects displayed wider step widths on the treadmill compared to the over ground system, although the last subject displayed slightly overlapping variation between the two conditions.
Table 14 shows the results of the Shapiro-Wilk test for normality for the biomechanical variables. If $p < 0.05$, then statistical significance was found, and the assumption of normal distribution was rejected. Normal distribution was rejected for peak contact force medial portion treadmill ($p = 0.038$) and peak contact force lateral portion over ground ($p = 0.016$). Normal distribution was also rejected for total mean contact loading forces medial compartment over ground ($p = 0.049$) and peak external knee rotation angle over ground ($p = 0.008$). This meant the non-parametric Wilcoxon test was used to examine these variables further in Table 15, Table 16 and Table 17. If normal distribution was assumed, the parametric paired $t$-test was used in the same tables.
### Table 14: Shapiro-Wilk test for normality on the biomechanical variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Over ground</th>
<th>Treadmill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step width (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak knee adduction angle (deg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak knee abduction angle (deg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external knee abduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external knee adduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak contact force medial portion (N/BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak contact force lateral portion (N/BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak hip adduction angle (deg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external hip adduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak hip abduction angle (deg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external hip adduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external ankle abduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external ankle adduction moment (N.m/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mean contact loading forces medial compartment (N/BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mean contact loading forces lateral compartment (N/BW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak knee flexion angle (°)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak internal knee rotation angle (°)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak external knee rotation angle (°)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant

Table 15 shows step width while walking on a treadmill was significantly wider than over ground with an average of 9cm greater width (p= 0.002). Statistical significance was found for all measured variables between treadmill and over ground walking. These were peak knee adduction angle (p= 0.013), peak knee abduction angle (p= 0.017), peak external knee abduction moment (p= 0.007) and peak external knee adduction moment.
(p= 0.010). Also peak contact force medial portion (p= 0.028) and peak contact force lateral portion (p= 0.028).

The maximum calculated forces at the tibiofemoral joint interface generally showed that the medial and lateral compartments are loaded more equally on the over ground system as seen in Table 15. On the treadmill, the contact force distribution was altered. The peak lateral compartment offloading for treadmill walking compared to over ground walking was substantial at 54.3%. A loading trend assumes peak treadmill compartment lateral force divided by peak over ground compartment lateral force and represented as a percentage. The remainder of the percentage represents the offloading trend, calculated as 100-((0.63/1.38)x100). Medial compartment contact force increased by 21%, whilst knee adduction angle and eKAM increased. Peak external knee abduction moment is also featured to complement the eKAM data.

Table 15: Mean, standard deviations and significance levels are provided for key knee biomechanical parameters for the first half of the stance phase only

<table>
<thead>
<tr>
<th>Variable</th>
<th>Over-ground</th>
<th>Treadmill</th>
<th>Significance (Paired t-test)</th>
<th>Significance (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step width (m)</td>
<td>0.07 (±0.03)</td>
<td>0.16 (±0.03)</td>
<td>p= 0.002*</td>
<td>-</td>
</tr>
<tr>
<td>Peak knee adduction angle (deg)</td>
<td>2.72 (±0.50)</td>
<td>5.24 (±1.78)</td>
<td>p= 0.013*</td>
<td>-</td>
</tr>
<tr>
<td>Peak knee abduction angle (deg)</td>
<td>-0.16 (±0.36)</td>
<td>-0.80 (±0.30)</td>
<td>p= 0.017*</td>
<td>-</td>
</tr>
<tr>
<td>Peak external knee abduction moment (N.m/kg)</td>
<td>0.16 (±0.09)</td>
<td>0.006 (±0.03)</td>
<td>p= 0.007*</td>
<td>-</td>
</tr>
<tr>
<td>Peak external knee adduction moment (N.m/kg)</td>
<td>0.39 (±0.08)</td>
<td>0.64 (±0.11)</td>
<td>p= 0.010*</td>
<td>-</td>
</tr>
<tr>
<td>Peak contact force medial portion (N/BW)</td>
<td>2.05 (±0.26)</td>
<td>2.48 (±0.30)</td>
<td>-</td>
<td>p= 0.028*</td>
</tr>
<tr>
<td>Peak contact force lateral portion (N/BW)</td>
<td>1.38 (±0.38)</td>
<td>0.63 (±0.22)</td>
<td>-</td>
<td>p= 0.028*</td>
</tr>
</tbody>
</table>

* Statistically significant

Where negative numbers are in the opposite direction to those stated

Using Figure 43 as the motivation for which external moments at the knee, hip and ankle need to be investigated (knee adduction, hip abduction and ankle abduction), three subsequent figures can be plotted and examined.

Figure 47 shows the median maximum eKAMs between treadmill and over ground gait.

It is worth noting that once again, that median treadmill eKAMs are generally higher per
individual than the values produced through over ground gait, and the difference between the two scenarios was statistically significant (p= 0.010).

Figure 47- Maximum external knee adduction for treadmill and over ground gait in N.m/kg (p= 0.010)

Figure 48 displays similar information to that shown in Figure 47 but is instead focusing on external hip abduction moments. It is interesting to observe that the median treadmill external abduction moments are lower than their over ground counterparts, and actually, in some cases, go into external adduction. The peak external hip abduction moment, which is equivalent to the internal hip adduction moment, was statistically significant between the treadmill and over ground scenarios (p= 0.009).

Figure 48- Maximum external hip abduction for treadmill and over ground gait in N.m/kg (p= 0.009)

Figure 49 is once again related to Figure 47 and Figure 48 and displays the external ankle abduction moments, or simply the eversion moments. As with Figure 48, it is worth noting that the median treadmill external ankle abduction moments are slightly smaller
than those for the over ground situation, however, these results were not statistically significant ($p=0.103$).

Figure 49: Maximum external ankle abduction for treadmill and over ground gait in N.m/kg ($p=0.103$)

Table 16 shows the key kinematic and kinetic parameters for the knee, hip and ankle, remembering that these analyses were for the first half of stance only. The ankle adduction and abduction angles have not been mentioned as they were minimal across subjects (all in the range of $10^{-4}$). Considering the peaks for the hip and ankle moments in this period, the peak external hip abduction moment which is of particular interest should occur near the start of the stance phase (Alkjaer et al. 2006). The peak external hip adduction moment should occur just under halfway through the stance phase (Alkjaer et al. 2006). The peak external ankle abduction moment, also of interest and known as the eversion moment, should be at the very start of the stance phase (de David et al. 2015). The peak external ankle adduction (or inversion) moment should occur in the first quarter of the stance phase (de David et al. 2015). Therefore, for the hip and ankle, all moment peaks are at quite similar points in the first half of stance, particularly for the external abduction moments at the hip and ankle which are of specific interest.

As can be seen, statistical significance was found for peak external hip abduction moment ($p=0.009$), peak external hip adduction moment ($p=0.009$) and peak external ankle adduction moment ($p=0.009$). Statistical significance was also found for total mean contact loading forces medial compartment ($p=0.028$) and total mean contact loading forces lateral compartment ($p<0.001$).
Table 16: Mean, standard deviations and significance levels are provided for hip and ankle kinematics and kinetics, and knee total mean contact loading for the first half of the stance phase only

<table>
<thead>
<tr>
<th>Variable</th>
<th>Over ground</th>
<th>Treadmill</th>
<th>Significance (Paired t-test)</th>
<th>Significance (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak hip adduction angle (deg)</td>
<td>8.14 (±2.53)</td>
<td>6.22 (±1.50)</td>
<td>p= 0.271</td>
<td>-</td>
</tr>
<tr>
<td>Peak external hip abduction moment (N.m/kg)</td>
<td>0.22 (±0.14)</td>
<td>0.003 (±0.08)</td>
<td>p= 0.009*</td>
<td>-</td>
</tr>
<tr>
<td>Peak hip abduction angle (deg)</td>
<td>1.75 (±1.65)</td>
<td>0.89 (±2.26)</td>
<td>p= 0.321</td>
<td>-</td>
</tr>
<tr>
<td>Peak external hip adduction moment (N.m/kg)</td>
<td>0.77 (±0.10)</td>
<td>1.11 (±0.20)</td>
<td>p= 0.009*</td>
<td>-</td>
</tr>
<tr>
<td>Peak external ankle abduction moment (N.m/kg)</td>
<td>0.29 (±0.04)</td>
<td>0.24 (±0.10)</td>
<td>p= 0.103</td>
<td>-</td>
</tr>
<tr>
<td>Peak external ankle adduction moment (N.m/kg)</td>
<td>0.001 (±0.01)</td>
<td>0.03 (±0.01)</td>
<td>p= 0.009*</td>
<td>-</td>
</tr>
<tr>
<td>Total mean contact loading forces medial compartment (N/BW)</td>
<td>19.44</td>
<td>24.34</td>
<td>-</td>
<td>p= 0.028*</td>
</tr>
<tr>
<td>Total mean contact loading forces lateral compartment (N/BW)</td>
<td>14.06</td>
<td>5.00</td>
<td>p&lt; 0.001*</td>
<td>-</td>
</tr>
<tr>
<td>Ratio total mean contact loading forces (medial: lateral)</td>
<td>58:42</td>
<td>83:17</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Statistically significant

Utilising the knowledge gained from the external adduction knee moments, the external abduction hip moments and the external abduction ankle moments, Figure 50 demonstrates what is occurring for the average individual during the wider step width on a treadmill. The external abduction moments at the hip decrease substantially. As statistical significance was not found for the external abduction moment of the ankle, this was demonstrated on the figure as a question mark. The knee, in contrast, is exposed to a much larger increased external adduction moment. This is hard to understand due to the simplified figure but would mean that there is a much greater internal stress on the hip joint as the knee is attempting to adduct but the hip is attempting to adduct more too. That would also mean that there is a lot of internal tension at the medial portion of the tibiofemoral joint of the knee too.

This can also be summarised as there are statistically significantly increased adduction moments in all joints of the lower body when using a treadmill due to the increased step width. It is therefore important to take care if observing increased external adduction moments in patient individuals that are measured using a treadmill-based motion.
analysis system as they cannot be compared to normative data collected from an over-ground-based setup. These patient individuals must be compared to healthy data that has also been collected on a treadmill-based motion analysis system.

![Figure 50](image)

Figure 50: Representation of the right knee frontal position with an initial assumption of joint moments (left) and the actual joint moment interaction (right) for an increased step width on a treadmill

* Statistically significant

As this chapter primarily considers the knee in the frontal plane, Table 17 shows the biomechanical results on the knee in the sagittal and transverse planes. This is to support further analysis of the frontal plane results in the discussion in section 5.4.5. No statistical significance was found with these results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Over ground</th>
<th>Treadmill</th>
<th>Significance (Paired t-test)</th>
<th>Significance (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak knee flexion angle (°)</td>
<td>25.68 (±0.70)</td>
<td>25.02 (±0.56)</td>
<td>p= 0.724</td>
<td>-</td>
</tr>
<tr>
<td>Peak internal knee rotation angle (°)</td>
<td>4.11 (±0.69)</td>
<td>2.42 (±0.88)</td>
<td>p= 0.101</td>
<td>-</td>
</tr>
<tr>
<td>Peak external knee rotation angle (°)</td>
<td>3.87 (±1.26)</td>
<td>5.44 (±0.65)</td>
<td>-</td>
<td>p= 0.345</td>
</tr>
</tbody>
</table>

* Statistically significant

In summary, this research chapter aimed to compare the biomechanical differences between gait in over ground and treadmill scenarios, with a particular focus on examining if there were any step width changes. Most of the results displayed statistical significance in the differences between over ground and treadmill settings, and an increase in step width and increased external adduction moments, or decreased internal abduction moments, for the hip, knee and ankle on a treadmill.
5.4 Discussion
5.4.1 Overview
Consistent differences were observed between treadmill and over ground walking, with an increased step width for treadmill walking, with a mean increase of 9cm between all the subjects, or an increase of 129% (p=0.002). It is worth noting this increase is 229% of the over ground value. For treadmill walking, there were also increased peak knee adduction angles (mean increase of 92%, p=0.013), decreased peak external knee abduction moments (total 96.25%, p=0.007), and increased peak eKAMs (mean increase of 64%, p=0.010). There were increased peak contact forces in the medial compartment (mean increase of 21%, p=0.028) while lower peak contact forces in the lateral compartment (mean decrease of 54%, p=0.028) for treadmill walking. Paired t-test statistical significance was found with the step width and all knee parameters. These included the peak knee adduction and abduction angles, and peak external knee abduction and adduction moments. There was also statistical significance for peak contact forces on the medial and lateral compartments, as well as total mean contact loading forces on medial (a mean increase of 25%, p=0.028) and lateral (a mean decrease of 64%, p<0.001) compartments. There were some statistically significant values within hip and ankle moments, with 3 of the 4 moments being statistically significant, though the hip angles were not in themselves statistically significant.

5.4.2 Hypotheses
The null hypothesis can be rejected, and the alternative hypothesis accepted when considering the biomechanical variable of step width which is statistically significantly wider for treadmill gait compared to over ground gait at the same speed (p=0.002). The same is true for the peak knee adduction external moment, which was statistically significantly higher at the same speed on a treadmill (p=0.010). In fact, external adduction moments at the hip and ankle were also statistically significantly higher (both p=0.009). This suggests that the three joints in the frontal plane should all be represented as adduction moments for clarity, rather than the ‘z’ configuration suggested in section 5.1.2.2. Additionally, the null hypothesis can be rejected and the alternative hypothesis considering medial loading accepted; the peak contact force for the medial portion is higher on a treadmill (p=0.028) than on an over ground setting for the same speed. Additionally, the total mean contact loading forces for the medial compartment were higher on a treadmill and statistically significant (p=0.028).
5.4.3 Step Width
Figure 46 shows the step width differences between treadmill and over ground walking for the six subjects. Every subject's result shows that treadmill walking causes a subject to walk with a wider step width. However, there is a large standard deviation for every result. Additionally, subject 6 displayed a slight overlap between standard deviations for over ground and treadmill walking, suggesting a transitory participant that could fall into a different gait style category.

Previous literature suggests that increased step width reduces the eKAM (Simic et al. 2011; Stief et al. 2021). However, this was not observed in this study, and Table 15 shows that the eKAM increased statistically significantly by 64%.

5.4.4 Hip and Ankle
As can be seen from Figure 47, Figure 48 and Figure 49 displaying the knee, hip and ankle moments, it could be presumed that with a wider step width, you would increase the knee varus angle. It could be presumed in turn that the abduction moments from the hip and ankle, and the adduction moments for the knee would increase. However, the knee adduction moments were higher on the treadmill, but the hip and ankle external abduction moments were lower on the treadmill (the latter not significantly), suggesting a more complex strategy.

5.4.5 Biomechanical Discussion
It is worth noting that the parameters collected in this study were not from the same time instant and were mostly sampled as maximum values from the first half of stance, as the loading response component of the stance phase is of particular interest in this research. In terms of the moments, the maximum hip abduction and ankle abduction occur at very similar times during the early stance phase as mentioned in section 5.3, whilst external adduction of the knee occurs at a similar time of one-quarter of the way through the stance phase (Preece et al. 2016; Meireles et al. 2017). These three moment timings are specifically chosen as they represent the frontal plane moments of the stick figure as shown in Figure 50. Depending on the speed, the average duration of the stance phase is 0.59 to 0.67 seconds long (Murray et al. 1964), meaning that for the worst-case scenario, these three particular moment values comprising the stick figure in Figure 50 are 0.17 seconds apart.

Knowing that step width was wider for a treadmill-based setup but eKAM was higher in this scenario leads to the first query of what is happening in the frontal plane biomechanically for this to occur when it is not expected from some literature (Simic et al. 2011). This is clearly due to the varus angle at the knee increasing the moment arm.
for the eKAM calculation. However, the increase in eKAM could be due to a lack of trunk
lean in the frontal plane which was not considered in this research. Healthy individuals
should produce a small degree of trunk lean through gait and no trunk lean is known to
increase the eKAM for both a normal step width and a wide step width, with a frontal
trunk lean of as little as 6 degrees lowering the eKAM peaks dramatically (Anderson et
al. 2018). This is because the CoM is more laterally displaced in a wider step width as it
shifts during gait (Anderson et al. 2018), and a lateral trunk lean over the supporting leg
during the stance phase makes the resultant GRF pass closer to the knee, meaning the
knee moment arms, and hence the eKAM, would reduce. It is also important to note
that whilst the aforementioned systematic review suggested a wider step width would
reduce knee eKAM (Simic et al. 2011), it only actually considered two studies that
discussed step width. Both studies were from the same research team that developed a
model from data from only one individual, who crucially had OA (Fregly et al. 2008;
Reinbolt et al. 2008). The model was not used with data from more individuals, or from
individuals without OA for a baseline comparison (Fregly et al. 2008; Reinbolt et al.
2008).

The second query that requires exploration is why is the external knee adduction
moment a lot higher for treadmill loading, and subsequent medial loading is also
increased but the external abduction moment for the hip is decreased (the external
abduction for the ankle is not explored as statistical significance was not established). In
Figure 50 it is not very clear due to the simplified representation of how the angle and
moment of the knee can be higher for treadmill walking whilst the knee is in varus, but
the abduction moment of the hip is lower. Considering the joints not in a ‘z’
configuration, but a configuration which considers the moments around the GRFs shows
that the knee and hip are more adducted on the treadmill. This means that there is a lot
of strain on the knee joint, as the hip moment is forcing the hip in the opposite direction
of movement to the knee. This could mean that a lot of force is heavily directed through
the medial compartment of the knee because the knee is at a greater varus angle.
Additionally, there will be more strain on the hip particularly on the treadmill as it is
‘straighter’ in angle than for the over ground scenario yet has to aid the formation of a
greater knee angle and moment. It is also worth noting that the increased varus angle
for the knee on the treadmill is a subtle increase, and acceptable within the range of
reasonable movement predicted by the model (Yang and King 2016).

It was assumed that changing the step width, which is a frontal plane consideration,
would change the biomechanics mainly in the frontal plane. This study did not take into
account how movement adjustments may be made in the sagittal or transverse plane to
compensate, such as increased flexion in the lower limb joints. Therefore, Table 17 was then formed to investigate the biomechanics in other planes during this increase in varus angle. As can be seen, there is no statistical significance for the peak knee sagittal angle, therefore the knee is not flexing more when the varus angle occurs. However, when the peak internal and external knee rotation angles were examined for the two gait scenarios, the knee was less internally rotated and more externally rotated during the treadmill gait compared to the over ground gait, though not statistically significantly. This could explain this varus angle, as when the knee is more externally rotated, it is forced into more of a varus alignment, with there being precedence of more external rotation with a varus knee compared to a neutral alignment (Bennett et al. 2017), and explains how the hip and ankle can, at the same time, be quite straight in the frontal plane. Additionally, a varus angle is known to increase the eKAM compared to neutral gait (Bennett et al. 2017) linking the first and second queries together more clearly; there are clear frontal plane kinematic (angle) and kinetic (moment) changes in the knee. Whilst there are frontal plane kinetic or moment changes in the hip, the kinematic or angle changes for the hip and ankle are less obvious.

5.4.6 Loading of the Tibiofemoral Joint
Due to the large size of the effect of treadmill walking on the peak contact forces, even though this was representative of the ‘worst case scenario’, this was not representative of loading patterns during the whole of the stance phase as was discussed previously in Chapters 2, 3 and 4 (Dabbs et al. 2015; Rasnick et al. 2016). Therefore, the area under each loading graph was integrated and the difference between these two scenarios was additionally considered; these ratios clearly show a relationship similar to those peak values.

The medial compartment load increases by 48% when on a treadmill when compared to over ground, whilst the lateral compartment load decreases by 60%, supporting the knowledge of wider step widths causing higher eKAMs and in turn, increasing medial knee forces. As discussed in this chapter, this is due to the knee producing more of a varus angle, and at these wider step widths, there is the possibility that the biomechanics change in other planes too. This increase in varus knee angle increasing the load on the medial tibiofemoral compartment with the same musculoskeletal model is supported by other research (Van Rossom et al. 2019). Possible external rotation could be considered moving towards malalignment of the knee, and indeed varus combined with external rotation is known to increase medial compartment loads (Norman et al. 2017). Furthermore, higher medial compartment loads occur from pure frontal changes than pure transverse changes (Van Rossom et al. 2019). This is because
changes in transverse positioning are less impactful than changes in frontal positioning (Van Rossom et al. 2019).

5.4.7 Study Limitations
Due to potential variations in the abilities of those using the GRAIL system at any time, it is policy within the research laboratory to ensure a subject is wearing a harness and attached to rigging to support the user in case of a fall. The wearing of such a harness may be thought to disturb the natural gait and hence the kinematics and kinetics of a subject, though literature supports that this is not the case (Stout et al. 2016).

Some studies support a familiarisation period to treadmill walking; with step width variation normalising in one minute and step width itself normalising after two minutes (Oude Lansink et al. 2017). Interestingly, second visits can record a narrower initial step width, with step width normalising after only one minute suggesting retained familiarisation with the treadmill (Oude Lansink et al. 2017). Another study suggested a normalisation period of up to 5 minutes (Zeni and Higginson 2010). The subjects in this study have all either experienced the GRAIL before or were given a short period of time before recording began to familiarise themselves.

Once on the treadmill, the treadmill speed was controlled and mimicked the average of the over ground walking speed to reduce any confounding effects from the variance of speed. Confounding effects have happened when comparing between two investigated factors in other studies (Saucedo and Yang 2017).

Step width is often widened by those who feel instability during gait that cannot compensate in other ways (Krebs et al. 2002). Initial adjustments include slowing gait speed or decreasing the variation between the centre of pressure and the centre of gravity in all planes (Krebs et al. 2002). Decreasing the centre of gravity is considered an increase in leg stiffness in this research. Hence, there is a potential confounding factor in this study as individuals could not slow down the gait speed and so may have decreased their centre of pressure and gravity variation or widened their step width.

Also, toe-in or toe-out considerations were not made in this study, though they do influence frontal knee alignment (Bennett et al. 2017).

Considering the psychological reasons for a wider step width, treadmill walking in this study was collected from a dual-belt treadmill system, where each foot had an individual belt and hence an individual force plate. This is necessary to calculate kinetic information for each side of the body without the influence of the contralateral side. However, there is a very small between-belt gap. The subjects were encouraged not to place their foot on this gap or indeed, to plant the foot on the wrong treadmill belt. Even though there was a period of time for the subject to acclimatise to the treadmill so as to
bring their feet closer together, this initial instruction may have had some bearing, with a subject purposely placing their feet extra far apart so as not to approach the gap. Additionally, the experience of coming to a purpose-built facility and being monitored from a data collection desk not in the participant’s eyeline may have a small influence on the subject’s behaviour whilst in the laboratory.
5.5 Conclusions
Due to the different frontal plane biomechanical outcomes between treadmill and over ground walking, researchers need to take into account the setting used. Researchers may even apply a tolerance to their calculations if they are recording information from a treadmill-based setup with little acclimatisation time for the subject, especially when there are comparisons to data collected from an over ground setting. If researchers are required to compare a similar motion from an over ground setting to a treadmill movement, the varus knee angle must be analysed initially. If a near-to-doubling of the maximum varus angle occurs as does in this study, an extra variable has been introduced into the comparison and the resultant loading will be changed significantly. If the resultant loading is the item of interest, a tolerance of up to 25% of the medial loading results should be returned back to the lateral compartment. If only comparing treadmill data to each other, this caution does not necessarily need to be followed, though stating any changes in frontal plane mechanics, particularly the varus angle, is useful when concluding information for a certain population. It would be beneficial to analyse this theory in a future study, with other individual adjustments such as toe-in and toe-out gait studied.

Evidently, analysing the effect of step width on frontal plane kinetics is important when comparing treadmill walking to normative over ground data, crucially suggesting increases in eKAM and medial loading could give false indications suggesting poorer knee health for otherwise healthy individuals. However, if using treadmill-based data collection to study knee health in individuals in two groups, the constant of treadmill data collection should not interfere with a valid between-group comparison. Caution must be employed when comparisons are drawn between data collected in two different settings.
6. The Differences between Modelled Muscle Activations and Collected EMG Readings for Healthy Volunteers related to an ACLr Patient Group

6.1 Introduction and Additional Literature Review
This literature review investigates additional literature to that of the main literature chapter and is a result of the queries raised at the end of the last chapter and is divided into two sections; muscle modelling and EMG background, and processing techniques to compare two signals. These two sections seek to establish if the model used in this research successfully predicts muscle activations for healthy and patient individuals.

6.1.1 Muscle Modelling and EMG Background
Many other models in use are driven dynamically in forward simulation by EMG data to predict kinematic results. As acceleration can affect the angle and velocity movements, a feedback loop is used, implementing static optimization to influence the original inputs, a process termed Computed Muscle Control (CMC) (Leuven 2017). If however static optimization is used at the beginning, and not as a feedback loop, this direction is called Inverse Dynamics, and is performed after the Scaling and Inverse Kinematics have been established (National Center for Simulation in Rehabilitation Research 2020). Through the use of static optimization, calculations can be performed at each time frame, giving each muscle a certain weighting, to understand the activation of each muscle to keep the system in balance at any point (Heintz 2006). The results are termed Muscle Force Distribution (MFD) and form the basis for how the muscle activations are derived in this research.

As the modelled muscle activations in this research used a non-EMG-based input model, there is an interest in understanding the difference between collected EMG and the modelled muscle activations, especially as the model used was not adapted for a patient population. Hence this study could identify how the model acts for the post-ACLr subjects and how the muscle outputs change. Recent literature is in support of this method; a robust validation method would be to validate modelled muscle activations against collected EMG data (Trinler et al. 2018). This would require comparison in 4 ways:
(1) healthy EMG to healthy modelled muscle activations, so that the validity of modelling muscles without other variables can be examined, which is termed HEvHM in this study
(2) patient EMG to patient modelled muscle activations, so that how the model works with a patient’s altered kinematic and kinetic data can be examined, along with the validity of modelling muscles can be analysed, which is termed PEvPM in this study

(3) EMG of healthy to EMG of patients, so that the difference in collected muscle activation data can be examined before and after an injury and subsequent surgery, which is termed HEvPE in this study

(4) modelled muscle activations of healthy compared to modelled muscle activations of patients, so that the same factors as part (2) can be explored; how the model works with a patient’s altered kinematic and kinetic data, along with the validity of modelling muscles generally, which is termed HMvPM in this study.

Accurate knowledge of muscle activation patterns in an injured individual can inform targeted treatments, though modelling approaches are still used for research and are not timely or validated enough to be regularly used in clinical practice (Trinler et al. 2018). Additionally, knowledge of modelled muscle activation patterns at different speeds is not widely known in the current literature (Trinler et al. 2018).

Surface EMG is a popular way to collect muscle activation information during a movement. The comparison of amplitudes of surface EMG for similar muscles is a valid way to represent muscle force production (Vigotsky et al. 2017; Whiteley et al. 2021), though the difference is important to note. The term muscle activation is linked to force a muscle actively produces, though are different concepts (activation only deals with active components to produce force, is a scaling factor between potential active force and active force itself, and activation does not consider fibre length and velocity whereas force does) (Vigotsky et al. 2017). Complexities aside, EMG is a usual muscle activation collection tool to use to validate modelled muscle activation data.

EMD is the principle that there is a short period of time between the voltage in a muscle to the resultant reaction by the muscle and/or movement. This can vary depending on the type of muscle contraction occurring, the joint angle, effort level, the effect of fatigue, and age and gender (Zhou et al. 1995). Males have been suggested to have shorter EMD times (Bell and Jacobs 1986; Winter and Brookes 1991), though this is debated, with others finding no significant differences between genders (Troy Blackburn et al. 2009; De Ste Croix et al. 2015). EMD can widely vary depending on the muscle and the type of contraction. The hamstrings have the following variability: biceps femoris, semimembranosus and semitendinosus displaying maximum values between 27ms and 63ms (De Ste Croix et al. 2015), biceps femoris long head having an average of 126ms between the genders (Troy Blackburn et al. 2009) and approximately around 50ms for
both males and females before a fatiguing task (Minshull et al. 2007). But also for the hamstrings, no gender differences for medial or lateral EMD at different speeds have been found, so all muscles are equally acting to stabilise the joint (De Ste Croix et al. 2015). Furthermore, EMD can be prolonged for those who have received an ACLr (Kaneko et al. 2002). For simplicity and comparability between healthy and patient groups (adding a longer EMD to those with ACLr could be a confounding factor in not being able to identify the success of the model for patients), this research will use one EMD value of 40ms as it is in the middle of the aforementioned values of EMD. In summary, analysis of the collected EMG compared to the modelled muscle activation information could be used to understand the effectiveness of modelling muscles using the model in this research. Furthermore, the model can used to understand how it responds to those with an ACLr. Additionally, comparisons between EMG for healthy individuals and EMG for patients, and modelled muscle activations for healthy individuals and modelled muscle activations for patients can be conducted, to further examine the robustness of the model.

6.1.2 Processing Techniques to Compare Two Signals

From these queries, an appropriate way to establish the validity of the model against collected EMG needs to be identified. Frequently for this research, activation curves will need comparing to see how similar they are in terms of the peak, the phase shift (how offset left or right they are) and how similar the spread of data is.

To establish a relationship between two variables and the strength of the relationship, a correlation coefficient can be used (Schober et al. 2018), which can address the peak and lag of the data. The two most popular correlation coefficients are the Pearson coefficient and the Spearman coefficient (Schober et al. 2018). These two types of correlation coefficients are best used for linear or monotonic relationships (Schober et al. 2018), though interestingly a previous study investigating EMG against modelled muscle activations did use the Spearman correlation coefficient (Trinler et al. 2018). Another measure, linear regression, is similar to a Pearson correlation, but has one independent and one dependent variable allowing a regression line to be formed to establish one variable from the other but has no information on the strength of the correlation (Schober et al. 2018); therefore, this would not be good at establishing the strength of the relationship between the two signals in this study. Furthermore, Pearson and Spearman are not to be used for sinusoidal, known as waveform, relationships (Schober et al. 2018).

As for other ways of determining correlation, the Intraclass Correlation Coefficient (ICC) can be used to compare sinusoidal results (Iosa et al. 2014), though can be quite
complicated to calculate correctly, as there are 10 different versions with different distinct assumptions and calculations (Koo and Li 2016). Another two types of correlation coefficient that are often discussed are autocorrelation and the cross-correlation coefficient (Rowell 2008). Autocorrelation measures the self-similarity of a waveform, whilst cross-correlation investigates the cross-similarity between two waveforms (Rowell 2008). The cross-correlation coefficient is a well-established measure to compare EMG signals and is the most appropriate measure for this required situation, and produces a cross-correlation value between 0 and 1, where completely similar curves would have a correlation of 1 (Wren et al. 2006) and hence a vector of all the variation is produced along with the timing value of the signal (or the lag). A more traditional interpretation of cross-correlation results marks all data over 0.5 as very strongly correlated (Cohen 1992), therefore sometimes a more subtle interpretation is required. How to interpret the size of a correlation coefficient in this research is therefore shown below in Figure 51.

<table>
<thead>
<tr>
<th>Size of Correlation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>.90 to 1.00 (-.90 to -1.00)</td>
<td>Very high positive (negative) correlation</td>
</tr>
<tr>
<td>.70 to .90 (-.70 to -.90)</td>
<td>High positive (negative) correlation</td>
</tr>
<tr>
<td>.50 to .70 (-.50 to -.70)</td>
<td>Moderate positive (negative) correlation</td>
</tr>
<tr>
<td>.30 to .50 (-.30 to -.50)</td>
<td>Low positive (negative) correlation</td>
</tr>
<tr>
<td>.00 to .30 (.00 to -.30)</td>
<td>negligible correlation</td>
</tr>
</tbody>
</table>

*Figure 51- How to Interpret the Size of a Correlation Coefficient*

(Mukaka 2012) from the earlier source (Hinkle et al. 2003)

As a vector result is produced by the Cross-Correlation Coefficient, it can be somewhat difficult to compare between different results. The Coefficient of Variation calculation can then be performed on the cross-correlation coefficient resultant vector to aid understanding of the relationships. The Coefficient of Variation is the standard deviation divided by the mean and is a unitless measure (UCLA Statistical Consulting Group 2020), however as the inputs in this research are percentages (between 0 and 1 for the cross-correlation coefficient), it is considered that the outcome is a percentage also. A Coefficient of Variation calculation will not be performed on the cross-correlation lag values, as these results will wrap zero, causing negative and positive results, which cannot be analysed using this method (UCLA Statistical Consulting Group 2020). Additionally, in model evaluation studies, the Root Mean Squared Error (RMSE or RMS) and the Mean Absolute Error (MAE) are both popular analysis methods (Chai and
Draxler 2014). Both calculations investigate the line of best fit of the two combined data curves and measure the vertical direction of each individual result from the line of best fit. RMS takes the MAE calculation, squares the errors before it before averaging it (a slightly different step to how the MAE is taken) and square roots it. While a comprehensive study in this area of research used MAE (Trinler et al. 2018) and another investigating changes for those with knee OA (Mau-Moeller et al. 2017), it is recommended to use RMS over MAE if large errors are particularly undesirable (Chai and Draxler 2014; Medium 2020). Furthermore, when considering model error sensitivities, RMS is recommended over MAE (Chai and Draxler 2014).
6.2 Methods and Data Processing

6.2.1 Introduction
These sections define the study design, data collection, data processing workflow, ethics, data and safety, and statistics sections.

6.2.2 Study Design
This study was a quantitative, cross-sectional, observational, analytical study (Centre for Evidence-Based Medicine 2021), and all data was collected in the RCCK laboratory at the Ty Dewi Sant building in the School of Healthcare Sciences at Cardiff University, Cardiff, Wales. The subjects analysed in this study are the same analysed in Chapter 4, and the ethical approval is outlined in that Chapter.

6.2.2.1 Sample Size
A sample size of 19 applies to this study. 9 healthy participants and 9 participants with ACLr were recruited, and these 18 subjects are the same subjects analysed for Chapter 4.

6.2.2.2 Methods of Assessment
This study is focused on the EMG data that was recorded on each individual while walking at four different increasing speeds, as mentioned in further detail in Chapter 4. Gluteus medius was recorded due to it being included in a recommended EMG setup, however as the literature review progressed, it was clear it was not a closely examined value for the CCI and was then omitted.

6.2.3 Data Collection
The Equipment, Vicon and D-Flow and OpenSim, SIMM and The University of Wisconsin-Madison Model sections are discussed in Chapter 3.

6.2.3.1 Study Protocol
The study protocol is outlined in full in Chapter 4, however, the main premise was that each subject was asked to walk at an increasing self-selected steady-state speed for 4 periods of time to represent what each individual felt was slow, normal, fast and very fast walking.

6.2.4 Data Processing Workflow
Due to slight differences in the way the patient and healthy group steps were initially ‘cut’ in Vicon, steps 2 to 9 were analysed, therefore 8 steps were produced. As 8 steps...
for each of the 4 walking speeds, for 18 subjects, for 7 muscles and in 2 different muscle representations (EMG and modelled), a total of 8,064 files were analysed.

6.2.4.1 Calculations
All calculations are the same as for Chapter 4. Protocols were strictly adhered to to gather MVCs from each volunteer in different static positions. However, it was clear from the analysis that higher voltages were generated through gait itself, with other previous literature confirming that more than one position, or comparable to a dynamic movement is needed to establish the maximal activation (Hébert-Losier et al. 2011; Rutherford et al. 2011; Al-Qaisi and Aghazadeh 2015; Schwartz et al. 2020). Each EMG reading from a muscle was normalised to the maximum for that muscle from the whole walking category, to create a percentage value, with a maximum of 1. The modelled muscle activation values were already generated to create a value between 0 and 1. Considering EMD, and due to the large number of variables to analyse, an EMD value of 40ms will be applied to all collected EMG data, as taking gender differences into account is not required. It is important to note that the modelled muscle activation results do not apply any kind of EMD to their results. The modelled muscle activations had already been produced in the previous chapters, did not require further processing and were ready for analysis.

During the analysis, the Coefficient of Variation was calculated as mentioned in section 6.1.2. Standard deviations for the cross-correlation coefficient and the RMS values are unable to be established due to the way the data was analysed. Instead, standard deviations of the cross-correlation maximums were taken.

The sample rates of the EMG (1000Hz) and the modelled muscle activations (200Hz) were different, so all data was aligned at 99 data points per second. Therefore, one lag represents approximately 0.01 seconds. Additionally, cross-correlation is measured between -1 and 1, and 0 and 1 if the waveforms appear in the same y direction.

6.2.4.2 Matlab and Data Analysis
Figure 52 shows the data processing and analysis workflow for the EMG and modelled muscle activations study. Please see Appendix E for more details on these steps and examples of codes and outputs.

As discussed in section 6.1.2, the maximum of the cross-correlation coefficient, the lag and the RMS values are the main measures of interest between EMG and modelled muscle activation information for both healthy and patient groups. This is examined in 4 different ways as mentioned in the introduction of this chapter, section 6.1.1. The coefficient of variation can be used to quantify the cross-correlation coefficient vector,
in this case the cross-correlation maximums due to the way the data was constructed, so that the results can be more directly compared.

The EMG muscle data for each of the 8 steps was averaged, so that one curve represented one muscle, and the same protocol was developed for the modelled muscle activation data produced by the musculoskeletal model. Both sets of data (EMG and modelled muscle activations) could then be compared for both the healthy and patient groups, forming 4 separate analyses.

Due to noise from collected EMG, whilst Notch filters are sometimes discouraged for physiological data due to removing some interesting physiological data at this frequency (Tankisi et al. 2020), it is required for this research to remove any additional noise. The EMG and GRFs were filtered similarly as before.

6.2.5 Ethics, Data and Safety
All ethical application, recruitment, informed consent, data collection and storage procedures, as well as safety protocols, are the same as for Chapter 4.
6.2.6 Statistics
As the data examined in this study was the same as collected in Chapter 4, the same sample size is required, 19. The calculation is discussed in section 4.2.6.
A test for normality within the data was also performed using the Shapiro-Wilk test (Ghasemi and Zahediasl 2012). Shapiro-Wilk was selected over the Kolmogorov-Smirnov test, due to the latter having a lower power than the former (Ghasemi and Zahediasl 2012).
As detailed in section 4.2.6, if the data was non-parametric, an Aligned Rank Transform (ART) occurred so that an ANOVA could be performed, so that within- and between-subject information could be examined together (Conover and Iman 1981; Mansouri 1998; Leys and Schumann 2010; Wobbrock et al. 2011).
The software package SPSS was used to establish a repeated measures ANOVA with either Sphericity Assumed or Greenhouse-Geisser (depending on the results of Mauchly’s Test for Sphericity) on the data to understand the cross-correlation maximums, the cross-correlation lags and the RMS results (Statistics 2020; Tutorials 2020). Statistical significance was found if p<0.05, or if p>0.1 for the Mauchly’s test. Additionally, a repeated measure ANOVA was not possible for the examination of the cross-correlation coefficient of the EMG for the healthy and patient groups, or modelled muscle activations for the healthy and patient groups, due to the way the data was constructed. Instead, the Cross-Correlation Coefficient (maximum and lag), Root Mean Squared Error (RMS) and Coefficient of Variation were examined to establish the reliability of the data.

6.2.7 Research Hypotheses
Hypothesis 3 H₁: There is a difference in computing the co-contraction patterns of an ACLr population with a musculoskeletal model compared to that produced for a healthy population, and the model results are supported by co-contraction data collected from EMG.

With the null hypothesis (H₀) of:
Hypothesis 3 H₀: There is no difference in computing the co-contraction patterns of an ACLr population with a musculoskeletal model compared to that produced for a healthy population, and the model results are not supported by co-contraction data collected from EMG.
6.3 Results
Table 18 and Table 19 show the cross-correlation coefficient results comparing EMG to modelled muscle activations for healthy and patient groups respectively. All cross-correlation coefficient results in these two tables show at the least, a moderately positive correlation using Figure 51.

Table 18 shows that at a vector’s maximum, a comparison between a healthy participant’s EMG and modelled muscle activations at four speed categories finds between a moderately positive correlation and a highly positive correlation for tibialis anterior and lateral biceps femoris. There is also a highly positive correlation for gastrocnemius lateral head, vastus lateralis, rectus femoris, medial biceps femoris, and a very highly positive correlation for soleus. The lags in this table also show that the tibialis anterior and gastrocnemius lateral head peak wrap zero. Additionally, the peak for the vastus lateralis, medial biceps femoris and soleus are slightly negative which means the healthy EMG peak occurs before the healthy modelled peak. The peaks for the rectus femoris and the lateral biceps femoris were very negative which means the healthy EMG occurs much earlier than the healthy modelled peak.

<table>
<thead>
<tr>
<th>XCORR HEvHM</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cor</td>
<td>Lag (10ms)</td>
<td>Cor</td>
<td>Lag (10ms)</td>
</tr>
<tr>
<td>Tib Ant</td>
<td>0.71</td>
<td>(0.12)</td>
<td>-2</td>
<td>(23)</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.83</td>
<td>(0.09)</td>
<td>-4</td>
<td>(3.35)</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.86</td>
<td>(0.09)</td>
<td>-4</td>
<td>(30)</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.84</td>
<td>(0.06)</td>
<td>-16</td>
<td>(19)</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.76</td>
<td>(0.09)</td>
<td>-24</td>
<td>(27)</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.81</td>
<td>(0.12)</td>
<td>-2</td>
<td>(11)</td>
</tr>
<tr>
<td>Sol</td>
<td>0.93</td>
<td>(0.06)</td>
<td>-2</td>
<td>(12)</td>
</tr>
</tbody>
</table>

Key: XCORR HEvHM= Cross-correction coefficient for healthy EMG versus healthy modelled muscle activations; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 19 shows that at the maximum cross-correlation for comparison of a patient individual between EMG and modelled muscle activations for four different speed categories, there is between a very highly positive correlation and a highly positive correlation for soleus. There is also a highly positive correlation for tibialis anterior, gastrocnemius lateral head, vastus lateralis and medial biceps femoris. Additionally,
there is between a moderately positive correlation and a highly positive correlation for rectus femoris and lateral biceps femoris. The lags in this table show that the peak of the tibialis anterior and medial biceps femoris are positive which means the patient EMG occurs after the modelled peak whilst the peak of the gastrocnemius lateral head wraps zero. Additionally, the peaks of vastus lateralis and soleus are slightly negative, whilst the peaks for the rectus femoris and lateral biceps femoris were highly negative which means the patient EMG occurs considerably before the modelled peak.

<table>
<thead>
<tr>
<th>Table 19: Maximum cross-correlation coefficient and lag for patient subjects comparing EMG to modelled muscle activations</th>
</tr>
</thead>
<tbody>
<tr>
<td>XCORR PEvPM</td>
</tr>
<tr>
<td>Cor</td>
</tr>
<tr>
<td>Tib Ant</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
</tr>
<tr>
<td>Vas Lat</td>
</tr>
<tr>
<td>Rec Fem</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
</tr>
<tr>
<td>Med Bic Fem</td>
</tr>
<tr>
<td>Sol</td>
</tr>
</tbody>
</table>

**Key:** XCORR PEvPM= Cross-correction coefficient for patient EMG versus patient modelled muscle activations; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 20 shows the cross-correlation coefficient results for the EMG comparison between healthy and patient subjects. The correlation for all muscles in all walking categories shows a very high positive correlation. The lags between data also show no variance for all muscles, apart from slightly for the gastrocnemius lateral head and the soleus at fast and very fast speeds. As these are negative values, and the healthy data was input first into the cross-correlation calculation, means that the peak for the healthy EMG occurs before the peak for the patient EMG in these four lags.
Table 20—Maximum cross-correlation coefficient and lag for EMG comparing healthy subjects to patient subjects

<table>
<thead>
<tr>
<th>XCORR HEvPE</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cor</td>
<td>Lag (10ms)</td>
<td>Cor</td>
<td>Lag (10ms)</td>
</tr>
<tr>
<td>Tib Ant</td>
<td>0.99</td>
<td>0</td>
<td>0.99</td>
<td>0</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.97</td>
<td>0</td>
<td>0.98</td>
<td>-3</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.98</td>
<td>0</td>
<td>0.97</td>
<td>0</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.98</td>
<td>0</td>
<td>0.97</td>
<td>0</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.99</td>
<td>0</td>
<td>0.98</td>
<td>0</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.99</td>
<td>0</td>
<td>0.98</td>
<td>0</td>
</tr>
<tr>
<td>Sol</td>
<td>0.97</td>
<td>0</td>
<td>0.97</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: XCORR HEvPE= Cross-correction coefficient for healthy EMG versus patient EMG; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 21 shows the cross-correlation coefficient results for the modelled muscle activation comparison between the healthy and patient participants. The correlation is not as strong as it was for the previous table however, a very high positive correlation can be found for gastrocnemius lateral head, vastus lateralis and soleus. Between high positive and very high positive correlation can be found for tibialis anterior, rectus femoris, lateral biceps femoris, and medial biceps femoris. The lags are much more irregular for this comparison too; the healthy peak is equal with or before the patient peak for tibialis anterior and vastus lateralis, the healthy peak is equal or after the patient peak for lateral biceps femoris, medial biceps femoris and soleus. The healthy and patient peaks swap which is first and which is last for gastrocnemius lateral head and rectus femoris.

Table 21—Maximum cross-correlation coefficient and lag for modelled muscle activations comparing healthy subjects to patient subjects

<table>
<thead>
<tr>
<th>XCORR HMvPM</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cor</td>
<td>Lag (10ms)</td>
<td>Cor</td>
<td>Lag (10ms)</td>
</tr>
<tr>
<td>Tib Ant</td>
<td>0.87</td>
<td>0</td>
<td>0.98</td>
<td>-2</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.98</td>
<td>2</td>
<td>0.97</td>
<td>-1</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.95</td>
<td>-3</td>
<td>0.75</td>
<td>2</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.94</td>
<td>0</td>
<td>0.92</td>
<td>0</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.96</td>
<td>1</td>
<td>0.87</td>
<td>1</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.94</td>
<td>1</td>
<td>0.99</td>
<td>3</td>
</tr>
<tr>
<td>Sol</td>
<td>0.99</td>
<td>3</td>
<td>0.99</td>
<td>3</td>
</tr>
</tbody>
</table>

Key: XCORR HMvPM= Cross-correction coefficient for healthy modelled muscle activations versus patient modelled muscle activations; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 22 shows the RMS results for healthy EMG compared to healthy modelled muscle activation data. The lower the value of the RMS indicates a better relationship between the two data components being compared. However, there is no quantifiable definition of what a good relationship between the RMS data is as it depends on the data set that
is being analysed (Schermelleh-Engel et al. 2003). Hence the cross-correlation definition has been used in reverse to interpret the RMS results. Henceforth, data between 0.5 and 0.3 can be considered a moderate relationship, 0.3 and 0.1 can be considered a good relationship and 0.1 to 0 a very good relationship. Therefore, between healthy EMG and modelled muscle activations, a good relationship can be seen for tibialis anterior, gastrocnemius lateral head, vastus lateralis, lateral biceps femoris, medial biceps femoris and soleus. A moderate to good relationship can be seen for rectus femoris.

Table 22 - RMS for healthy subjects comparing EMG to modelled muscle activations

<table>
<thead>
<tr>
<th>RMS (S.D.) HEvHM</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tib Ant</td>
<td>0.24 (0.08)</td>
<td>0.28 (0.07)</td>
<td>0.27 (0.05)</td>
<td>0.27 (0.06)</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.24 (0.11)</td>
<td>0.24 (0.09)</td>
<td>0.26 (0.09)</td>
<td>0.27 (0.10)</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.28 (0.09)</td>
<td>0.27 (0.07)</td>
<td>0.26 (0.05)</td>
<td>0.29 (0.04)</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.23 (0.09)</td>
<td>0.27 (0.09)</td>
<td>0.30 (0.06)</td>
<td>0.32 (0.06)</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.21 (0.10)</td>
<td>0.19 (0.08)</td>
<td>0.25 (0.10)</td>
<td>0.30 (0.13)</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.20 (0.08)</td>
<td>0.18 (0.08)</td>
<td>0.16 (0.11)</td>
<td>0.19 (0.13)</td>
</tr>
<tr>
<td>Sol</td>
<td>0.16 (0.04)</td>
<td>0.18 (0.05)</td>
<td>0.19 (0.03)</td>
<td>0.20 (0.05)</td>
</tr>
</tbody>
</table>

Key: RMS HEvHM = Root mean square for healthy EMG versus healthy modelled muscle activations; Tib Ant = Tibialis Anterior; Gastroc Lat Head = Gastrocnemius Lateral Head; Vas Lat = Vastus Lateralis; Rec Fem = Rectus Femoris; Lat Bic Fem = Lateral Biceps Femoris; Med Bic Fem = Medial Biceps Femoris; Sol = Soleus

Table 23 shows the RMS values for patient EMG compared to patient modelled muscle activations. Using the same identifier as mentioned above to understand the RMS results, a good relationship can be established for the soleus and a moderate to good relationship can be seen for tibialis anterior, rectus femoris, lateral biceps femoris, and medial biceps femoris. Finally, a moderate relationship is found for the gastrocnemius lateral head and the vastus lateralis.
Table 23- RMS for patient subjects comparing EMG to modelled muscle activations

<table>
<thead>
<tr>
<th>RMS (S.D.) PEvPM</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tib Ant</td>
<td>0.33 (0.21)</td>
<td>0.30 (0.19)</td>
<td>0.30 (0.19)</td>
<td>0.29 (0.17)</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.38 (0.18)</td>
<td>0.35 (0.18)</td>
<td>0.36 (0.20)</td>
<td>0.30 (0.22)</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.36 (0.21)</td>
<td>0.36 (0.17)</td>
<td>0.35 (0.17)</td>
<td>0.34 (0.21)</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.27 (0.18)</td>
<td>0.32 (0.13)</td>
<td>0.40 (0.12)</td>
<td>0.39 (0.14)</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.33 (0.18)</td>
<td>0.35 (0.16)</td>
<td>0.39 (0.14)</td>
<td>0.41 (0.16)</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.36 (0.16)</td>
<td>0.36 (0.17)</td>
<td>0.34 (0.15)</td>
<td>0.30 (0.20)</td>
</tr>
<tr>
<td>Sol</td>
<td>0.25 (0.13)</td>
<td>0.28 (0.12)</td>
<td>0.26 (0.12)</td>
<td>0.27 (0.14)</td>
</tr>
</tbody>
</table>

Key: RMS PEvPM= Root mean square for patient EMG versus patient modelled muscle activations; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 24 shows the RMS results for healthy EMG versus patient EMG, which is relevant as it can be compared to the modelled muscle activations between healthy and patient groups to understand the differences as the model is not adapted to represent a reconstructed ACL. As can be seen, the RMS is much smaller, and hence the two data components are more closely related. There is a very good relationship for tibialis anterior, between a good and very good relationship for gastrocnemius lateral head, rectus femoris, lateral biceps femoris, and soleus, and a good relationship for vastus lateralis and medial biceps femoris.

Table 24- RMS for EMG comparing healthy subjects to patient subjects

<table>
<thead>
<tr>
<th>RMS HEvPE</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tib Ant</td>
<td>0.08</td>
<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.15</td>
<td>0.11</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.13</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.07</td>
<td>0.10</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.12</td>
<td>0.11</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.15</td>
<td>0.17</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Sol</td>
<td>0.12</td>
<td>0.15</td>
<td>0.09</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Key: RMS HEvPE= Root mean square for healthy EMG versus patient EMG; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 25 shows the RMS values for healthy modelled muscle activations versus patient modelled muscle activations. Again, very low RMS values are displayed suggesting a good association between the two compared variables. A very good relationship can be seen for tibialis anterior, vastus lateralis, and medial biceps femoris, between a good
and a very good relationship can be seen for lateral biceps femoris, and a good relationship can be seen for gastrocnemius lateral head, rectus femoris, and soleus.

### Table 25- RMS for modelled muscle activations comparing healthy subjects to patient subjects

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Slow</th>
<th>Normal</th>
<th>Fast</th>
<th>Very Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tib Ant</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Gastroc Lat Head</td>
<td>0.22</td>
<td>0.25</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Vas Lat</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Rec Fem</td>
<td>0.10</td>
<td>0.14</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Lat Bic Fem</td>
<td>0.03</td>
<td>0.04</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>Med Bic Fem</td>
<td>0.01</td>
<td>0.03</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>Sol</td>
<td>0.18</td>
<td>0.20</td>
<td>0.20</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Key:** RMS HMvPM= Root mean square for healthy modelled muscle activations versus patient modelled muscle activations; Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Figure 53 demonstrates the ensemble average for the healthy versus patient EMG or the healthy versus patient modelled results. This figure has been created so that standard deviation results can be visualised as these results cannot be represented in a boxplot format, as it would be comparing one individual from the healthy participants with one individual from the patient participants, rather than taking an average across the group. The subsequent figures comprised of Figure 54, Figure 55 and Figure 56 examine the healthy model versus healthy EMG or the patient model versus patient EMG analyses in further detail.
Figure 54 shows the plots of the maximum cross-correlation coefficients across the four walking categories for the healthy EMG versus healthy modelled results, and for the patient EMG versus patient modelled results, so that direct visual comparisons can be made. In general, all individual graphs show good correlations approaching 1, though there are generally larger standard deviation error bars for the patient analyses. For tibialis anterior and vastus lateralis, the comparison between the patient EMG and patient modelled appears stronger than for the healthy EMG and healthy modelled. The opposite is true for rectus femoris. However, for gastrocnemius lateral head, lateral and medial biceps femoris and soleus, it appears that both analyses seem similar.
**Figure S4-** Maximum cross-correlation coefficients for the 7 analysed muscles

Key: HEvHM = Healthy EMG versus healthy model; PEvPM = Patient EMG versus patient model
Figure 55 shows the cross-correlation coefficient lag for the healthy EMG versus healthy modelled muscles, and the patient EMG versus patient modelled muscles of across the 7 muscles. As can be seen, some of the error bars are extremely large and there a few outlying values too. As the y-axis scale has been adapted to observe these error bars, some of the data is hard to observe. However, the smaller the lag, the more promising the fit between the data is; therefore, the largest lag differences are present for the rectus femoris and lateral biceps femoris.
Figure 55- Lag for the 7 analysed muscles

(modified image) (Daley 2017)

Key: HEvHM = Healthy EMG versus healthy model; PEvPM = Patient EMG versus patient model
Figure 56 shows the RMS values for healthy EMG versus healthy modelled results and for patient EMG versus patient modelled results for the seven muscles visually. As the smaller the RMS value, the better, the healthy EMG compared to healthy modelled muscle activations have resulted in a consistently lower range for the seven muscles compared to the patient EMG compared to the patient modelled muscle activations, though the median values vary. There are also larger error bars for the patient EMG compared to patient modelled muscles analysis.
Figure 56- RMS for the 7 analysed muscles
(modified image) (Daley 2017)

Key: HEvHM= Healthy EMG versus healthy model; PEvPM= Patient EMG versus patient model
Table 26 shows the Shapiro-Wilk test for normality between the first two analyses; comparing the healthy EMG versus healthy modelled muscle activation results with the patient EMG versus the patient modelled muscle activation results, otherwise known as HPEvM. The second two analyses (healthy EMG versus patient EMG, and healthy modelled muscle activations versus patient modelled muscle activations) have not been statistically analysed in this way.

If $p > 0.05$, statistical significance was not found and the data was assumed to be normally distributed. This then led to the data being examined with a repeated measures ANOVA. If $p < 0.05$, the data was assumed to be not normally distributed and the ART method was utilised before examination with a repeated measures ANOVA (Conover and Iman 1981; Mansouri 1998; Leys and Schumann 2010). Many results were found to be statistically significant and hence are not individually listed in the text here.
**Table 26- Shapiro-Wilk test for normality on the study variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Shapiro-Wilk Test</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slow</td>
<td>Normal</td>
</tr>
<tr>
<td>XCORR TibAntHPEvM Healthy</td>
<td>p= 0.082</td>
<td>p= 0.133</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.373</td>
<td>p= 0.666</td>
</tr>
<tr>
<td>GastrocLatHPEvM Healthy</td>
<td>p= 0.926</td>
<td>p= 0.083</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.720</td>
<td>p= 0.555</td>
</tr>
<tr>
<td>VasLatHPEvM     Healthy</td>
<td>p= 0.540</td>
<td>p= 0.985</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.006*</td>
<td>p= 0.029*</td>
</tr>
<tr>
<td>RecFemHPEvM     Healthy</td>
<td>p= 0.149</td>
<td>p= 0.999</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.002*</td>
<td>p= 0.422</td>
</tr>
<tr>
<td>LatBicFemHPEvM  Healthy</td>
<td>p= 0.274</td>
<td>p= 0.427</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.954</td>
<td>p= 0.669</td>
</tr>
<tr>
<td>MedBicFemHPEvM  Healthy</td>
<td>p= 0.016*</td>
<td>p= 0.380</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.318</td>
<td>p= 0.585</td>
</tr>
<tr>
<td>SolHPEvM        Healthy</td>
<td>p= 0.004*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.267</td>
<td>p= 0.082</td>
</tr>
<tr>
<td>Lag             TibAntHPEvM Healthy</td>
<td>p= 0.002*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>Patient</td>
<td>p&lt; 0.001*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>GastrocLatHPEvM Healthy</td>
<td>p= 0.715</td>
<td>p= 0.009*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.014*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>VasLatHPEvM     Healthy</td>
<td>p= 0.010*</td>
<td>p= 0.019*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.165</td>
<td>p= 0.172</td>
</tr>
<tr>
<td>RecFemHPEvM     Healthy</td>
<td>p= 0.002*</td>
<td>p= 0.190</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.078</td>
<td>p= 0.017*</td>
</tr>
<tr>
<td>LatBicFemHPEvM  Healthy</td>
<td>p= 0.069</td>
<td>p= 0.011*</td>
</tr>
<tr>
<td>Patient</td>
<td>p&lt; 0.001*</td>
<td>p= 0.004*</td>
</tr>
<tr>
<td>MedBicFemHPEvM  Healthy</td>
<td>p&lt; 0.001*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>Patient</td>
<td>p&lt; 0.001*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>SolHPEvM        Healthy</td>
<td>p&lt; 0.001*</td>
<td>p&lt; 0.001*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.105</td>
<td>p= 0.326</td>
</tr>
<tr>
<td>RMS             TibAntHPEvM Healthy</td>
<td>p= 0.665</td>
<td>p= 0.708</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.012*</td>
<td>p= 0.051</td>
</tr>
<tr>
<td>GastrocLatHPEvM Healthy</td>
<td>p= 0.015*</td>
<td>p= 0.002*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.215</td>
<td>p= 0.233</td>
</tr>
<tr>
<td>VasLatHPEvM     Healthy</td>
<td>p= 0.955</td>
<td>p= 0.027*</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.015*</td>
<td>p= 0.061</td>
</tr>
<tr>
<td>RecFemHPEvM     Healthy</td>
<td>p= 0.345</td>
<td>p= 0.484</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.583</td>
<td>p= 0.263</td>
</tr>
<tr>
<td>LatBicFemHPEvM  Healthy</td>
<td>p= 0.816</td>
<td>p= 0.760</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.344</td>
<td>p= 0.060</td>
</tr>
<tr>
<td>MedBicFemHPEvM  Healthy</td>
<td>p= 0.453</td>
<td>p= 0.180</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.152</td>
<td>p= 0.135</td>
</tr>
<tr>
<td>SolHPEvM        Healthy</td>
<td>p= 0.844</td>
<td>p= 0.193</td>
</tr>
<tr>
<td>Patient</td>
<td>p= 0.012*</td>
<td>p&lt; 0.001*</td>
</tr>
</tbody>
</table>

*Sig <0.05 Statistically significant

Key: HPEvM= The healthy EMG versus healthy modelled muscle activation results (HEvHM) compared with the patient EMG versus the patient modelled muscle activation results (PEvPM); Tib Ant= Tibialis Anterior;
Table 27 shows the repeated measures ANOVA between the first two analyses, HPEvM. Any non-parametric data utilised the ART method before being examined with the same repeated measures ANOVA and is marked accordingly in the table. Green boxes represent statistically significant results whilst red boxes represent no statistical significance, and the colour coding is only present in this table due to the number of statistical results reported. As can be seen, for the most comprehensive statistical test, the within-subject effects for both speed and group, only soleus was statistically significant for the lag comparison \((p= 0.017)\), and tibialis anterior for the RMS comparison \((p= 0.036)\).
<table>
<thead>
<tr>
<th>HEvHM and PEvPM between group interaction</th>
<th>Mauchly’s test of sphericity</th>
<th>Tests of Within-Subject Effects</th>
<th>Tests of Between-Subject Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig.</td>
<td>Sphericity assumed</td>
<td>Greenhouse-Geisser</td>
</tr>
<tr>
<td>XCORR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TibAntHPEvM †</td>
<td>p= 0.211</td>
<td>p= 0.103</td>
<td>-</td>
</tr>
<tr>
<td>GastrocLatHPEvM †</td>
<td>p= 0.026</td>
<td>-</td>
<td>p= 0.544</td>
</tr>
<tr>
<td>VastLatHPEvM †</td>
<td>p= 0.014</td>
<td>-</td>
<td>p= 0.298</td>
</tr>
<tr>
<td>RecFemHPEvM †</td>
<td>p&lt; 0.001</td>
<td>-</td>
<td>p= 0.008</td>
</tr>
<tr>
<td>LatBicFemHPEvM †</td>
<td>p= 0.007</td>
<td>-</td>
<td>p= 0.126</td>
</tr>
<tr>
<td>MedBicFemHPEvM †</td>
<td>p= 0.445</td>
<td>-</td>
<td>p= 0.141</td>
</tr>
<tr>
<td>SolHPEvM †</td>
<td>p&lt; 0.001</td>
<td>-</td>
<td>p= 0.817</td>
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<tr>
<td>Lag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TibAntHPEvM †</td>
<td>p= 0.705</td>
<td>p= 0.009</td>
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</tr>
<tr>
<td>GastrocLatHPEvM †</td>
<td>p= 0.272</td>
<td>p= 0.002</td>
<td>-</td>
</tr>
<tr>
<td>VastLatHPEvM †</td>
<td>p&lt; 0.001</td>
<td>-</td>
<td>p= 0.055</td>
</tr>
<tr>
<td>RecFemHPEvM †</td>
<td>p= 0.037</td>
<td>-</td>
<td>p= 0.046</td>
</tr>
<tr>
<td>LatBicFemHPEvM †</td>
<td>p= 0.296</td>
<td>p= 0.140</td>
<td>-</td>
</tr>
<tr>
<td>MedBicFemHPEvM †</td>
<td>p= 0.004</td>
<td>-</td>
<td>p= 0.606</td>
</tr>
<tr>
<td>SolHPEvM †</td>
<td>p= 0.038</td>
<td>-</td>
<td>p= 0.201</td>
</tr>
<tr>
<td>RMS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TibAntHPEvM †</td>
<td>p= 0.197</td>
<td>p= 0.853</td>
<td>-</td>
</tr>
<tr>
<td>GastrocLatHPEvM †</td>
<td>p= 0.040</td>
<td>-</td>
<td>p= 0.696</td>
</tr>
<tr>
<td>VastLatHPEvM †</td>
<td>p= 0.085</td>
<td>-</td>
<td>p= 0.638</td>
</tr>
<tr>
<td>RecFemHPEvM †</td>
<td>p= 0.658</td>
<td>p= 0.001</td>
<td>-</td>
</tr>
<tr>
<td>LatBicFemHPEvM †</td>
<td>p= 0.007</td>
<td>-</td>
<td>p= 0.022</td>
</tr>
<tr>
<td>MedBicFemHPEvM †</td>
<td>p= 0.022</td>
<td>-</td>
<td>p= 0.120</td>
</tr>
<tr>
<td>SolHPEvM †</td>
<td>p= 0.513</td>
<td>p= 0.347</td>
<td>-</td>
</tr>
</tbody>
</table>

† Data which used the ART method

Key: HPEvM= The healthy EMG versus healthy modelled muscle activation results (HEvHM) compared with the patient EMG versus the patient modelled muscle activation results (PEvPM); Tib Ant= Tibialis Anterior; Gastroc Lat Head= Gastrocnemius Lateral Head; Vas Lat= Vastus Lateralis; Rec Fem= Rectus Femoris; Lat Bic Fem= Lateral Biceps Femoris; Med Bic Fem= Medial Biceps Femoris; Sol= Soleus

Table 28 shows the comparison between the EMG and modelled muscle activations coefficient of variation for the cross-correlation coefficient, for either healthy or patient individuals. This was derived using the maximum of the cross-correlation coefficient at the four different speed categories. This was performed to establish if the peaks were similar to each other to aid deductions. This is true for healthy individuals, apart from the medial and lateral biceps femoris, which appear to be more varying for healthy individuals than for patient individuals. Lags cannot be analysed through the Coefficient of Variation as they have both positive and negative integers.
Table 28- Coefficient of Variation for the cross-correlation coefficient between EMG and modelled muscle
activation results

<table>
<thead>
<tr>
<th>Coefficient of Variation (S.D.)</th>
<th>Healthy</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis Anterior</td>
<td>3.83% (0.03)</td>
<td>6.98% (0.06)</td>
</tr>
<tr>
<td>Gastrocnemius Lateral Head</td>
<td>1.68% (0.01)</td>
<td>3.59% (0.03)</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>2.27% (0.02)</td>
<td>3.00% (0.03)</td>
</tr>
<tr>
<td>Rectus Femoris</td>
<td>4.74% (0.04)</td>
<td>11.32% (0.08)</td>
</tr>
<tr>
<td>Lateral Biceps Femoris</td>
<td>7.34% (0.06)</td>
<td>3.00% (0.02)</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td>5.66% (0.04)</td>
<td>4.55% (0.03)</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.54% (0.00)</td>
<td>1.66% (0.02)</td>
</tr>
</tbody>
</table>

Table 29 shows the coefficient of variation for the cross-correlation coefficient between healthy and patient results for either EMG or modelled muscle activations. Standard deviation values are not available in this analysis as the mean result of healthy individuals is compared to the mean result of patient individuals. The coefficient of variation for the maximum cross-correlation coefficient is much less varied for EMG for healthy individuals compared to EMG of patients, with all muscles being under 2%. The coefficient of variation for healthy modelled muscle activations compared to patient modelled muscle activations is higher, but all values are under 10%. For the same muscle, the modelled muscle activation comparison is always higher than for the EMG comparison.

Table 29- Coefficient of Variation for the cross-correlation coefficient between healthy and patient results

<table>
<thead>
<tr>
<th>Coefficient of Variation (S.D.)</th>
<th>EMG</th>
<th>Modeled muscle activations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis Anterior</td>
<td>0.51%</td>
<td>5.77%</td>
</tr>
<tr>
<td>Gastrocnemius Lateral Head</td>
<td>0.51%</td>
<td>1.56%</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>0.98%</td>
<td>1.96%</td>
</tr>
<tr>
<td>Rectus Femoris</td>
<td>1.90%</td>
<td>9.57%</td>
</tr>
<tr>
<td>Lateral Biceps Femoris</td>
<td>0.51%</td>
<td>3.20%</td>
</tr>
<tr>
<td>Medial Biceps Femoris</td>
<td>1.54%</td>
<td>9.01%</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.59%</td>
<td>1.44%</td>
</tr>
</tbody>
</table>

In summary, this research chapter aimed to compare EMG results to modelled muscle activation results to understand how similar the ‘real world’ results were to the ‘calculated’ results. As this was coupled with comparisons for healthy and patient
groups, a four-way analysis of the data occurred. All results generally show strong relationships in the four directions of analysis, suggesting that the fundamental question of whether modelled muscle activations for healthy or patient individuals satisfy their associated EMG is indeed true. Cross-correlation coefficient maximums examined the amplitude between two curves, cross-correlation coefficient lags examined the timing between two curves, whilst the RMS examined the line of best fit between two curves. All four analyses between EMG and modelled muscle activations for healthy and patient individuals despite their differences appear strong, with no cross-correlation coefficient maximums or RMS values falling below a 50% association, and for the most part, over 70% association. This means the activation timings, amplitude of frequencies and line of best fit between two results were often extremely similar. The deeper analysis of these four comparisons and how they rank is analysed in the discussion.
6.4 Discussion
6.4.1 Overview
This chapter aimed to establish the relationship between the collected EMG and the modelled muscle activation information to establish knowledge on the validity of the musculoskeletal model for modelling patients who have relatively recently received an ACLr. Patient data was compared to their uninjured counterparts, to identify how the impulses compare to each other in magnitude (cross-correlation), phase difference (cross-correlation lag) and similarity between waves (RMS).

Analysis was conducted in four different ways; firstly, the healthy EMG was compared to the healthy modelled muscle activations. Then the patient EMG was compared to the patient modelled muscle activations. Thirdly, the EMG for the healthy and patient individuals was analysed. Fourthly, the modelled muscle activations for the healthy and the patient groups were examined. Finally, a repeated measures ANOVA was conducted to compare the first two types of analysis to each other to examine if there were statistically significant differences.

Generally, it appears from the results that the EMG versus model for either healthy individuals or for patients is extremely comparable. The model could be used to predict muscle forces in a post-ACLr population within the time period used in this research (6 years post-injury), or less since injury and subsequent ACLr. Additionally, the comparisons of EMG for both groups and modelled muscle activations for both groups are extremely similar. There were not many statistically significant results in this study, and a lack of statistical significance is required to support that the musculoskeletal model is sufficiently predicting muscle activation patterns. There is the possibility that the patient group do not perform differently than the healthy group as it is too soon to identify changes in movement patterns.

6.4.2 Hypotheses
The hypotheses may appear incorrect, but this is due to this research chapter trying to establish similarities not differences in the data. Considering a repeated measures ANOVA that analysed the first two analyses (HEvHM and PEvPM), the cross-correlation maximum value, the cross-correlation lag and the RMS value (between the EMG and modelled muscle activations) were compared to each other between the healthy and patient groups, which equated to 21 statistical comparisons. Considering the most central test, tests of within-subject effects (for both speed and group), only two displayed statistical significance, the lag for the soleus (p= 0.017) and the RMS for tibialis anterior (p= 0.036). Therefore, there was not enough statistical significance from these tests, meaning a lot of similarity between the results was found. Therefore, the null
hypothesis can be rejected and the alternative accepted. However, there is the absence of a formal test to demonstrate statistically significant equivalence for the second two analyses comparing the healthy EMG to patient EMG, and healthy modelled muscle activations with patient modelled muscle activations. This is because due to the way the data was collated, a repeated measures ANOVA was not possible. Therefore the parameters of the cross-correlation maximum and lag, RMS, and coefficient of variation can be examined instead.

For the EMG comparison, Table 20 can be analysed for the maximum cross-correlation coefficient with values ranging between 0.99 and 0.94, a change of 5%. Meanwhile, the coefficient of variation in Table 29 shows that all values are between 0.51% and 1.90% different. Examining the lags in Table 20 shows that there is a difference between 0 and -4, which is a maximum of 4% variation. Examining Table 24 for the RMS values, between very good and good relationships can be established for all muscles. For the modelled muscle activations, Table 21 shows that for the maximum cross-correlation coefficient, values range between 0.99 and 0.76, a change of 20%. Additionally, examining Table 21, there are lag values between 7 and -6, which is a 13% variation. Examining Table 25 for the RMS values shows between a very good and good relationship for all muscles. Considering both of the last two analyses, the Coefficient of Variation to analyse the cross-correlation coefficient maximums in Table 29 suggests that modelled muscle activations were generally more than for the collected EMG values. Comparing these values to the Coefficient of Variation for the first two studies have comparable ranges in the Coefficient of Variation. RMS is generally a little higher for the last two analyses compared to the first two analyses, but lags are a lot lower. The null hypothesis can be rejected, and the alternative accepted for the last two analyses.

6.4.3 Further Examination
Due to the rejection of the null and acceptance of the alternative hypothesis, it is also important to understand what the data means in more depth. The peak cross-correlation coefficient is straightforward, as the nearer the peak of two curves is to each other, the higher the value. The lower the RMS value the better the result. However, lags are slightly harder to understand; when one curve is compared to another, if there is a misalignment in the peak on the x-axis, a lag is created. Therefore, a negative lag suggests that the first variable occurs before the second variable. A positive lag suggests that the first variable occurs after the second variable. The order the variables are analysed is always written in the top left box of each table for consistency.

Considering all of the results, the strongest associations can be seen between the EMG for healthy and patient individuals. At least very highly positive maximums of the cross-
correlation vector were found, with a maximum lag for the cross-correlation coefficient of -4, a minimum good relationship for RMS, and a 1.39% range of coefficient of variation percentages with a maximum coefficient of 1.9%. This suggests that the healthy and patient individuals are highly similar in a ‘real world setting’, in terms of amplitude, timing of signals, and how close the data is to a line of best fit (RMS) between two signals.

The second strongest association is for the modelled muscle activations for the healthy individuals compared to the patient individuals. At least a highly positive maximum for the cross-correlation coefficient was found, a maximum cross-correlation lag of -6, a minimum good relationship for RMS, and a range of 8.13% for the coefficient of variation range with a maximum of 9.51%. This is to be expected as they are comparisons of the same technology, though there is some slight variance as the physiological changes in the patients could not be added to the model.

Comparisons between the healthy EMG and the healthy modelled muscle activations are closer than for their patient counterparts, suggesting either some post-injury changes have occurred or that the modelled muscle activation is less representative in patients. Therefore, the healthy EMG versus healthy modelled muscle activations were the third strongest association with a minimum moderately positive maximum cross-correlation coefficient. The healthy EMG versus healthy modelled muscle activations also had an associated maximum lag of -36, a minimum moderate relationship for RMS, and a range of coefficient of variation percentages of 6.8% with an associated maximum of 7.34%. The weakest relationship was for the patient EMG versus the patient model. There was a minimum moderately positive maximum for the cross-correlation coefficient, an associate maximum lag of -33, at least a moderate relationship for RMS and a range of coefficient of variation percentages of 9.66% with a maximum of 11.32%. Lags for both the third and fourth associations are particularly large suggesting there are large timing differences in when a muscle is activated in the model compared to in the real world. This seems associated particularly with rectus femoris and lateral biceps femoris. The way the muscle force distribution is solved in the University of Wisconsin-Madison model may need small modifications for these two muscles in terms of their activation timings before the modelled muscle activation outputs are used in further research. Understanding that patient physiological changes could not be added to the model perhaps suggests why the patient EMG compared to the patient modelled muscle activations comes in fourth position. However, it is worth noting that all four analyses despite their differences appear strong, with no cross-correlation coefficient maximums
or RMS values falling below a 50% association, and for the most part, over 70% association.

In terms of specific muscle results, the soleus muscle appears to be the muscle that varies the least in terms of the coefficient of variation in Table 28 and Table 29. However, the soleus is only one of two muscles to gain statistical significance for within-subject effects for speed and group, for lag only. However, this lack of statistical significance in other measures could well be due to this lack of variation. For the cross-correlation coefficient maximums or RMS values for soleus, at least a 70% association was established. Therefore, soleus in particular shows some very strong values through cross-correlation and RMS but shows a statistical difference for lag too.

6.4.3.1 Cross-Correlation Coefficient
The cross-correlation coefficient peak compares the peak between two signals and is measured between 0 and 1 if the waveforms are both in the same direction on the y-axis. Completely similar curves would have a correction of 1. Examining any noticeable differences between the EMG and modelled muscle activations for either the healthy or patient groups, three muscles sit in different correlation definitions as discussed in section 2.1.3.3, namely rectus femoris, soleus and tibialis anterior. For the patients, it seems that the tibialis anterior is more strongly correlated, whilst both the soleus and rectus femoris are less strongly correlated when compared to their healthy counterparts. When understanding what this means, it cannot be stated that one muscle is more or less activated for one group, as it is the correlation that is being discussed and not an absolute measurement of activation. It is difficult to distinguish the EMG differences between healthy and patient individuals as each muscle is very highly correlated between the groups. Likewise, the model activations were at least highly correlated between healthy and patient groups. The model possibly overcalculates the tibialis anterior, hence ‘increasing’ it up to a higher correlation of between high and very high correlation than is in the ‘real life’ situation of EMG. There is also the possibility that the model undercalculates the soleus and the rectus femoris, hence causing less of a correlation between the EMG and model results.

There is ever so slightly more correlation for the healthy group EMG and modelled muscle activations than for patients, but both groups are very similar. This suggests that the model appears robust in its modelled muscle activation calculations in terms of maximums as the correlation is very similar to the patient EMG.

An interesting side note about the model versus model comparison is stronger correlation was found for gastrocnemius, vastus lateralis and soleus being very highly correlated than was found for tibialis anterior, rectus femoris and medial and lateral
biceps femoris being highly correlated. All these findings are directly corroborated by previous research comparing EMG with modelled approaches for healthy individuals (Trinler et al. 2018). It was suggested that there was a better understanding of the muscle characteristics for each muscle in the lower leg compared to the muscle characteristics of the muscles of interest in the thigh section (Trinler et al. 2018). The aforementioned study appears to use similar modelling approaches as this research; the OpenSim lower leg model and muscle activations were calculated through both static optimization (SO) and computed muscle control (CMC) (Trinler et al. 2018). The findings in this chapter are particularly pertinent for future use of the University of Wisconsin-Madison model. It is worth at this point remembering that this research study is interested in the similarity between activation curves, and statistical significance is interested in looking at the difference, a somewhat juxtaposition.

Considering all statistical significance found within this study though not considering lag statistical significance as that is expanded on below, for within-subject effects for just speed, there was significance for cross-correlation for rectus femoris and medial biceps femoris, and rectus femoris and lateral biceps femoris for RMS. Furthermore, tests of between-subject effects for group only, suggest statistical significance for cross-correlation for lateral biceps femoris, and RMS for lateral and medial biceps femoris and soleus. Therefore, considering where any statistical significance was found more than once during the repeated measures ANOVA, there is more interest in rectus femoris, medial biceps femoris and lateral biceps femoris. This could also mean that the ACLr group did not change their activation patterns significantly compared to healthy individuals for the other muscles, namely tibialis anterior, gastrocnemius lateral head, vastus lateralis, and soleus. This could be because, as previously mentioned in this section, rectus femoris, medial biceps femoris and lateral biceps femoris are all upper leg muscles and there could be an effect of the model here on the statistical outcomes.

6.4.3.2 Cross-Correlation Lag
The cross-correlation lag examines how offset each of the two compared waveforms is in the x-axis. When examining the cross-correlation lags in Figure 55, it is difficult to understand the lag differences for the patient and healthy groups when comparing the model to the EMG. This is because the lags are not consistently positive or negative, though there seems to be a common correlation for vastus lateralis, rectus femoris and lateral biceps femoris, with all similar-sized negative lags between the patient or healthy group, though the latter two muscle lag figures are generally quite high. There are slightly more lags nearer zero for the healthy group. Positively, the lags being so close to zero suggests the peaks align, and the EMD value used for the EMG data was quite
accurate. The most central statistical test, tests of within-subject effects for speed and group, only suggests lag significance in the lag for the soleus (p = 0.017). However, soleus also had statistical significance for its lag between groups (p = 0.047). This suggests that there is a statistically different offset between the two waves for soleus and this may be linked to the findings in Chapter 4, where the soleus was acting in a statistically significantly different way between ACLr and healthy individuals.

It is of interest to examine which muscles have the most lags comparing healthy participants EMG and modelled muscle activations, or patients EMG to modelled muscle activations. In this case, and as mentioned in the paragraph above, the lags for the rectus femoris and the lateral biceps femoris seem the most problematic. Considering just the lateral biceps femoris first, the lag becomes increasingly negative as speed increases, and this is true for the healthy and patient participants. This means that the point of the peak of the EMG lateral biceps femoris is increasingly earlier compared to the modelled lateral biceps femoris. There could be a need for a changing EMD for the lateral biceps femoris, depending on the speed. However, the same EMD for all individuals would be required and not changing per individual, as that would add a confounding factor. The model perhaps does not manage the speed change in adapting the timing of the activations correctly; the modelled muscle activation results do not include an EMD, but the model could be proposing the activation of the muscle at a slightly incorrect time point. However, there is no clear understanding of whether the EMG peak is moving earlier or indeed if the modelled peak is moving to a later point in time. For the rectus femoris, the lag increases until fast walking and then decreases at very fast walking. This is indicative of some of the other more parabolic style behaviours discussed in Chapter 4, suggesting that activation of the rectus femoris changes when getting into an area of walking that seems more complicated and a separate gait style, somewhat nearer a jog behaviour.

For the correlation lags for the healthy EMG versus the patient EMG, there is very little difference in the lags, something that was not fully expected. Therefore, the healthy and patient groups have similar voltage timings in the different muscle groups, and an ACLr does not affect the rate at which different muscles react to control gait. The amplitudes are extremely similar for the cross-correlation coefficients too. However, for model versus model, there are some small discrepancies between the lags (both positive and negative), suggesting the modelled muscle activations calculate slightly more or less lag than is really present in the EMG. This could be driven by pre-set values in the model files needing a slight adjustment for each individual muscle, with these changes being highly speed-dependent, and a potential suggestion that the model is not fully
accounting for this speed variation in muscle activation timings. However, there is also the option that the kinematic and kinetic variations in movement patterns by patient individuals are influencing these slight differences in calculated muscle results and coupled with the model not being able to be adapted to reflect the ACLr, this is why this slight difference occurs. One muscle of interest, the gastrocnemius lateral head showed the greatest variation for modelled comparisons, either healthy started 20ms late or 60ms too soon in comparison to patients; for fast and very fast speeds this was -60ms and -30ms respectively. However, this greater variation could be caused by the slight lag present in the EMG between healthy and patient individuals. For fast (-30ms) and very fast (-20ms) walking, the gastrocnemius lateral head reaches its peak for healthy individuals earlier than for patients which were 20ms and 30ms earlier respectively. The gastrocnemius lateral head could be activating later in a patient group at faster speeds, which would explain the findings in the healthy to patient model comparison.

6.4.3.3 RMS
Root Mean Square (RMS) investigates the line of best fit between two combined data curves to examine how far each result is from the line of best fit. Figure 56 can be examined to explore the RMS results. A lower RMS result suggests data that more closely sits on the line of best fit and therefore is more representative of a population. The healthy to patient EMG and healthy to patient model results show they are closer to the line of best fit than for the healthy EMG versus model or the patient EMG versus model results. For all muscles, the healthy individuals’ model versus EMG RMS values are lower than that for the equivalent of the patient group, suggesting that for RMS values, the modelling of the muscles may be slightly more robust for a not-injured population, due to the RMS values and the standard deviation bars being much lower. Alternatively, it could be argued that as RMS represents the deviation from the line of best fit, it could just mean that the patient population is a more varied cohort and does not produce such a clear line of best fit.

6.4.3.4 Coefficient of Variation
As the results from the cross-correlation coefficient are in vector form, the Coefficient of Variation can be utilised to further analyse the vector and calculated as the standard deviation divided by the mean. The coefficient of variation results support this discussion; the EMG results between healthy and patient groups vary minimally with no variation over 2%. Modelled muscle activations vary slightly more so; soleus, gastrocnemius lateral head and vastus lateralis have lower variation for the modelled muscle activations than the other four muscles. This influence can be seen when
comparing either healthy EMG to modelled muscle activations or patient EMG to
modelled muscle activations as soleus, gastrocnemius lateral head and vastus lateralis
have the least variation, for both healthy and patient comparisons.
The approaches used in this research appear to be valid and patients did not change a
lot in their timing or amount of activation compared to healthy individuals. This could be
because the patients are too early from their injury and mal-adaptations have not
occurred yet. There is literature that supports that there are no changes in co-
contraction for affected and unaffected limbs 12 months post-ACLr during gait and one-
legged squats (Bee et al. 2016). However, other individuals with ACLr approximately 12
months post-surgery, demonstrate different muscle amplitudes when exposed to
arguably more complex tasks such as stepping down, dual tasks and perturbations
(Smeets et al. 2021). As the patients examined in this thesis are a few years post-ACLr,
there is an argument that these individuals should be showing more muscular changes.
This can be counterargued that because they were a generally young and active group,
they recovered better, however, the aforementioned reference where those 12 months
post-ACLr were exposed to complex tasks (Smeets et al. 2021) were all athletes. At this
point, it could be proposed that it is the complexity of the task that influences the
change in results. Another study for individuals 6 months to 5 years post-ACLr, who
were all young and relatively fit, found co-contraction differences during the relatively
uncomplicated task of gait, which supports the findings found in Chapter 4 but does not
explain the results here (Blackburn et al. 2019). It is at this point that it is worth
remembering that Chapter 6 has sought to identify the similarities between two
amounts of data, not the differences, and herein lies the difference: a difference in
analysis. Whilst there are a few literature articles that discuss changes in co-contraction
for individuals with ACLr, there are few that discuss fundamental changes in specific
muscles in terms of wave comparisons.
As the cross-correlation coefficient for example only discusses the similarity between
the peak in two curves, and not the fundamental value generated by the healthy and
patient groups, there can actually be wave changes for the patient group. Crucially, the
pattern of the waves in terms of the symmetry and peak are very similar between
healthy and patient individuals. This research suggests that patient individuals still
demonstrate muscular activations at similar timings and magnitudes as they did pre-
injury, but co-contraction results demonstrate more clarity on what impact this has in a
real-world environment.
6.4.4 Limitations
The largest concern in this study was the EMG equipment as the signal presented with considerable noise or occasional ‘drop-out’. Simple modifications during data collection were implemented as required such as replacing certain leads or EMG pads to improve the data. There also was some voltage resonance in the patient data, which was not present for the healthy group. Rather than it being a feature of the group, this occurred from equipment issues. Upon visual inspection, any EMG data with noise or resonance was filtered to improve the quality, and discarding of any trials due to significant anomalies was not required.

The interaction between the results from this study paired with the co-contraction results from Chapter 4 could also be considered a limitation. The co-contraction indices used in Chapter 4 paired at least two muscles together, and the statistics performed examined this interaction. Therefore, it is difficult to separate the findings from this chapter where single muscles were examined with the other chapters. Also, the statistics used for Chapter 4 for the co-contraction indices were inherently trying to find differences, whilst this chapter was involved in examining similarities. This may also be why so few components for the repeated measures ANOVA achieved statistical significance, as the input parameters being examined had to do with correlation.

It is extremely important to note an individual’s level of daily activity, their knee joint health, their general muscle condition as well as the speed they walk as it will influence the EMG results (Limbird et al. 1988). These natural variations are important to gain an appropriate sample from across a population and something that could have been explored with qualitative questionnaires such as the Knee injury and Osteoarthritis Outcome Score (KOOS) (Roos and Lohmander 2003). Additionally, the patient data could have been split into different gait modulation styles, so that more knowledge could have been formed on particular rehabilitation programmes for those demonstrating a particular gait ‘style’ (Roberts et al. 2017).

It is also worth noting the very commonly discussed issues regarding surface EMG, such as soft tissue movements and artifacts. Although effort was made to be clear with pad placement, there will always be some concern over noise and collecting results from additional or incorrect muscles (Konrad 2006; Seniam 2021). Especially for, working laterally to medially, due to the close positioning of the biceps femoris short head and long head and the large size of the EMG pads, there was a concern that the semitendinosus and semimembranosus might also be influencing the data (Gray and Lewis 1918; Konrad 2006). All participants had EMG recorded on both legs regardless of whether a certain leg was of interest to the research to avoid influencing changes in movement patterns by the subject.
It is also worth considering that there may have been some effects on the EMG or gait patterns, and hence modelled muscle activations, due to a participant being in a new environment. The lab can be observed as quite high-tech and the self-paced application, as well as a treadmill in general, can be a lot to understand for an individual. This is why recruitment required people who knew how to use a treadmill and why additional time was given for people to understand how to use the self-paced application effectively.
6.5 Conclusions
Modelling muscle activations through inverse dynamics and comparing to collected EMG shows that during gait for lower limb muscles, healthy and patient cohorts appear to act extremely similarly, in terms of phase error, peak error and RMS values. The least close analysis was for patient EMG compared to patient modelled muscle activations, possibly due to the lack of opportunity to adapt the ligament component of the model for an ACLr to more accurately predict results. There appear to be closer connections between lower leg muscles than upper leg muscles for EMG to model comparisons, which is supported by previous findings.

Whilst the patient group demonstrate very similar muscle timings and amplitudes, caution should be used in interpreting these results to a real-world scenario, and measurements like CCI provide more understanding of how a patient is performing in a task such as gait to identify any mal-adaptations.
7. Discussion

7.1 Introduction

This research aimed to collect biomechanical data from healthy and post-ACLr patients, coupled with step width differences and EMG versus modelled muscle activation differences, to understand dynamic knee stability and how it is affected by ACLr, through the use of musculoskeletal modelling. This chapter discusses the prior knowledge linked with the interpretations garnered from the previous research chapters, to understand the contribution this research gives to knowledge and future research, and its real-world implications for clinical practice.

Whilst there is existing knowledge on movement parameters for healthy individuals such as angles and moments, particularly for gait, much less is known when linking it to other gait quality indicators such as stiffness, co-contraction and joint surface loading (defined in this research as the representatives of dynamic knee joint stability). The same is true for individuals with ACLr. Prior literature discussed the link between ACL injury/ACLr and OA development (Risberg et al. 2016; van der List and DiFelice 2017; Sharifi et al. 2018; Vaishya et al. 2019; Snoeker et al. 2020), through increased co-contraction and other changes in biomechanics (Georgoulis et al. 2010; Blackburn et al. 2019), and how ACLr may not be the best option for restoring pre-injury function (Georgoulis et al. 2010). However, little has been analysed in changes after ACL injury in terms of analysing dynamic knee joint stability (Rudolph et al. 2000; Chmielewski et al. 2002). There appears to be no literature defining dynamic knee joint stability holistically after knee injury, especially when coupled with the use of musculoskeletal modelling.

However, there is one recent study investigating components of dynamic knee joint stability in healthy individuals, but not defining the concept of dynamic knee joint stability in absolute terms, and not using musculoskeletal modelling including joint loading to do so, but instead spring-like mathematical modelling (Akl et al. 2020). There is also no clear knowledge of knee joint contact forces at different speeds for individuals with ACLr using musculoskeletal modelling, though knee joint contact force knowledge is available for an average walking speed (Saxby et al. 2016b), or for general lower limb musculoskeletal injuries (Lenton et al. 2018).

There is a need for musculoskeletal modelling to resolve queries around dynamic knee joint stability too. Whilst a knee’s function can be recorded through a static test utilising a kinematic dynamometer or even through a simple repetition movement such as a lunge, the knee spends much of its day working dynamically mainly through the cyclic movement of gait with subtleties of surface texture, speed, surface stability and inclination to overcome (Andriacchi et al. 2004; Fernandes et al. 2016). A kinematic
dynamometer can establish the knee strength, or the lunge can establish the knee range, but it does not gauge what the knee is doing most often in a day and how, and what impact that has on the joint in return. As it cannot be established in real-time through the marker and GRF information alone what scale and pattern of forces travel through each of the knee’s compartments (List et al. 2017), it falls to musculoskeletal modelling to estimate these unknowns (Hicks et al. 2015).

Speed is an interesting variable to consider, due to the link between speed decline and pathological gait (Fukuchi et al. 2019a). Studying the effects of speed on dynamic joint stability measures, would raise questions such as: would a subject’s knee become stiffer when walking at a speed faster than normal? Would their co-contraction and joint loading increase? The interest of this research was to establish how people with an ACL injury adapt their movement control strategies, if these adaptations can suggest knee health decline and how this works to understand the link between ACL injury and early OA development.

To account for the different speeds produced, the results were divided into the four main speed categories of slow, normal, fast and very fast. These four categories aimed to understand how healthy and patient individuals controlled speed changes and the categories were defined to the individual as a leisurely stroll around a park, normal daily walking, late for an appointment and the fastest you can walk without running. It was important to define them in realistic scenarios, though as was discussed particularly in Chapter 4, some individuals varied these speeds a lot, so that ‘fast’ for one person, was ‘slow’ for another, suggesting the speed categories could have been better defined to the individual. Furthermore, it was still important to remove inclination, surface texture changes like concrete versus grass differences, and surface stability issues, such as a slippery or rocky surface requiring more internal balance, to try and provide as similar a scenario between participants as possible. This is because destabilising terrain can drastically affect the resultant biomechanics (Foster et al. 2020), which was easily resolved as all subjects were utilising the same laboratory-based treadmill. Literature information provided knowledge that all participants needed to wear similar shoes whilst under analysis (Roman de Mettelinge et al. 2015) and participants needed regular breaks to ensure the muscles were well rested. Well rested muscles were also applicable for MVC measurements, and MVCs needed to be taken at the start of the session (Konrad 2006). However, MVCs did not produce values anywhere near as high as those performed during gait itself, thus validating the cruciality of dynamic not static data collection, as found in other research (Al-Qaisi and Aghazadeh 2015).
7.2 Stiffness
Firstly, it is worth examining the role of stiffness for healthy and patient participants; with the examination starting with knee stiffness. As garnered in the discussion of Chapter 4, knee stiffness was examined in less depth due to its lack of statistical significance and caution around some of the results producing either negative or positive values. As found in Chapter 4, these negative and positive values were assumed to be an individual’s style and whilst statistical significance was not found, the fact that the knee stiffness average results did not vary greatly for most of the healthy or patient individuals in Figure 32, is of interest. Please note that whilst there is less range in these values for the patient individuals compared to the healthy individuals which was to be expected, there is perhaps more interest in the fact that both groups displayed no clear directional trends in knee stiffness. These results appear to suggest that knee stiffness does not change with different speeds but instead regulates to keep near constant.

Analysing recent existing literature to understand if there have been previous links between knee stiffness measurements, preferably during different gait speeds, with a near-constant knee stiffness value, establishes one recent study which deduces the same (Akl et al. 2020). As this thesis chose knee stiffness to be deduced from IC to maximum flexion angle, knee stiffness can actually be considered knee flexion quasi-stiffness (Shamaei et al. 2013c). A further subsection from maximum flexion angle to maximum extension angle is deemed knee extension quasi-stiffness. The two measurements averaged together to make the overall knee quasi-stiffness in the weight acceptance phase (Shamaei et al. 2013c).

Additionally, the discussion in Chapter 4 discussed quasi-stiffness in terms of leg stiffness, but the term quasi-stiffness can be used whenever lower limb joints and spring-like behaviour are being considered (Shamaei et al. 2013c). One paper states that it considers the knee flexion quasi-stiffness phase, however continues to discuss the results as if they are representing the knee quasi-stiffness for the full weight acceptance phase (Akl et al. 2020). Another paper considers the full weight acceptance phase, or knee quasi-stiffness in the weight acceptance phase (Shamaei et al. 2013c). Neither of these two papers derive any clear directional trends for knee quasi-stiffness during normal gait, though it is deemed that knee flexion quasi-stiffness and knee extension quasi-stiffness are similar at preferred gait speeds, and knee flexion quasi-stiffness does increase at very high speeds (Shamaei et al. 2013c). Furthermore, Shamaei et al. (2013c) establish that knee flexion quasi-stiffness, and in turn knee quasi-stiffness, for the average adult does not display any clear trend between 1.2m/s and 2.0 m/s, and similar results were found across all adults. The combination of participants selecting their own preferred gait speeds in this thesis, coupled with no clear trends across normal gait
speeds for the two aforementioned papers (Shamaei et al. 2013c; Akl et al. 2020) support the lack of statistical significance found in this research.

It appears that a more subtle control strategy is occurring at general walking speeds, not considering very slow or very fast speeds. It raises the query of why there are near-constant behaviours for knee stiffness at normal gait speeds and how this occurs.

Firstly, it is worth remembering that knee joint stiffness is comprised of the components of the difference between knee joint moments and the difference between knee joint angles, with the boundaries of the calculation for this research being between IC and the maximum moment at flexion angle time (Zeni and Higginson 2009b). Due to the mathematical foundation of the knee joint stiffness calculation, if the moment difference increases, knee joint stiffness increases, and the reverse happens if the angle difference increases. Examining the flexion angles and moments for a healthy individual indicates that both increase as speed increases and that if both increase by a similar factor, very little knee joint stiffness change will be present at different speeds.

Understanding that those with ACLr produce less knee angle ROM (Slater et al. 2017), it is acceptable that knee joint stiffness will be increased even if moments do not increase; Table 8 in section 4.3 shows that both peak knee flexion angle decreases and peak flexion moments at angle time increase for patients, assuming that IC moments and angles are similar.

Secondly, it is worth considering previous literature investigating surrounding lower limb joints. One argument is knowing that just hip (Jin and Hahn 2018), or hip and ankle joint stiffness increases with increasing speeds (Akl et al. 2020) perhaps the hip and ankle joint stiffnesses adjust to help maintain knee joint stiffness. Examining the ankle in particular quasi-stiffness sections appears more complex, with no clear speed-related trend in another study (Shamaei et al. 2013a). Another study examining quasi-stiffness, this time for the hip, appears to suggest that hip flexion quasi-stiffness increases with speed for most individuals (Shamaei et al. 2013b), which could complement the aforementioned knee extension quasi-stiffness increasing with speed (Shamaei et al. 2013c), hence demonstrating a spring-like gait at the hip joint. However, hip extension quasi-stiffness can vary at different speeds and not show a clear pattern (Shamaei et al. 2013b), which could supplement the deduction that knee flexion quasi-stiffness displays no real trend in this research. Additionally, knowing that stance phase gait energy tends to absorb proximally in the lower extremity joints at increasing speeds (Jin and Hahn 2018), it has been plausible that the hip is the more likely factor for stiffness regulation of the leg. However, understanding from this research that soleus and gastrocnemius lateral head have significant differences for patient individuals, suggests changes more
distally in the lower extremity joints. Next, it is worth exploring why the regulation of knee joint stiffness is important.

It could be argued that the knee stiffness considered in this research, deemed knee flexion quasi-stiffness (Shamaei et al. 2013c), is exposed to a lot of factors during the gait cycle. An individual is required to bear the largest GRFs during the gait cycle, deaccelerate/dampen their movement and maintain the stability of an unstable joint, all at once (Schrijvers et al. 2019). Therefore, it is an extremely beneficial strategy to try to ‘shift’ some of the burden onto surrounding joints, particularly the hip as it is a more stable joint with a large amount of surrounding muscle to dampen deacceleration and the associated force components (Jin and Hahn 2018).

Next, leg stiffness in this research requires further examination. Statistical significance was established for increasing speeds for the healthy group ($p= 0.007$), but not for the patient group ($p= 0.068$), though this in itself could be a finding, as has been seen from the knee joint stiffness results. Additionally, as the patient group was very close to statistical significance, a future study may find significance between these factors. Considering the same middle segment of speeds as for the knee joint stiffness discussion, the main query would be how the parabolic leg stiffness results in this segment for healthy individuals interact with the linearity of the knee joint and hip joint stiffnesses to produce movement control strategies.

Once again, the components of the calculation need consideration. The maximum GRF during stance, which is approximately when the knee is in maximum flexion for maximum ‘spring’ compression, is divided by the change in the vertical component of the whole-body CoM between the maximum and minimum values from the whole of the stance phase. The maximum and minimum vertical components of the whole-body CoM are approximately mid-stance and IC respectively. Comparing the start and finish of the calculation, or the boundaries of the calculation, with the joint stiffness boundaries means that both consider the full period of the first half of the stance phase, through to mid-stance approximately. Though joint stiffness considers the flexion component more as mentioned earlier, the leg stiffness calculation would consider the whole period across knee flexion and knee extension quasi-stiffness. The knee flexion quasi-stiffness does not change considerably, but the knee extension quasi-stiffness as well as the hip flexion quasi-stiffness decreases as speed increases. It is reasonable to assume that these components are therefore linked to the changes in the leg stiffness results decreasing with an increased speed. Therefore the factors influencing this change need examining. Understanding that either the difference in the knee extension moment is decreasing or the difference in the knee extension angles is increasing, there is not
enough published data from the referenced study to derive the component causing the change at different speeds (Shamaei et al. 2013c). It would be of interest in future research to investigate which component defining joint quasi-stiffness, the difference in moment or angle values for both the knee and hip, is interacting with the leg stiffness results. A deeper understanding of the control strategy for individuals with ACLr could then be sought.

7.3 Co-Contraction
Leading on from the discussion about stiffness, the biomechanical implications on the lower leg system from an internal input, namely co-contraction, can be considered. The main question that has arisen from this research is do certain muscle synergies (in this case, each CCI) indicate a certain movement mechanism during the stance phase of gait? Whilst there has been some exploration in the literature using limb dynamics to define limb movements, little links co-contraction with limb movements (Na and Buchanan 2019). This is a purely exploratory discussion, due to the lack of statistical significance between the results from the healthy and patient cohorts, though the lack of difference in co-contraction has been found in other studies previously too (Collins et al. 2014).

This lack of statistical significance is possibly due to the ease of a movement such as unchallenged gait (Na and Buchanan 2019), with more difficult movements, such as step-down tasks, demonstrating clearly altered neuromuscular responses between groups (Smeets et al. 2021). It is also possible that limb dynamics such as linear acceleration and jerk, and by association, co-contraction, vary more with challenging walking alone than for other influencing factors, such as OA (Na and Buchanan 2019). However, challenging movements, such as movements exposed to perturbation training, appear to aid motor learning, and lower co-contraction in time, aiding a more coordinated muscle strategy (Chmielewski et al. 2005a), though importantly, this is for copers only. False-negative results can occur with a small sample size (Peterson and Foley 2021). Therefore, a larger future cohort could demonstrate a trend change between groups. Also, as mentioned above, while there appears to be a strategy for copers to improve, less is known about how to improve the muscle strategy for non-copers (Chmielewski et al. 2005b).

Whilst this discussion could dissect each muscle pair or group forming each CCI calculation and how that would change the biomechanical positioning, this may be too simplistic. Previous research has established that between different strides for healthy individuals during self-paced steady-state gait, one muscle alone can be expected to have up to five different activation modalities during the gait cycle, and these five
modalities also stand true for the stance phase (Strazza et al. 2017). This means that neuromuscular strategies are not strictly repeatable, even when the same movement is being performed cyclically with no challenges making it more difficult such as perturbations (Sheffler and Chae 2015). The traditional activation patterns for each muscle doing exactly the same during each stance phase, as discussed previously in the literature review for Chapter 4, do not apply (Sheffler and Chae 2015). There are however, more common patterns for activation than others (Strazza et al. 2017), so an average scenario can be discussed, but there would be beneficial future work in analysing these different activation modalities for common muscles in a post-ACLr cohort, to be able to separate the neuromuscular control pattern changes for these individuals.

For CCI Five, it is understandable that there is a between subject effect for group (p=0.010) and that the co-contraction is higher for the patient individuals as the quadriceps femoris components (rectus femoris and vastus lateralis) form knee extension in early stance and synergistic preparation for weight bearing during early stance (Strazza et al. 2017). If there is a lack of confidence in the weight-bearing activity as there can be for patient individuals, then the increased co-contraction can be understood; co-contraction of the hamstrings and quadriceps is known to reduce ACL elongation (Serpell et al. 2015). This could be interpreted to cause increased co-contraction to offset the deficit from a reconstructed ACL. Interestingly, this concept only works if the medial hamstring-quadriceps components outweigh the lateral component (Serpell et al. 2015), and this, along with an assumption that there is increased co-contraction to offset the deficit from the reconstructed ACL, could also explain the increased co-contraction values found in CCI One (between subject effects for group p=0.019). Additionally, the increased activation of the hamstrings increases the knee flexion angle to restrain anterior tibial translation (Mengarelli et al. 2018), and the ACL restrains anterior tibial translation (Grood et al. 1984). Hence an ACL insufficiency could produce the same outcome, which is supported by the knee flexion findings for patients in this research (between subject effects for group p=0.010), which is also supported by the knowledge that hamstring activation protects the ACL (Adouni et al. 2016). However, the other muscle components contributing to CCI Five must also have increased, otherwise, co-contraction would not increase overall; CCI One has already been fully analysed as they both use components of the quadriceps femoris and the hamstrings, whilst CCI Three did not establish enough significance. For CCI Five, this is namely the gastrocnemius lateral head. The gastrocnemius acts to flex the knee joint, as the hamstring component does but acts more like the quadriceps component in the fact that the gastrocnemius
acts as an ACL antagonist and loads the ACL (Adouni et al. 2016). So why would the gastrocnemius component be more activated in patients rather than less if it has a negative effect on a reconstructed ACL? It could be that the gastrocnemius is acting like the quadriceps in more of a stabilising role rather than an ACL-protective one, and the other muscles in the synergies increase their activations accordingly to try to offset the damaging impact on the reconstructed ACL. Next, CCI Two requires examination (between subject effects for group p= 0.001). Interestingly gastrocnemius lateral head paired with components of the quadriceps femoris (vastus lateralis, the same as in this research) can cause increased ACL strain if either muscle activates without the other muscle (Mengarelli et al. 2018). This could very simply explain once again why co-contraction is greater for these two muscles; the ACLr has not successfully restored full pre-injury function and these two muscles activate more to reduce the strain on the ACLr. Finally, CCI Four can be examined (within subject effects for speed and group p= 0.039). In this research, it has been the most interesting of the five index calculations due to the differing nature of the soleus for patients across different speeds. The increase in activation of the soleus for patients was deduced in the previous discussion sub-sections to occur due to its role as an ACL agonist (Mulligan 2012). Interestingly it is known that increased co-contraction with tibialis anterior and gastrocnemius will cause the soleus to otherwise increase its activation (Wang and Gutierrez-Farewik 2014), suggesting a specific synergistic interaction.

7.4 Loading
A few loading queries have arisen during the results for the healthy and patient individuals which require further analysis. In Chapter 4, the results discussed higher loading results for the second half of stance rather than for the first peak during gait for both medial and lateral tibiofemoral compartments. This was an unexpected result due to the understanding that more loading occurs during deacceleration into stance with the knee experiencing contact forces as the lower limb loads whilst also trying to maintain its stability (Saxby et al. 2016b). It is therefore interesting to examine what influenced this result. The loading in the second peak increased as speed increased. There is previous literature that supports the lateral tibiofemoral 2nd peak load being higher than the 1st peak load for gait, but not for the medial condition (Lenton et al. 2018). However, another literature study for gait comparing instrumented loading, generic modelling and subject-specific modelling using CT scans on tibiofemoral contact forces, found that second-peak lateral loading could often be higher than the first peak (Dumas and Moissenet 2020). There was the possibility that occasionally the second
peak for the medial loading was higher too using any of the three measurement techniques (Dumas and Moissenet 2020). It was deduced that generic kinematic constraints produce a ‘fair’ comparison to instrumented measurements, with ‘minor’ improvements to be expected (Dumas and Moissenet 2020), even though the model used was not from the same source as used in this research. Additionally, comparing the results from this research to previous research using the same model demonstrated when the knee is in the correct knee alignment, it is expected for the second peak medial tibiofemoral forces to slightly exceed the first peak (Van Rossom et al. 2019). The aforementioned study appears to show that for the lateral compartments, the first peak is slightly higher than the second peak (Van Rossom et al. 2019). This possibly shows that the musculoskeletal modelling has deduced that some of the first peak contact forces are not in the medial compartment (as would have previously been thought from generic research) but have possibly shifted to the lateral compartment during the first half of stance and the loading response (Van Rossom et al. 2019).

It is therefore concluded that the results from the data show acceptable results for both medial and lateral tibiofemoral compartments having higher peaks for the 2nd peak compared to the 1st and demonstrate an individual’s style. However, there is an argument for utilising fluoroscopy-informed adjustments for those in a patient cohort, particularly those with signs of join mal-alignment and OA, due to the adjustment in the contact points for both medial and lateral compartments in the tibiofemoral joint (Dumas and Moissenet 2020). Knee OA of the tibiofemoral joint causes clear physical changes to the interacting surfaces in the tibiofemoral joint (Andriacchi et al. 2004) and hence, if considering individuals with significant signs of OA ‘wear’, it would not be legitimate to use a musculoskeletal modelling process with ‘healthy’ measurements as its basis.

Another query that has arisen during this thesis, is the link between the knee loading results and the knee joint moment results. In Chapter 2, the importance of the link between the eKAM as a surrogate measure for the tibiofemoral joint loading was discussed (Hunt et al. 2008; Yocum et al. 2018). Chapter 5 found statistically significant results for the peak eKAM between over ground and treadmill walking (p= 0.010) and how that impacted the joint loading results (peak medial and lateral both p= 0.028, total medial p= 0.028, total lateral p< 0.001). However, there is an argument that sagittal moments should also be considered when investigating medial contact loading (Creaby 2015). A repeated measures ANOVA in Chapter 4 found statistical significance for group and speed for knee flexion moment (p= 0.003), and there was statistical significance between groups for medial and lateral total loading (p= 0.001 and p= 0.019).
respectively). There could be a link between knee loading and moments in the frontal and sagittal planes in this research and future statistical examination directly between these components is required.

It would also be interesting to expand on this research in the future by finding the maximum for internal extension moments so that the link between knee joint moment values and knee loading could be explored further. There has been some previous research which ascertained that patients after ACLr have smaller maximum external knee flexion moments which caused lower tibiofemoral joint contact forces in patients (Saxby et al. 2016b). This could also explain why the tibiofemoral contact forces are lower for patients in this research too when both the lateral and medial results are added together as can be seen in Table 9 in section 4.3. However, it is interesting that the medial tibiofemoral joint compartment is less loaded but the lateral tibiofemoral compartment is more loaded for patients, possibly suggesting that the ‘absorbing’ type of gait that patients have is shifting the loading laterally, which could be a potentially extremely damaging result (Horita et al. 2002).

7.5 Impact of ACLr on Patient Strategy
As outlined so far in the discussion, healthy and patient individuals have similar knee joint stiffness results, though there are changes at different speeds for patient individuals (p= 0.036). Leg stiffness changes more for speed than for different groups (within-subject effects for speed p= 0.001). Co-contraction is statistically higher for patients in comparison to CCI One, Two and Five (p= 0.019, p= 0.001 and p= 0.010 respectively). There are significant changes for CCI Four between speeds and group (p= 0.039), where there appear to be changes for soleus. Medial tibiofemoral joint forces are lower for patients than healthy individuals (p= 0.001), but lateral tibiofemoral joint forces are higher (p= 0.019). This information together can inform what sort of dynamic knee stability strategy the patients are developing in this research.

It appears that whilst patients produce higher peak knee flexion angles and lower peak knee flexion moments, this is not reflected in between subject effects for knee stiffness. Patients’ knees are more angularly flexed through stance and stiffer in terms of their peak internal knee flexion moment, though the leg stiffness results specifically do not show this. This may mean that patients appear to produce a less ‘bouncy’ gait, and in fact, possibly more of an ‘absorbing’ type gait (Horita et al. 2002). This would explain why the knee joint was more flexed for the patient group during stance, as when an inadequate landing with the ground occurs, there is a deep knee flexion after IC; this knowledge is developed from drop jumping literature, but this information is still
relevant (Horita et al. 2002). More recently, an increased flexion angle during stance for destabilised individuals is also present during gait (Foster et al. 2020), further supporting this research. There is also the suggestion that an absorbing type of motion rather than a bouncing type of motion is less efficient for forward motion and more fatiguing (Horita et al. 2002). Increases in flexion angles with decreases in flexion moments found in this research demonstrate the patients’ attempt to increase their shock-absorbing mechanism (Foster et al. 2020), due to less reliance on the stabilisation of the reconstructed ACL. However, less knee range of excursion can mean that the dampening and shock absorbing of the GRFs is ineffective, and it falls to proximal joints to address, causing more issues to the hip (with hip joint quasi-stiffness discussed earlier), as well as the spine (Holt et al. 2003). There can also be increased eccentric contractions causing muscle damage (Horita et al. 1996) as CCIs One, Two, Four and Five were higher for patients compared to healthy individuals in this research, which could lead to delayed muscle onset soreness (Holt et al. 2003). Therefore, future research investigating muscle soreness coupled with an expansion on this research would be beneficial in corroborating the theory that a post-ACLr cohort attempts to increase the shock-absorbing mechanism of the lower leg to compromise for their lack of fully-restored ACL function.

7.6 Real-World Impact
The opinion of the NHS derived from surgical papers of ACLr establishes successful restoration of function in 80% of cases (NHS 2020) but this does not tally with the findings in this research. This research has sought to establish the links between knee injury, subsequent knee ACLr and unusual gait biomechanics possibly leading to OA development.

Joint deterioration is somewhat limited historically, and knee OA care can improve by being more proactive with treatment plans, such as weight loss or pain medication, considering the individual over a longer term and increasing the attention on psychosocial aspects (Teo et al. 2020). More focus should be placed on preventive measures gained from early to intermediate outcomes in trials (Hunter and Bierma-Zeinstra 2019), and surgery to address issues such as OA should be seen as a last resort (Hunter and Bierma-Zeinstra 2019). Therefore, there is a real need to establish knowledge and support ACL reconstructed individuals through recovery and rehabilitation before altered loading patterns and subsequent knee OA occur. Considering the findings in this research, there needs to be clinical input into ensuring individuals with ACLr maintain a healthy range of knee angles during the stance phase of
gait. This is to ensure that a more efficient ‘bouncy’ type gait pattern is occurring, as supported by literature when training for drop landings, which is still applicable in this situation (Tsai and Powers 2012). This would therefore increase the internal knee flexion moment, and work towards aiding less co-contraction, ensuring the knee worked in a more flexible manner rather than just as a lower limb stabiliser. It is possible that with the development of technology, some rehabilitation could occur through home-based mobile application methods using image tracking or feedback to aid in increased engagement from patients (Shepherd et al. 2016).

Musculoskeletal modelling is becoming widely used in research but remains less used in medical device design and clinical settings due to caution around accuracy and reliability (Hicks et al. 2015). Particularly for clinical settings, it is also due to the lack of easy-to-use interface with quick results, and historically slow technology adoption in healthcare (Smith et al. 2021). There is a possibility however, that with the speed of musculoskeletal development currently, that in a few years, musculoskeletal modelling could be utilised by the clinician with contribution from the patient, further aiding patient participation and responsibility of the individual in the management of their health condition.

7.7 Limitations
One of the major limitations in this research however was modelling these patients with an un-adapted model to reflect the injury and subsequent repair. In terms of co-activation patterns, there seems to be minimal change between groups, though the model shows significant kinematic and kinetic differences between groups. It would be useful in future research to apply adaptations to the model in terms of positional changes, material property changes or any other physical adaptations to the model deemed necessary to understand subtler differences between injured and uninjured groups.

A clear theoretical framework for dynamic knee joint stability was not confirmed in this research, due to lack of statistical significance in some of the differences between healthy and patient participants. Whilst extremely interesting patterns and deductions were found, it is hoped that a future research project could gain more statistical significance in the differences by recruiting a larger sample size and increasing the power of the research (Peterson and Foley 2021). Additionally, it is beneficial to report the confidence intervals in future research to establish the magnitude of the relationship between the groups (Nead et al. 2018; Peterson and Foley 2021).
A small limitation of this research was the speed of the gait produced; it could have been fixed in this research. However, there was the fear that if a fixed speed was implemented, the subtleties of what a subject normally does, when not under the pressure of speeding up or slowing down to are narrow speed requirement for research purposes, would not be produced. This issue has been addressed in previous research (Strazza et al. 2017), where it has also been argued that naturally selected pace allows for better EMG repeatability, as well as for other general gait variables too (Kadaba et al. 1989).

Traditionally in biomechanics, opposite movement directions are discussed, such as flexion compared to extension, both in terms of angles and moments, and the latter of which is often coupled with the addition of internal and external moments. In this research, it could be mistaken that the results did not consider opposing movement directions, as while this research discusses internal knee flexion moments, internal knee extension moments are not explored. The mistake could then be made that this research has not deduced knee joint stiffness correctly, as it is discussed in the literature (section 2.1.3.2.2) that external flexion moments are required, otherwise known as internal extension moments. However, the methods (sections 4.2, 5.2, 6.2 and the Appendices) mention that the internal knee flexion moment at maximum flexion angle time was sampled. This is because the musculoskeletal model used in this research produces only one angle and moment column of data per plane, and for the knee sagittal knee plane, is named the knee flexion angle and moment. Hence, when sampling the internal knee flexion moment at maximum flexion angle time is discussed, what is meant is that the general sagittal knee moment was sampled at the maximum flexion angle time. If the result was positive, an internal flexion moment occurred, and if the result was negative, an internal extension moment occurred. The moment and knee stiffness calculations were conducted correctly, though there may be some confusion with the naming protocol.

Gait was the primary movement analysed. Further research would benefit from analysing an array of functional movements for uninjured and injured groups.
8. Conclusion
This research addressed how those with an ACLr had altered knee functionality compared to uninjured individuals. Three studies, comprised of one observational study and two smaller study chapters, were implemented to identify gait differences between groups using measurements representing dynamic joint stability. Firstly biomechanical and dynamic joint stability factors were analysed between healthy and patient populations. Interestingly, it was found that there was lateral offloading of the tibiofemoral joint during the stance phase for these healthy individuals. This fuelled the investigation of the next chapter, where frontal plane kinematics were examined between treadmill and over ground settings to understand if step width changes between settings were behind the lateral offloading results. Finally, the third study investigated the muscle activation differences between collected EMG and modelled muscle activations to determine if there were changes in the musculoskeletal model for a patient population considering that the model was not adapted to reflect the reconstructed ACL.
Medial loading during gait is less for patients than it is for healthy participants, potentially due to data collection occurring on a treadmill setup which causes statistically wider step widths, and a more varus knee angle, altering the loading pattern and eKAM results significantly. The result on total mean contact loading for those on a treadmill means a shift of up to 25% of the lateral loading moving onto the medial compartment of the tibiofemoral joint, assuming of course that the individual displays wider step width on a treadmill setup, as they did for this research study. This meant that while both healthy and injured individuals used the same treadmill setup and hence the direct comparison is possible in ratio or percentage terms, some caution is required when analysing medial and lateral loading in fundamental terms when the results were garnered from a treadmill situation.
Additionally, between healthy and patient participants there was statistical significance for the change in CCI Four. This is specifically because of the muscle soleus, which is not considered in the other CCIs. Soleus is known to act in a stabilising capacity by preventing anterior tibial translation, hence is considered to be an agonist of the ACL. More focus in the future is required on the soleus for an ACLr cohort, with it possible that the soleus could indicate how successful an ACLr has been by observing the amount of non-linear activation, particularly for higher speeds.
Comparing EMG and modelled muscle results, patient participants were not experiencing muscular activation effects from the ACLr, either because the analysis was conducted on patients too soon post-injury, or possibly because the cohort was young.
and fit, was not as affected in terms of changes in their muscle activation patterns. In some form, statistical significance was found across all muscles in different analyses, but soleus, vastus lateralis and gastrocnemius lateral head displayed the smallest variations, as demonstrated by the coefficient of variation, across all four sub-analyses. The musculoskeletal model was not adapted for an injured and reconstructed ACL and yet, it seems, that patient modelling was very accurate when compared to patient EMG. However, EMD requires future analyses, with each muscle requiring an individually prescribed EMD, and possibly longer EMD values for patient individuals.

This research looks to address the differences between an uninjured and ACLr patient group, 6 years post-surgery in terms of dynamic knee joint stability during gait, with particular focus on knee stiffness, leg stiffness, 5 different co-contraction indices and medial and lateral tibiofemoral knee joint loading. The strongest statistically significant representative of dynamic knee joint stability is the peak internal knee flexion moment and CCI Four (soleus and gastrocnemius lateral head versus tibialis anterior). Individuals with ACLr are more flexed during the stance phase of gait and yet the ACLr knee does not appear to produce such a wide range of motion at an uninjured counterpart, which can be supported by the peak internal knee flexion moment being lower. This is indicative of an absorbing gait style, looking to maintain stability as its primary focus rather than flexibility, and is supported by higher lateral contact forces, and higher values for CCI One, Two and Five. However, patients have lower medial loading than their healthy counterparts. There is particular interest in the soleus acting as a surrogate support for a not fully restored ACL. These changes are all suggestive of early biomechanical changes in patient individuals.

8.1 Recommendations
It is recommended for healthcare settings to focus more on post-injury support, with particular focus on individuals with ACLr maintaining a large knee flexion range of motion and maintaining faster gait speeds as slower speeds have such strong corroboration with poor indications of joint and overall health. The soleus activation patterns could be focused on as a measure of ACLr success; with greater activation suggesting that the ACLr has not been as successful at preventing tibial anterior translation. It is however also understood how difficult it is for all therapeutic settings to engage this population, so the recommendations may be best served as independently available advice for those that want to rehabilitate further in their own setting.
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Appendix A - Marker Layout

(All White Background 2017)
<table>
<thead>
<tr>
<th>No.</th>
<th>Marker</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LFHD</td>
<td>Left front head</td>
</tr>
<tr>
<td>2</td>
<td>RFHD</td>
<td>Right front head</td>
</tr>
<tr>
<td>3</td>
<td>LBHD</td>
<td>Left back head</td>
</tr>
<tr>
<td>4</td>
<td>RBHD</td>
<td>Right back head</td>
</tr>
<tr>
<td>5</td>
<td>C7</td>
<td>On the 7th cervical vertebrae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The most noticeable bony prominence on the back of the neck is either C7 or T1. Feel for the first bony prominence (down the spine) that does not ‘sink’ when the subject is asked to extend or raise their head.</td>
</tr>
<tr>
<td>6</td>
<td>LT10</td>
<td>Left transverse process on the T10 vertebrae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If the subject is lying prone, use the lateral side of your hand to move upwards from the pelvis to feel when the heel side of the hand ‘drops’, this is L5. Count upwards until T10.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Or for standing, palpate from the lateral side the floating ribs T11 and T12, then trace back to the spinous processes and count up two.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Or follow T10 the first full rib around from the front of the subject, remembering that the transverse processes for the T10 are approximately 2-3cm higher than the spinous process because of the downwards curve of the spinous process at this point.</td>
</tr>
<tr>
<td>7</td>
<td>RT10</td>
<td>Right transverse process on the T10 vertebrae</td>
</tr>
<tr>
<td>8</td>
<td>CLAV</td>
<td>On the jugular notch of the sternum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palpate the depression at the base of the throat. Just below where the clavicles meet at the start of the bone from soft tissue is the jugular notch.</td>
</tr>
<tr>
<td>9</td>
<td>STRN</td>
<td>Xiphoid process of the sternum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A projection on the most inferior point of the sternum. Palpate the first firm mass in between the ribs.</td>
</tr>
<tr>
<td>10</td>
<td>LSHO</td>
<td>On the left acromion process</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palpate the acromion on the most lateral edge of the shoulders until you feel the ‘drop’ to the humerus. Place the marker just before the ‘drop’ on the superior edge. Ensure that you do not mark the most prominent part of the clavicle by accident.</td>
</tr>
<tr>
<td>11</td>
<td>LUA1</td>
<td>Middle of the left upper arm</td>
</tr>
<tr>
<td>12</td>
<td>LELB</td>
<td>Lateral epicondyle of the left elbow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Look for a dimple on the lateral side of the arm and near this palpate the most distal bony prominence on the humerus. Ask the subject to flex their elbow to confirm. You can also palpate down the humerus until the flare of the distal end is found. If a subject leans against a wall on their upper arm it is the area that will blanch first.</td>
</tr>
<tr>
<td>13</td>
<td>LLA1</td>
<td>Middle of the left lower arm</td>
</tr>
<tr>
<td>14</td>
<td>LWRA</td>
<td>Radial styloid process of the left arm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palpate the distal end of the radius and place the marker on the distal medial portion of the bone and not within the anatomical snuffbox.</td>
</tr>
<tr>
<td>15</td>
<td>LWRB</td>
<td>Ulnar styloid process of the left arm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Place the marker on the clear bony prominence on the distal end of the ulnar.</td>
</tr>
<tr>
<td>16</td>
<td>LFIN</td>
<td>Base of the third metacarpal on the left hand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palpate proximally down the third metacarpal until you feel a small notch where it joins the start of the carpals. This should be approximately two-thirds down the back of the hand; anymore and it is within the carpals (capitate being the most prominent).</td>
</tr>
<tr>
<td>17</td>
<td>RSHO</td>
<td>On the right acromion process</td>
</tr>
<tr>
<td>18</td>
<td>RUA1</td>
<td>Middle of the left upper arm</td>
</tr>
<tr>
<td>19</td>
<td>RELB</td>
<td>Lateral epicondyle of the right elbow</td>
</tr>
<tr>
<td>20</td>
<td>RLA1</td>
<td>Middle of the left lower arm</td>
</tr>
<tr>
<td>21</td>
<td>RWRA</td>
<td>Radial styloid process of the right arm</td>
</tr>
<tr>
<td>22</td>
<td>RWRB</td>
<td>Ulnar styloid process of the right arm</td>
</tr>
<tr>
<td>23</td>
<td>RFIN</td>
<td>Base of the third metacarpal on the right hand</td>
</tr>
<tr>
<td>24</td>
<td>LASI</td>
<td>Left anterior superior iliac spine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bring both hands around the medial sides of the waist of the subject and over the iliac crests. The thumbs should be able to locate the bony prominences of the anterior superior iliac spine.</td>
</tr>
<tr>
<td>25</td>
<td>RASI</td>
<td>Right anterior superior iliac spine</td>
</tr>
<tr>
<td>26</td>
<td>RPSI</td>
<td>Right posterior superior iliac spine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>They can be identified as the dimples on the back of the subject. Otherwise, palpate the iliac crest backwards and drop downwards with your thumbs.</td>
</tr>
<tr>
<td>27</td>
<td>LPSI</td>
<td>Left posterior superior iliac spine</td>
</tr>
<tr>
<td>28</td>
<td>LILC</td>
<td>Centre of the left iliac crest</td>
</tr>
<tr>
<td>Number</td>
<td>Code</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>29</td>
<td>RILC</td>
<td>Centre of the right iliac crest</td>
</tr>
<tr>
<td>30</td>
<td>LGTR</td>
<td>On the centre of the left greater trochanter</td>
</tr>
<tr>
<td>31</td>
<td>LTHI</td>
<td>The anterior superior marker on the left thigh cluster</td>
</tr>
<tr>
<td>32</td>
<td>LTHI2</td>
<td>The posterior superior marker on the left thigh cluster</td>
</tr>
<tr>
<td>33</td>
<td>LTHI3</td>
<td>The inferior marker on the left thigh cluster</td>
</tr>
<tr>
<td>34</td>
<td>LKNE</td>
<td>Lateral epicondyle of the left knee</td>
</tr>
<tr>
<td>35</td>
<td>LKNEmed</td>
<td>Palpate down the femur until you feel a flare outward on the lateral side and apply the marker to the prominence of the flare (roughly level with the top half of the kneecap).</td>
</tr>
<tr>
<td>36</td>
<td>LTIB</td>
<td>Medial epicondyle of the left knee</td>
</tr>
<tr>
<td>37</td>
<td>LTIB2</td>
<td>The anterior superior marker on the left tibial cluster</td>
</tr>
<tr>
<td>38</td>
<td>LTIB3</td>
<td>The inferior marker on the left tibial cluster</td>
</tr>
<tr>
<td>39</td>
<td>LANK</td>
<td>Centre of the lateral malleolus of the left ankle</td>
</tr>
<tr>
<td>40</td>
<td>LANKmed</td>
<td>Palpate the clear bony prominence on the distal end of the fibula.</td>
</tr>
<tr>
<td>41</td>
<td>LHEE</td>
<td>Centre of the left heel at the same height as the toe</td>
</tr>
<tr>
<td>42</td>
<td>LTOE</td>
<td>Tip of the left big toe</td>
</tr>
<tr>
<td>43</td>
<td>LLatFoot</td>
<td>The base of the 5th metatarsal bone on the left foot</td>
</tr>
<tr>
<td>44</td>
<td>RGTR</td>
<td>On the centre of the right greater trochanter</td>
</tr>
<tr>
<td>45</td>
<td>RTHI</td>
<td>The anterior superior marker on the right thigh cluster</td>
</tr>
<tr>
<td>46</td>
<td>RTHI2</td>
<td>The posterior superior marker on the right thigh cluster</td>
</tr>
<tr>
<td>47</td>
<td>RTHI3</td>
<td>The inferior marker on the right thigh cluster</td>
</tr>
<tr>
<td>48</td>
<td>RKNE</td>
<td>Lateral epicondyle of the right knee</td>
</tr>
<tr>
<td>49</td>
<td>RKNEmed</td>
<td>Palpate down the femur until you feel a flare outward on the medial side of the femur. Positioning this marker is slightly higher than its counterpart. Palpate the patellar tendon underneath the patella. Moving horizontally medially across the bony surface you feel the tibial plateau as you first move past the femoral condyles. When you reach the medial side, palpate upwards onto the femur and you will feel a flare, this is the medial epicondyle. The marker should be approximately level with the top half of the kneecap.</td>
</tr>
<tr>
<td>50</td>
<td>RTIB</td>
<td>Medial epicondyle of the right knee</td>
</tr>
<tr>
<td>51</td>
<td>RTIB2</td>
<td>The anterior superior marker on the right tibial cluster</td>
</tr>
<tr>
<td>52</td>
<td>RTIB3</td>
<td>The inferior marker on the right tibial cluster</td>
</tr>
<tr>
<td>53</td>
<td>RANK</td>
<td>Centre of the lateral malleolus of the right ankle</td>
</tr>
<tr>
<td>54</td>
<td>RANKmed</td>
<td>Centre of the medial malleolus of the right ankle</td>
</tr>
<tr>
<td>55</td>
<td>RHEE</td>
<td>Centre of the right heel at the same height as the toe</td>
</tr>
<tr>
<td>56</td>
<td>RTOE</td>
<td>Tip of the right big toe</td>
</tr>
<tr>
<td>57</td>
<td>RLatFoot</td>
<td>The base of the 5th metatarsal bone on the right foot</td>
</tr>
</tbody>
</table>
Appendix B- Healthy Participants Data Processing Workflow in Detail

Matlab

Step 1- The first script “Masterscript2016healthygroup” is opened in Matlab. The original names for the scripts are provided here for reference for clarity with the script examples in the Appendices. Much of the scripts are automated, with checks and pauses built in by the author. If there is an issue, the script often errors and will not continue until the issue is resolved manually.

- Loads the .c3d file for a participant and converts the marker and force plate data ready for the simulations (.forces, .mot, and .trc extensions).
- Events the data so that IC and TO can be found and used as the defining time parameters for the simulations. Any crossing of treadmill belts or unusual GRFs need to be noted, and the ‘cut off’ frequency of eventing of steps should be noted and raised as required. Steps may even need to be removed at this point.
- Generates the executable files for the Scale, Inverse Kinematics, and Muscle Force Distribution (MFD) cmd as required for SIMM
- Generates the executable files for the Inverse Dynamics and Contact functions cmd
- Can remove the ‘bad frames’ if required- where the log file from the MFD simulation outlines the frames where the calculations were not satisfactory and can be input into the removal command (see step 4 for more information).

Executable files

Once a command file for SIMM is produced, it draws on the files for the right leg University of Wisconsin-Madison Model, adapted by KU Leuven and modified as recommended by KU Leuven. Those files are (1) program executable files, (2) parameters in text files and (3) geometry files for the type of analysis being conducted. The results can be viewed in OpenSim for validation reasons if there were any issues.

Step 2- Run the command file (.cmd) for Scale, Inverse Kinematics and MFD (this can take approx. 3 hours per participant, per walk)

Step 3- Run the command file for Inverse Dynamics and Contact (this can take approx 1 hour per participant, per walk)

Return to Matlab

Step 4- Identify any specific steps that did not run correctly (i.e. terminated) or short files (i.e. only ‘x’ frames were available for analysis). This stage is extremely important to analyse, and any difficult simulations must be re-run, as often this can resolve the issue. It was very easy to identify a failed file as the cmd would crash and would not close (as it would naturally once the model was complete) until forced closed manually. The log files were updated line-by-line as the model ran, so once the cmd was shut, the log files could be examined to identify the cause of the issue. This could be as simple as a typo,
or the computer was asked to run too many models all at once and could not cope, to something more complicated, such as an error in the conversion of the .c3d file.

**Step 5** Back in Matlab, any analyses that are required can now be performed. In this case, the second script “Stiffnessanglesmomentsforhealthy” Matlab file was written to (on a one-person, one-walk level):

- Obtain the right leg sagittal flexion angles for the full stance phase
- Obtain the right leg sagittal flexion moments for the full stance phase
- The joint and knee stiffness calculation variables can then be generated as discussed in the literature review earlier and are mentioned below
- Obtain the maximum flexion angle for the first half of stance
- Overlay this time point onto the flexion moment and obtain a corresponding instantaneous flexion moment
- Obtain the angular difference in flexion between the start of stance and the maximum flexion angle (previously identified)
- Obtain the moment difference in flexion between the start of stance and the maximum flexion angle time point (previously identified)
- Calculate the knee stiffness (for each step) as the difference in moments divided by the difference in angles
- Identify the maximum GRF for each step
- Identify the maximum and minimum whole body CoM y position from the inverse kinematics file and establish the difference between the values
- Calculate the leg stiffness (for each step) as the difference in CoM (previously identified) divided by the maximum GRF (previously identified)
- Export results for further analyses

**Step 6** In this stage if there were steps that did not correctly overlay onto the sagittal flexion angle and moment graphs, they need to be recorded and removed. Step 5 needs to be repeated with the steps causing the issue omitted at the start of the script. This also applies to other speeds for the same subject, as all speeds need the same length of steps, ready for the next stage.

**Step 7** The third script “Stiffness4speedsforhealthy” Matlab file plots all stiffness calculations for all considered subjects at all walks with standard deviations and an average line.

**Step 8** The fourth script “KJcontactsforhealthy” runs at a one-person, all walks level

- Plots the splined contact angles for all four walks
- Obtain the maximum flexion angle for the first half of stance (as before), so that it can be saved
- Plots the splined internal flexion moments for all four walks
- Overlay this time point onto the flexion moment and obtain a corresponding instantaneous flexion moment (as before), and save it
- Plots the average spline for all speeds, one each for medial, lateral and total forces, with standard deviation error bars calculated and plotted
- Plots the splined medial, lateral, and total forces per walk
- Plots the average spline of each speed of the medial and lateral sides
- Finds the area for the first half of stance under the graph for each of the average spline for each medial and lateral force at each speed (a better measure than instantaneous force values).
At the end of this script, it is possible to run many further analyses to analyse GRF components and plots of the instantaneous peak pressures through the script “figures_output_pressure_maps”, and further effects on the cartilage properties (Van Rossum et al. 2017). This script has been partially modified by the author to gain peak pressure information.

**Step 9** - The fifth script “Forceplotsforhealthy” has the areas for the first half of stance manually input so that all subjects can be plotted together. Either all 4 walks medial loading can be compared to all four walks lateral loading, or that medial can be compared to lateral for each of the 4 walks separately.

- Steps may need removal and recording if they overlay incorrectly at this stage. Step 9 then needs repeating.

**Step 10** - The sixth script “Analysingsyncinghealthy” is used to examine the trigger information between D-Flow force plate information (blue) and Vicon force plate information (red) in the x, y and z planes. This is a laborious step and requires analysis to see if too many triggers were made, or to even add artificial triggers if not enough were present (the latter can be produced through gap filling between two triggers that are known).

- Plots several graphs displaying the triggers, and their maximums, identifying which trigger goes with each cut Vicon file. Finally overlays the force plate information from the two sources together to ensure that the syncing calculations were correct so that the mean speed of the treadmill with the standard deviation of the speed can be established and saved. If there are any disparities, there is the ability to analyse and resolve them manually.

**Step 11** - The seventh script “EMGforhealthy” imports the raw EMG data and aligns it with IC and TO. The data is passed through a 50Hz Notch filter, then a Bandpass filter of 20-400Hz, then a 10Hz low pass Butterworth filter, through a script modified from an earlier source (Afschrift et al. 2019). 5 different CCI calculations are then made; initially, the different muscles are plotted with IC and TO for visualisation. All individual EMG channels are normalised to their individual maximum within the walk being analysed so that each channel is represented as a percentage. The EMG is then targeted to analyse the change between IC and the maximum knee moment for each step. All EMG within this window is summed and divided by its size, to produce an average. If they are grouped muscles, these averages are added together. The paired muscles are then compared, to obtain which is lower and which is higher, so that the CCI calculations can be made and saved, on a per-step basis. Though it may seem counter intuitive, obtaining the total intensity of the less and more active muscle is how the sum is recommended in the literature review. The average is then sought to produce a per-walk value.

**Step 12** - The eighth script “CCIplotsforhealthy” the averages for each person, per walk is then manually added to this script to generate figures. SD error bars are produced between all subjects for each walk, per CCI calculation from an Excel spreadsheet and added to the figures. The first three CCI calculations, which look at individual pairs are plotted on one figure, and the final two CCI calculations, which look at muscle groups, are plotted on another figure.
Appendix C- Treadmill Versus Over-Ground Data Processing Workflow in Detail

Step 1- All data was exported into the .c3d file format from Vicon Nexus and imported into Matlab for the first Matlab script (either “MasterScriptTMVOGovergroundgroup” or “MasterScriptTMVOGtreadmillgroup”). The files were converted into .forces, .mot and .trc extensions (for the forces information to contribute to eventing calculations, for the forces information in the correct orientation for the model, and for the coordinate data of the markers at every frame respectively). 3 steps collected from the over ground system were compared with 9 treadmill steps per subject for ease of analysis. Once the executable scripts were generated in Matlab, each subject could be modelled using the University of Wisconsin-Madison right leg model in SIMM.

Step 2- The first executable script scaled the model to fit each subject’s markers, ran inverse kinematics to generate angles at every time point, and then ran static optimisation to generate the muscle force distributions (with the results from each step informing the calculations for the next step).

Step 3- Then a second executable script was run to use the static optimisation results to calculate through inverse dynamics the joint moments and then the joint contact results. All calculated joint moments from SIMM are internal moments, so the results are occasionally reversed to discuss as external moments to support the biomechanical theory.

Step 4- The results were taken back into Matlab and opened back into the original Matlab script (titles of which were mentioned earlier) to remove any ‘bad’ frames or where the calculation determined convergence failed. The most reasonable value that was determined for that frame was over the threshold of 0.5. These ‘bad’ frames were not removed at an earlier stage to allow for the full variance of the data to be available, though the angles and moments analysed then remove the ‘bad’ frames data and spline between missing frames to allow for a better representation of the data. The joint contact results are not required to do this as the results are not influenced by the presence of ‘bad’ frames.

Step 5- A second Matlab script (either “KJcontactsTMVOOGOG” or “KJcontactsTMVOGTM”) generated the angles, moments and contact force results and obtained plots, with a third Matlab script (either “StepwidthsOG” or “StepwidthsTM”) obtaining the step widths for an individual. “TMVOGabstractallcalcs” was the fourth Matlab script which established the peak angles and moments at the knee for an individual in both walking scenarios. A fifth script “plottingforcescomparingallsubjectsTMVOG” establishes and plots the peak medial, lateral and total forces for all participants. The peak angles and moments for the hip and ankle for an individual were established through the sixth script “TMVOGhipaddmom” for the hip and the seventh script “TMVOGankaddmom” for the ankle for both walking scenarios. Finally, an eighth Matlab script (“TMVOGresultswidthscorrected”) plots the step widths for all individuals together for both walking scenarios, which was also adapted to plot all frontal joint moments (knee, hip and ankle) for all individuals (this last adapted script was not saved).
Appendix D- Patient Participants Data Processing Workflow in Detail

Step 1- “Masterscript2016ABgroupleft”

This script only converts data from the left leg. If the right leg is the injured leg, the old script for the healthy group can be used for conversion.

- Loads the .c3d file for a participant and converts the marker and force plate data ready for the simulations (.forces, .mot, and .trc extensions).
- Events the data so that IC and TO can be found and used as the defining time parameters for the simulations. Any crossing of treadmill belts or unusual GRFs need to be noted, and the ‘cut off’ frequency of eventing of steps should be noted and raised as required. Steps may even need to be removed at this point.
- Generates the executable files for the Scale, Inverse Kinematics, and Muscle Force Distribution (MFD) cmd
- Generates the executable files for the Inverse Dynamics and Contact functions cmd
- Can remove the ‘bad frames’ if required- where the log file from the MFD simulation outlines the frames where the calculations were not satisfactory (see step 4 for more information).

Executable files

Once a command file is produced, it draws on (1) program executable files, (2) parameters in text files and (3) geometry files for the type of analysis being conducted.

Step 2- Run the command file (.cmd) for Scale, Inverse Kinematics and MFD (this can take approx. 3 hours per participant, per walk)

Step 3- Run the command file for Inverse Dynamics and Contact (this can take approx 1 hour per participant, per walk)

Return to Matlab

Step 4- Identify any specific steps that did not run correctly (i.e. terminated) or short files (i.e. only ‘x’ frames were available for analysis). This stage is extremely important to analyse, and any difficult simulations must be re-run, as often this can resolve the issue.

Step 5- Back in Matlab, any analyses that are required can now be performed. In this case, the “StiffnessanglesmomentsforAB” Matlab file was written to (on a one-person, one-walk level), and can be for either the right or left leg:

- Obtain the left/right leg sagittal flexion angles
- Obtain the left/right leg sagittal flexion moments
- Obtain the maximum flexion angle for the first half of stance
- Overlay this time point onto the flexion moment and obtain a corresponding instantaneous flexion moment
- Obtain the angular difference in flexion between the start of stance and the maximum flexion angle (previously identified)
Obtain the moment difference in flexion between the start of stance and the maximum flexion angle time point (previously identified)

Calculate the knee stiffness (for each step) as the difference in moments divided by the difference in angles

Identify the maximum GRF for each step

Identify the maximum and minimum whole body CoM y position from the inverse kinematics file and establish the difference between the values

Calculate the leg stiffness (for each step) as the difference in CoM (previously identified) divided by the maximum GRF (previously identified)

Finally, and differently to the same script for the healthy group, the speed of each file is calculated from the foot markers and in conjunction with eventing, due to a lack of related D-flow files

Export results for further analyses

**Step 6**- In this stage if there are steps that do not correctly overlay onto the sagittal flexion angle and moment graphs, they need to be recorded and removed. Step 5 needs to be repeated with the steps causing the issue omitted at the start of the script. This also applies to other speeds for the same subject, as all speeds need the same length of steps, ready for the next stage.

**Step 7**- “Stiffness4speedsforAB” Matlab file plots all stiffness calculations for all considered subjects at all walks with standard deviations and an average line.

**Step 8**- “KJcontactsforAB” runs at a one-person, all walks level, and can be either for the right or left leg

- Plots the splined contact angles for all four walks
- Obtain the maximum flexion angle for the first half of stance (as before), so that it can be saved
- Plots the splined internal flexion moments for all four walks
- Overlay this time point onto the flexion moment and obtain a corresponding instantaneous flexion moment (as before), and save it
- Plots the average spline for all speeds, one each for medial, lateral and total forces, with standard deviation error bars calculated and plotted
- Plots the splined medial, lateral, and total forces per walk
- Plots the average spline of each speed of the medial and lateral sides
- Finds the area for the *first half of stance* under the graph for each of the average spline for each medial and lateral force at each speed (a better measure than instantaneous force values).

At the end of this script, it is possible to run further analyses and many graphs to analyse GRF components and plots of the instantaneous peak pressures through the script “figures_output_pressure_maps” (Van Rossom et al. 2017), which has been partially modified by the author.

**Step 9**- “ForceplotsforAB” has the areas for the first half of stance manually input so that all subjects can be plotted together. Either all 4 walks medial loading can be compared to all four walks lateral loading, or that medial can be compared to lateral for each of the 4 walks separately.

- Steps may need removal and recording if they look poor at this stage. Step 9 then needs repeating.
Step 10 - Is reserved for analysing the syncing between the D-flow and Vicon files, to output the average speed and standard deviation for each file. However, this step was not required in the study.

Step 11 - “EMGforAB” imports the raw EMG data and aligns it with IC and TO, with the option to select the leg of interest. The data is passed through a 50Hz Notch filter, then a Bandpass filter of 20-400Hz, then a 10Hz low pass Butterworth filter, through a script modified from an earlier source (Afschrift et al. 2019). 5 different CCI calculations are then made; initially, the different muscles are plotted with IC and TO for visualisation. All individual EMG channels are normalised to their individual maximum within the walk being analysed so that each channel is represented as a percentage. The EMG is then targeted to analyse the change between IC and the maximum knee moment for each step. All EMG within this window is summed and divided by its size, to produce an average. If they are grouped muscles, these averages are added together. The paired muscles are then compared, to obtain which is lower and which is higher, so that the CCI calculations can be made and saved, on a per step basis. The average is then sought to produce a per-walk value.

Step 12 - “CCIplotsforAB” the averages for each person, per walk are then manually added to this script to generate figures. SD error bars are produced between all subjects for each walk, per CCI calculation from an Excel spreadsheet and added to the figures. The first three CCI calculations, which look at individual pairs are plotted on one figure, and the final two CCI calculations, which look at muscle groups, are plotted on another figure.

Step 13 - “Healthyvpatient_load_stiff_CCI” enables scatter graphs to be plotted to compare loading, stiffness and CCI results between all healthy and patient individuals.

Step 14 - “Muscleswithspeed” script derives the average for each muscle (tib ant, gastroc and sol as these contribute to CCI Four which required further investigation) across 99 frames for each person, per walk, for either a healthy or patient individual and can display this in plot form. The max for each muscle is then derived (as a percentage with maximum contraction being 1). The information is then added to the overall Excel spreadsheet for reference. “Muscleswithspeedtogether” script plots the healthy and patient group max values on one scatter graph per muscle for further comparisons.
Appendix E- EMG Versus Modelled Muscles Data Processing Workflow in Detail

**Step 1**- The “EMGforAB” script filters and cuts the raw EMG data so that it is clearer and aligns with the IC and TO of the participant so that an EMG file for each of the 9 steps is created within each walking speed. As previously mentioned the data is processed with a 50Hz Notch filter, then a Bandpass filter of 20-400Hz and then a 10Hz low pass Butterworth filter, through a pre-existing script (Afschrift et al. 2019).

However, this script was now further modified to produce a mean EMG pattern for each muscle over 8 steps for each walking speed. Splining was used to ensure all files had the same length). Also, a mean muscle activation pattern from the modelled data over 8 steps was produced (for both patient and healthy subjects).

Additionally, a signal processing section was added, to include a cross-correlation coefficient and an RMS value of the modelled muscle activation versus the EMG pattern for either a healthy or patient subject. As the results from the cross-correlation coefficient produce a lag and a vector of correlation, the maximum of the vector was taken and used with the lag instead.

Whilst this establishes the statistics for the healthy EMG versus healthy modelled muscles, or the patient EMG versus the patient modelled muscles, it does not establish the statistics within either the healthy EMG versus the patient EMG, or the healthy modelled activations versus the patient modelled activations.

**Step 2**- “IntraanalysisEMGandMODEL” seeks to establish cross-correlation coefficient and RMS information for the EMG or the modelled activations for both the healthy and patient groups.

The mean EMG pattern and mean muscle activation pattern for each muscle were loaded (that were established in the last step), and a matrix was established, one for all healthy subjects, and one for all patient subjects, and a mean vector formed for each muscle.

**Step 3**- “IntraanalysisEMGandMODELXCORRandRMS” reloaded the mean vector for each muscle representing an average of all healthy or all patient subjects of either an EMG pattern or a modelled muscle pattern. Cross-correlation coefficient maximums with lags and RMS values are then established for both EMG versus EMG of both subject groups and model versus model of both subject groups. Due to the way this data is collated, standard deviation values are not able to be formed with these results. The coefficient of variation was found in Excel, using the cross-correlation maximums.
Title:

Knee contact loading differences during over ground and treadmill gait

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