DOCTORAL THESIS

Quadriceps strength asymmetry in transtibial amputees and able-bodied mechanisms and association with movement

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Award date:
2019

Awarding institution:
University of Roehampton

Quadriceps Strength Asymmetry in Transtibial Amputees and Able-Bodied: Mechanisms and Association with Movement

by

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A thesis submitted in partial fulfilment of the requirements for the degree of PhD

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University of Roehampton

2019

Abstract

Bilateral strength asymmetry of the quadriceps muscles has been implicated in a variety of special populations and movement performance deficits. However, while there is consensus of the main determinants of maximal and explosive strength, it is unclear to what extent these may determine strength asymmetries. The association between asymmetries in quadriceps strength and movement are also not well understood. This thesis aimed to determine the magnitude and variability of quadriceps maximum and explosive strength asymmetry, the underpinning neuromuscular determinants of these asymmetries, and the association of these asymmetries with movement. These aims were investigated in two populations distinct in their degree of habitual movement asymmetry during typical lower-body activities; able-bodied and individuals with unilateral transtibial amputations (ITTAs). This thesis also aimed to assess the effects of long-term muscle disuse, caused by habitual asymmetrical loading during movement in the ITTAs. The magnitudes of maximum and explosive strength asymmetry were similar (10% and 13%, respectively) in able bodied, and not explained by any systematic combinations of asymmetries in the neuromuscular determinants of strength (e.g. neural activation, muscle architecture and contractile properties). In both able bodied and ITTAs there was a greater variability in explosive strength asymmetry compared to that in maximum strength, which was associated with loading asymmetry in drop landings in able-bodied, and a good predictor of walking speed in ITTAs (r = -0.83). Additionally, greater strength asymmetry in ITTAs was associated with greater asymmetry in single support time (r = 0.60 – 0.83) and limb loading asymmetry (r = 0.54 – 0.89) at faster walking speeds. When utilising ITTAs as a model for long-term muscle disuse, we found substantially greater strength decrements than could be predicted from short-term disuse studies in otherwise young, healthy, and active adults. The reductions in maximum voluntary torque (MVT; -59%) were likely due to considerable declines in muscle thickness (-41%) and neural drive (~-44%), whilst reductions in explosive strength (-75%) appeared due to the decline in MVT, coupled with a slowing of the contractile properties. Overall, the results from this thesis suggest that asymmetries in explosive strength play a more important role in movement in both populations than maximum strength asymmetry. Furthermore, the novel findings from this thesis may help to inform the development of intervention programmes to target strength asymmetry in ITTA populations, and additionally, to combat the degenerative changes present in muscle as a by-product of long-term disuse.

Dissemination of Research

Oral Presentations

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2018) *Do Strength Asymmetries Explain Walking Gait Asymmetries in Unilateral Transtibial Amputees?* ISPO Annual Scientific Meeting, University of Southampton

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2017) *Neuromuscular Adaptations to Long-term Muscle Disuse in Unilateral Transtibial Amputees.* 23rd Annual Congress ECSS, Dublin

Poster Presentations

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2016) *Effects of Transtibial Amputation on Neuromuscular Function at the Quadriceps.* ISPO Annual Scientific Meeting, University of Cambridge

Other Oral Presentations

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2019) *Quadriceps Strength Asymmetry: Magnitude, Mechanisms, and the Relation to the Performance of Functional Movements.* Presented at: University of North Texas

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2017) *The Effect of Transtibial Amputation on Strength and Neuromuscular Function at the Quadriceps.* Presented at: University of Roehampton, London

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2017) *The Response of the Quadriceps Muscles to Unilateral Trans-tibial Amputation.* Presented at: University of Southern Denmark, Odense

Submissions

Sibley A.R., Strike S., Moudy S., Tillin N.A. (2019) The Effects of Long-Term Muscle Disuse on Neuromuscular Function in Unilateral Transtibial Amputees. *Experimental Physiology. In press.*

Acknowledgements

For Grandpa, whose irrepressible humour was reflected in the important life lesson you taught me aged eight: *nil illegitimi carborundum*. Your memory makes me smile every day.

I am very lucky to have a long list of people to whom I owe my thanks and gratitude for helping me on this journey. Firstly, to my supervisors, Dr Siobhán Strike and Dr Neale Tillin. I really couldn't have asked for a better supervisory team. Thank you for your guidance and support from day one. In particular, thank you Neale for a frankly astonishing level of patience and attention to detail – even though the latter has occasionally driven me to wine! I have learned so much from you about what it means to be a good scientist.

To the University of Roehampton, for providing me the means to feed and house myself for three years. I would not have been able to complete this thesis without the financial aid of the Vice-Chancellor's Scholarship.

To Dr Sarah Moudy, a.k.a. the second half of the amputee dream team. My Salad. One day I'll forgive you for finishing first.

To Alison Carlisle and Tom Reeves for all your technical help and letting me hide out in your office when I should have been working; to Ralph Gordon, for always giving up your femoral nerve in the name of science; to Jodie Moss, who never fails to make me laugh; and to the rest of the Sport Science team at UoR. It's been great fun working alongside you all.

To my fabulous colleagues at LSBU, Clare Deary and Mark Thomas. This last year would have been awful – and I would probably still be going(!) – had you not been so supportive.

To the participants, for giving your time and effort for what was a fairly gruelling data collection process. I am particularly grateful to all the amputees who took part, who often came from very long distances. And to those of you who brought chocolate!

To my poor, long-suffering mum and dad, Hilary and Paul, for your unfailing love and support, and believing in me always. To Beatrix and Ciarán, for the constant encouragement (read: telling me to hurry up and finish so I can talk about something else and stop boring you). To Grandma, for always insisting that you wanted a Doctor in the family, and someone had got to do it... To my friends, for not giving up on me after I essentially pulled a vanishing act for the last four years, and particularly Hatty, who knows that a jug of piña colada, leopard fleece and a BBQ will always cheer me up.

And finally, Nick. You have put up with so much over the course of this PhD and not complained once (well, hardly ever). I genuinely don't know if I could have got through it without you. You're the best.

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List of Abbreviations

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ACL	Anterior Cruciate Ligament	
ACSA	Anatomical Cross-Sectional Area	29
ADP	Adenosine Diphosphate	
AMP	Amputated limb	
ATP	Adenosine Triphosphate	
BAI	Bilateral Asymmetry Index	82
BAI_DIR	Directional Bilateral Asymmetry Index	84
ΔBAI	Difference in BAI from habitual to fast walking speeds	135
BM	Body Mass	
BSA	Bilateral Strength Asymmetry	82
BSA_DIR	Relative Bilateral Strength Asymmetry	84
Ca^{2+}	Calcium ion	
CMJ	Countermovement Jump	
CoM	Centre of Mass	
CON	Control limb	
CSA	Cross Sectional Area	
CV	Coefficient of Variation	
DHP	Dihydropyridine voltage receptors	
EMG	Electromyography	
EMG_{MVT}	Electromyography at Maximum Voluntary Torque	74
EMG_{0-100}	Electromyography from 0 – 100 ms of an explosive contraction	74
hGRF	Horizontal Ground Reaction Force	46
ICC	Intraclass Correlation Coefficient	
INT	Intact limb	
ITT	Interpolated Twitch Technique	21
ITTA	Individual with a Transtibial Amputation	
MHC	Myosin Heavy Chain	
M_{max}	Maximum M-wave	21

MRI	Magnetic Resonance Imaging	
MVC	Maximum Voluntary Contraction	21
MVF	Maximum Voluntary Force	8
MVT	Maximum Voluntary Torque	8
PCSA	Physiological Cross-Sectional Area	23
PT	Peak Torque	8
RF	Rectus Femoris	
RFD	Rate of Force Development	8
RMS EMG	Root Mean Square Electromyography	
RTD	Rate of Torque Development	8
TOV	Centre of Mass take-off velocity	110
TS	Temporospatial	
ULLS	Unilateral Lower Limb Suspension	
VA	Voluntary Activation	158
vGRF	Vertical Ground Reaction Force	
VL	Vastus Lateralis	
VM	Vastus Medialis	
V_{max}	Maximum shortening velocity	26
VMO	Vastus Intermedius	

Chapter 1

Introduction

Muscular strength is defined as the ability of a muscle to produce force in a given situation (Knuttgen & Komi 1992). It is an important component of health, as weak muscles may limit both a person's physical fitness and their capability to perform activities of daily living (Mizner et al. 2005, Mizner & Snyder-Mackler 2005, Maffiuletti 2010). Muscular strength can be subdivided into maximum, defined as the maximum force a muscle can produce (measured as Maximum Voluntary Torque, MVT) and explosive strength, defined as the muscle's capacity to rapidly exert force (commonly measured as Rate of Torque Development, RTD; Abernethy et al. 1995). At the quadriceps, persistent maximum strength deficits have been shown to impair dynamic knee stability, physical function and quality of life (Rice & McNair, 2010). In addition, weakness of these muscles has been linked to increased risk of knee joint injury and is thought to contribute to the development and progression of degenerative conditions (Hurley, Rees & Newham 1998).

Analyses of inter-limb asymmetries compare the performance of one limb in respect to another. Substantial quadriceps strength asymmetries have been found in the elderly (Skelton, Kennedy & Rutherford 2002), and particularly those prone to falling (Perry et al. 2007), as well as a variety of pathological conditions such as multiple sclerosis (Sandroff, Sosnof & Motl 2013), osteoarthritis (Suetta et al. 2007) and transtibial amputation (Lloyd et al. 2010). Additionally, strength asymmetries of the knee extensors have been extensively studied in populations with anterior cruciate ligament injuries, where inter-limb deficits of ≤ 10% have been linked to successful outcomes of return to activity (Grindem et al. 2016). Strength asymmetries are typically measured by quantifying the inter-limb difference in maximum strength; however, little is known about the extent of asymmetry in explosive strength, which may be more functionally relevant to human movement in situations when time to develop force is small (e.g. recovering from a perturbation, and sports-specific movements such as sprinting and jumping; Behan, Pain & Folland 2018, Tillin, Pain & Folland 2013a). In a healthy population, substantial strength asymmetries in maximum strength have been found at the quadriceps (Graham-Smith, Al-Dukhail & Jones 2015, Kobayashi et al. 2013), but the magnitude of explosive strength asymmetry is understudied. Furthermore, while there is consensus on the determinants of single limb maximal (neural drive, muscle architecture and size) and explosive strength (neural drive, intrinsic contractile properties and maximum force production capacity), it is unclear to what extent these neuromuscular mechanisms may underpin asymmetries in maximum and explosive strength.

While the effect of between-limb differences in movement on sporting performance is a popular topic in research literature, we currently have little understanding of the underpinning mechanisms of these asymmetries (Bishop, Turner & Read 2018). As previous research has linked significant lower limb maximum strength asymmetries to reduced jump performance (Bailey et al. 2013), and deficits in sport-specific skills such as kicking accuracy (Hart et al. 2014) and sprint cycling power (Rannama et al. 2015), it seems logical that quadriceps strength and neuromuscular asymmetries may play a role in overall movement performance. Jumping is a key skill in many sport and recreational activities. Importantly, the knee plays a key role in both the take-off and landing phases, with a large contribution from the quadriceps that is partially determined by maximal and, to a greater extent, explosive strength (Chang et al. 2015, de Ruiter et al. 2007, Sandler and Robinovitch 2001), which makes these movements an excellent means of assessing the relationships between movement and strength asymmetries.

Investigating the movement patterns of a population with known asymmetries in strength and movement allows a further opportunity to establish the relationships between these asymmetries in further detail. In individuals with unilateral trans-tibial amputations (ITTAs) who are characterised by the loss of the ankle joint and the surrounding musculature on one limb, there are decreases in maximal strength in the intact (-30%), and more severely, in the amputated (-60%) limb when compared to a non-injured population. This results in substantial inter-limb strength asymmetries of ~42% (Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Powers et al. 1996). However, deficits in explosive strength, and by extension, explosive strength asymmetry, have not been considered in this population. Furthermore, as ITTAs are increasingly encouraged to exercise for health and quality of life, understanding how asymmetries in strength result in changes in movement patterns is key to inform healthy movement prescription. In walking, which is vital for independent living, ITTAs have demonstrated reduced walking speed compared to able-bodied groups, suggesting a decline in gait function (Bohannan 1997, Oberg et al. 1993, Sanderson & Martin 1997). In addition, there is evidence of asymmetries in temporospatial (e.g. step length and single support time; Renstrom et al. 1983, Powers et al. 1996) and kinetic loading variables (e.g. ground reaction forces, joint moments and powers; Lloyd et al. 2010, Nolan et al. 2003). Through investigating the relationships between strength asymmetries and those in gait in ITTAs, we may be able to provide additional information to therapists to improve rehabilitation programmes and interventions in this population, particularly with regards to walking performance. This may be particularly important as muscular demand, and loading asymmetry, increases with increased walking speed (Nolan et al. 2003).

By investigating the neuromuscular characteristics of populations with known asymmetries, we can answer some broader physiological questions. Gait analyses in ITTAs has shown reductions in whole-limb and knee extensor loading of the amputated compared to intact limb while walking (e.g. Sanderson & Martin 1997, Silverman et al. 2008), therefore suggesting that they chronically disuse the knee extensor musculature of their amputated limb. The effects of long-term muscular disuse – a problem in multiple patient populations (e.g. individuals who are bedridden or have suffered a stroke) – on neuromuscular function are unclear. Current knowledge of the effects of muscle disuse on strength and neuromuscular function is based on short-term (typically < 120 days) bed rest or limb suspension studies (Narici & de Boer 2011); however, the short-term nature of these studies makes it difficult to draw conclusions about the long-term effect of disuse on skeletal muscle. Furthermore, the effects of disuse on explosive strength have not been studied. Given the apparent association between asymmetries in limb loading during locomotion and quadriceps muscle strength in ITTAs (Lloyd et al. 2010), a young, healthy ITTA population provides a novel model for investigating the long-term (> 120 days) effects of muscle disuse with the intact limb acting as an internal control.

The purpose of this thesis was therefore **to investigate strength asymmetry, its underpinning neural and mechanical determinants, and to determine its influence on movement asymmetry.** This was achieved by (1) quantifying the magnitude, direction and variability of knee extensor strength asymmetries and their potential underlying determinants in a healthy, active, control population; (2) investigating the influence of knee extensor strength asymmetries on movement asymmetries in two populations with different levels of inherent strength asymmetries;

and (3) utilising a known asymmetric ITTA population to assess the effects of long-term disuse on strength and neuromuscular function of the quadriceps muscles.

Chapter 2

Literature Review

2.1 Introduction

This literature review is structured as follows: first a brief overview of the determinants of strength, and how these impact maximal and explosive voluntary force production, is presented. This is followed by an exploration of strength asymmetry in various populations, and current understanding of its influence on movement performance. This will lead to a discussion of how strength and movement asymmetry may result in long-term disuse of the quadriceps in ITTAs. Finally, the changes in strength and neuromuscular function commonly found in short-term muscular disuse will be examined.

2.2 Strength and strength asymmetry

2.2.1 Muscular Strength

Muscular strength can be subdivided into maximum (the maximum force a muscle can produce) and explosive strength (the muscle's capacity to rapidly exert force; Abernethy et al. 1995). Maximal strength sets the upper limit of the system: theoretically, the greater an individual's maximal force production capabilities, the greater absolute forces they will be able to produce in any situation – be it a maximal, submaximal, fast or slow contraction. It is associated with the ability to perform daily ambulatory activities such as rising from a chair, walking at an appropriate speed, and ascending and descending stairs (Bassey et al. 1992, Buchner & Lateur 1991). Furthermore, reduced maximal strength has been identified as one of the most frequent factors accompanying degenerative loading diseases (Petterson et al. 2008), and has been implicated in populations showing increased limb loading during gait (Lloyd et al. 2010). As the time required to produce maximum force is generally >300 ms from onset in isometric and eccentric muscle contractions (Thorstensson et al. 1976, Tillin, Pain & Folland 2012a), explosive strength is considered especially important in situations where the time to develop muscular force is limited. In situations such as restabilising the body following a loss of balance or during explosive sports activities, the muscles must produce as much force as possible in time periods that could be as small as 50 – 250 ms, and high contractile RTD may be more important for performance than MVT (Aagaard et al. 2003, Izquierdo 1999, Maffiuletti et al. 2010, Pijnappels et al. 2008, Tillin, Pain & Folland 2013a). RTD has previously been shown to be an important determinant of the effectiveness of postural corrections following mechanical perturbations (Behan, Pain & Folland 2018). Additionally, in some populations such as the elderly, who, similarly to ITTAs, display strength impairments and reduced ability to adapt their gait pattern to environmental changes (Hofstad et al. 2006, Houdjik et al. 2012) knee extensor RTD appears associated with several typical tasks of daily life such as climbing stairs and walking (Bassey et al. 1992). Furthermore, relationships between asymmetry in RTD, but not MVT, and subjective knee function have been reported after total knee arthroplasty (Maffiuletti et al. 2010).

One of the most common methods of measuring strength experimentally is dynamometry, whereby strength can be measured in dynamic situations (isokinetically; e.g. Impellizzeri et al. 2007) or isometrically (e.g. Tillin, Pain, & Folland 2011). While maximum strength (Figure 2.1A) is defined by the greatest peak of the force-time curve, irrespective of when it occurs (isometric: maximum voluntary force/ torque [MVF/ MVT], isokinetic: peak torque [PT]; Abernethy et al. 1995), explosive strength can be measured in multiple ways, for example, as rate of force/ torque development (RFD/ RTD) (the slope of the force-time curve, as utilised in this thesis; Figure 2.1B [Aagaard et al. 2002]), the force produced at specific time points from the onset of the contraction, or as impulse beneath the force-time curve (Aagaard et al. 2002). Both isometric and isokinetic measures of maximum strength have high reliability (isometric MVT, Coefficient of Variation [CV] = 3 – 5%, Intraclass Correlation Coefficient [ICC] = 0.95 – 0.97%; isokinetic PT, CV 3 – 7%, ICC 0.97 – 0.99%; Impellizzeri et al. 2008, Maffiuletti et al. 2007, Tillin, Pain & Folland 2011). However, while the reduced ecological validity of assessing muscle function in a non-dynamic contraction is recognised, isometric measures of RTD are necessary to remove the confounding influence of joint angle changes that are inherent in isokinetic contractions (Maffiuletti et al. 2016).

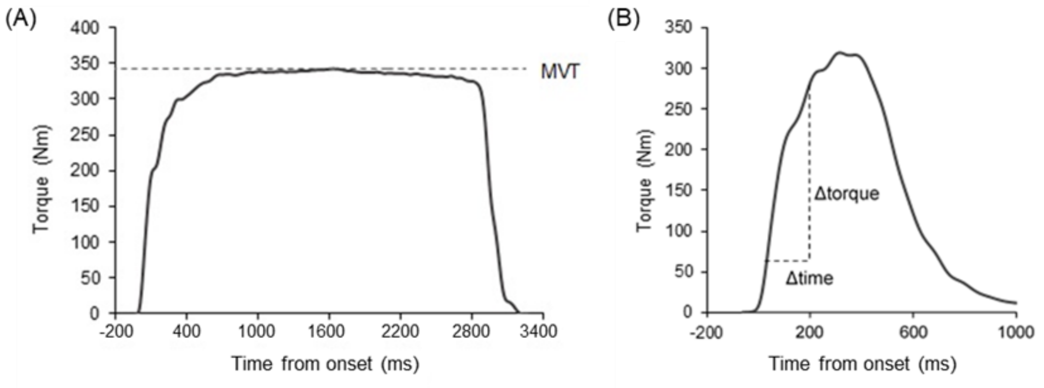


Figure 2.1. Torque-time curves recorded during (A) a maximal voluntary isometric contraction of the knee extensors and (B) an explosive voluntary isometric contraction of the knee extensors. The greatest peak torque recorded during an isometric maximal voluntary contraction is defined as Maximum Voluntary Torque (MVT). The method of measuring explosive strength referred to in this thesis is peak voluntary Rate of Torque Development (RTD), calculated using the peak slope of the torque-time curve (Δtorque/Δtime).

2.2.2 Strength Asymmetry

Inter-limb strength asymmetry refers to the concept of comparing the strength of one limb in respect to the other. This section of the review will focus on asymmetry of the lower limbs, and specifically, the quadriceps, given that quadriceps strength deficits have been shown to impair dynamic knee stability, physical function and quality of life (Rice & McNair, 2010). In addition, weakness of these muscles has been linked to increased risk of knee joint injury and is thought to contribute to the development and progression of degenerative conditions (Hurley, Rees & Newham 1998). Knee extensor strength asymmetries have been found in the elderly (Skelton, Kennedy & Rutherford 2002), those prone to falling (Perry et al. 2007), and pathological populations (Lloyd et al. 2010, Sandroff, Sosnof & Motl 2013, Suetta et al. 2007). Furthermore, asymmetries at the quadriceps have been linked to performance deficits (Hart et al. 2014, Rannama et al. 2015), and there is evidence that inter-limb differences >10% have a detrimental effect on the outcomes of return to activity following knee injuries (Grindem et al. 2016).

2.2.2.1 Calculation of Asymmetry

Before discussing current perspectives on strength asymmetry, it is important to understand how asymmetry indices are generated. Multiple equations exist for the calculation of asymmetry (Appendix A), and there is no systematic agreement amongst researchers on the best method. In non-injured groups, inter-limb asymmetries in strength have previously been calculated by comparing strong and weak, right and left, and dominant and non-dominant limbs. This makes it difficult to compare the results of the many studies that have assessed the magnitude of quadriceps strength asymmetries in a variety of healthy populations. One of the issues with calculating strength asymmetries between the left/ right, or dominant/ non-dominant limbs is that there is a tendency for the average difference to be around zero due to the variability in the direction of the asymmetry (Kobayashi et al. 2013, Newton et al. 2006, Schiltz et al. 2009). Indeed, when taking the direction (i.e. whether the asymmetry was positive or negative) as well as the extent of the measured asymmetry into account, Impellizzeri et al. (2007) found an average maximum lower-limb strength asymmetry of just 0.8%, but a range of -15 to 15%. For this reason, some studies have grouped limbs by strong vs. weak, in order to calculate absolute asymmetry. Calculated this way, the average lower-limb asymmetry reported by Impellizzeri et al. (2007) becomes 6%. Furthermore, two studies that compared asymmetry scores when calculated using strong/ weak vs. left/ right reported significant differences in the former, but not the latter, for all methods of asymmetry assessment (Jones & Bampouros 2010, Newton et al. 2006; Table 2.1). Jones & Bampouros (2010) postulated that this was because, despite all participants in their study being right-sided (i.e. being right handed and preferring the right limb in throwing and kicking tasks), some subjects were actually left leg dominant in strength, nullifying strength differences when averaged across the groups. This serves to indicate one of the problems with classifying limb dominance for the calculation of asymmetry (discussed further in Appendix A).

2.2.2.2 Maximum Strength Asymmetry

Most studies that have investigated inter-limb strength asymmetries at the quadriceps have used isokinetic, isotonic or isometric dynamometry to assess maximum strength (measured as PT [dynamic] or MVT [isometric]; Table 2.1), as this measure has been associated with the ability to perform daily ambulatory activities such as rising from a chair, walking at an appropriate speed, and ascending and descending stairs (Bassey et al. 1992, Buchner & Lateur 1991). Little research however, has focused on asymmetry in young, healthy, recreationally active adults, concentrating instead on special populations such as sports people, the elderly and those suffering from pathology. Studies investigating athletes from different sports, a variety of strength measures (both isometric and isokinetic), and a mix of genders and ages, have found an average magnitude of asymmetries of 7%, but a wide range of 1 – 18% (Table 2.1). Furthermore, Bell et al. (2014) found that the majority (79%) of athletes had peak force asymmetries of 0 – 10%, with 16% presenting in the 10 – 15% band, and 4% in the >15% band. The variability of strength asymmetry within each population, as evidenced by large ranges and standard deviations (e.g. Impellizzeri et al. [2007], who reported asymmetries of 0 – 15%) may stem from factors such as limb dominance (Sjöström et al. 1991), previous injury (Newton et al. 2006) and training background (Rahnama, Lees & Bambaecichi 2005, Theoharopoulos et al. 2000). Each of these factors are likely to introduce muscular adaptations at the affected limb that may influence strength. Training, for example, causes adaptations such as increased muscle thickness and enhanced neural drive (Narici et al. 1989, Tillin & Folland 2014), which contribute to both maximum and explosive strength (Sections 2.3 and 2.4). In an athlete that participates in a sport that has a more unilateral demand, it may be the limb that experiences increased loading more regularly experiences greater muscle adaptation, and therefore is capable of greater force production. This is supported by the work of Bell et al. (2014) who demonstrated that sports that involve greater demand on one lower limb than the other (i.e. American football and football [soccer], both of which involve kicking; and hockey) showed a greater proportion of athletes with larger strength asymmetries than those that require more symmetrical involvement of the lower limb (e.g. softball and volleyball).

Table 2.1. Summary of papers that have assessed strength asymmetries in the quadriceps. Asymmetry scores are as given in the paper cited. Calculations are explained in Appendix A. Values in brackets (if presented) are the mean absolute asymmetry score.

Reference	Subjects	Metrics Measured	Limb Groups	Asymmetry Score (%)
Coratella, Beato & Schena (2018)	U21 elite football players, n = 27	Concentric PT 30°·s^{-1}	S vs. W[1]	9
		300°·s^{-1}		8
		Eccentric PT 30°·s^{-1}		10
		300°·s^{-1}		9
Costa-Silva et al. (2015)	U20 footballers n = 22	Concentric PT 180°·s^{-1}	DL vs. NDL[2]	6
		Eccentric PT 180°·s^{-1}		10
Graham-Smith, Al-Dukhail & Jones (2015)	Mixed athletes, n = 145	Concentric PT 60°·s^{-1}	S vs. W[1]	18
Impellizzeri et al. (2007)	Male athletes, n = 59	Concentric PT 60°·s^{-1}	S vs. W[1]	8
Knapik et al. (1991)	College athletes, n = 38	Concentric PT 30°·s^{-1}	R vs. L[3]	5
Knezevic et al. (2014)	Pre-ACLR individuals, n = 20	Isometric MVT	Involved vs. uninvolved[1]	15
		Peak RTD		22
	4 months post-ACLR	Isometric MVT		32
		Peak RTD		41
	6 months post-ACLR	Isometric MVT		22
		Peak RTD		28
Kobayashi et al. (2013)	Male students, n = 11	Concentric PT 60°·s^{-1}	DL vs. NDL[4]	2
		180°·s^{-1}		1
Jones & Bampouras (2010)	Male college athletes, n = 33	Concentric PT 60°·s^{-1}	R vs. L[3]	5
		Eccentric PT 60°·s^{-1}		8
		Concentric PT 60°·s^{-1}	DL vs. NDL[4]	11
		Eccentric PT 60°·s^{-1}		12

PT, peak torque; MVT, maximum voluntary torque; ACLR, anterior cruciate ligament reconstruction; DL, dominant limb; NDL, non-dominant limb; S, strong limb; W, weak limb; R, right leg; L, left leg

[1] calculated using Bilateral Strength Asymmetry Index-1

[2] calculated using Bilateral Strength Asymmetry Index-2

[3] Bilateral Strength Asymmetry Index-1

[4] calculated using Limb Symmetry Index-2

Table 2.1 (continued). Summary of papers that have assessed strength asymmetries in the quadriceps. Asymmetry scores are as given in the paper cited. Calculations are explained in Appendix A. Values in brackets (if presented) are the mean absolute asymmetry score.

Reference	Subjects	Metrics Measured	Limb Groups	Asymmetry Score (%)
Lanshammar & Ribom (2010)	Women aged 20 – 39 years, n = 159	Concentric PT 90°·s^{-1}	DL vs. NDL[2]	5
LaRoche et al.	Women aged 65 – 80 years, n = 24	Isometric MVT	S vs. W[1]	20
		Peak RTD		28
Loturco et al. (2018)	Professional footballers, n = 24	Concentric PT 60°·s^{-1}	DL vs. NDL[5]	7
		300°·s^{-1}		7
Maffiuletti et al. (2010)	Individuals with total knee arthroplasty n = 31	Isometric MVT	Involved vs. uninvolved[1]	23
		Peak RTD		27
Maly et al. (2015)	Untrained boys, n = 22	Concentric PT 60°·s^{-1}	DL vs. NDL[1]	14
		120°·s^{-1}		13
		180°·s^{-1}		12
		240°·s^{-1}		10
		300°·s^{-1}		13
Menzel et al. (2013)	Professional male footballers, n = 46	Concentric PT 60°·s^{-1}	R vs. L[5]	-2 (9)
		180°·s^{-1}		-1 (6)
		300°·s^{-1}		2 (6)
Nakao et al. (2006)	Women aged 65 – 89 years n = 30	Isometric MVT	R vs. L[7]	10
Nunn & Mayhew (1988)	Male athletes, n = 24	Concentric PT 60°·s^{-1}	S vs. W[1]	8
		180°·s^{-1}		8
		300°·s^{-1}		8
		Isotonic PT		6
		Isometric MVT		9

PT, peak torque; DL, dominant limb; NDL, non-dominant limb; S, strong limb; W, weak limb; R, right leg; L, left leg

[1] calculated using Bilateral Strength Asymmetry-1

[2] calculated using Bilateral Strength Asymmetry Index-2

[5] calculated using Bilateral Strength Asymmetry-4

[7] calculated using Limb Symmetry Index-3

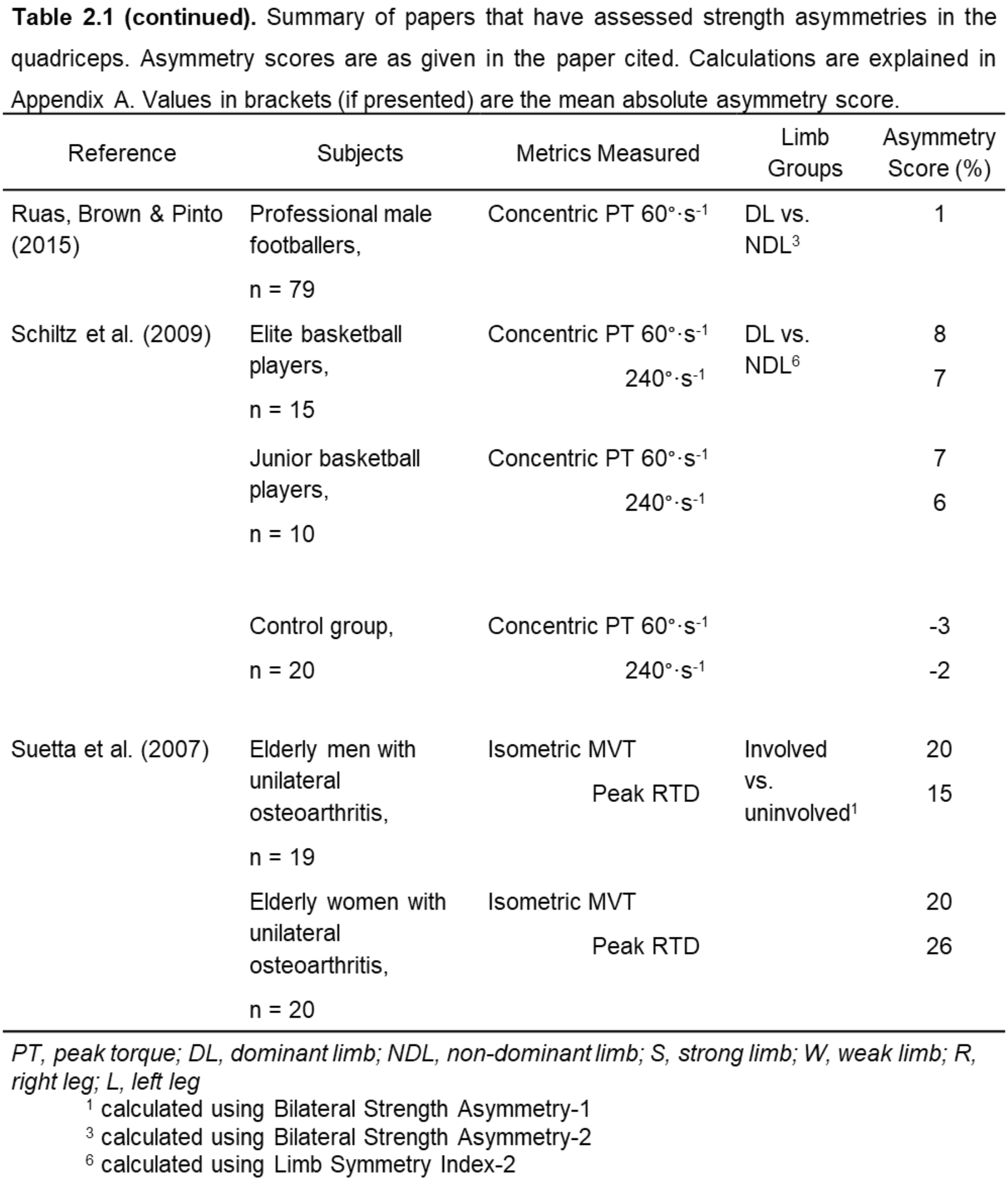


Table 2.1 (continued). Summary of papers that have assessed strength asymmetries in the quadriceps. Asymmetry scores are as given in the paper cited. Calculations are explained in Appendix A. Values in brackets (if presented) are the mean absolute asymmetry score.

Reference	Subjects	Metrics Measured	Limb Groups	Asymmetry Score (%)
Ruas, Brown & Pinto (2015)	Professional male footballers, n = 79	Concentric PT 60°·s^{-1}	DL vs. NDL[3]	1
Schiltz et al. (2009)	Elite basketball players, n = 15	Concentric PT 60°·s^{-1}	DL vs. NDL[6]	8
		240°·s^{-1}		7
	Junior basketball players, n = 10	Concentric PT 60°·s^{-1}		7
		240°·s^{-1}		6
	Control group, n = 20	Concentric PT 60°·s^{-1}		-3
		240°·s^{-1}		-2
Suetta et al. (2007)	Elderly men with unilateral osteoarthritis, n = 19	Isometric MVT	Involved vs. uninvolved[1]	20
		Peak RTD		15
	Elderly women with unilateral osteoarthritis, n = 20	Isometric MVT		20
		Peak RTD		26

PT, peak torque; DL, dominant limb; NDL, non-dominant limb; S, strong limb; W, weak limb; R, right leg; L, left leg

[1] calculated using Bilateral Strength Asymmetry-1
[3] calculated using Bilateral Strength Asymmetry-2
[6] calculated using Limb Symmetry Index-2

Evidence of maximum quadriceps strength asymmetry has also been found in ageing (Skelton, Kennedy & Rutherford 2002) and pathological populations such as, for example, those suffering from osteoarthritis (Suetta et al. 2007), multiple sclerosis (Sandroff, Sosnof & Motl 2013), and ITTAs, who display musculoskeletal asymmetry due to the loss of their ankle on one side, with resultant strength asymmetries of ~42% (see Section *2.5.2.1*; Isakov et al 1996a, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Renstrom, Grimsby & Larsson 1983). In females over the age of

65 years, substantial maximum strength asymmetries have been found (mean ~14%, range 0.6 – 30.7%; Nakao et al. 2006, Skelton, Kennedy & Rutherford 2002). Furthermore, Schimidt et al. (2014) found significant differences (i.e. asymmetries, calculated using a dominant/ non-dominant limb split) in knee extensor MVT in older participants, but not in young adults or children. They postulated that this was because of the higher daily involvement of younger individuals in bilateral actions, which they theorised contribute to similar force-production abilities between limbs. In contrast, Perry et al. (2007) found no effect of age on quadriceps strength asymmetry, although elderly fallers exhibited greater asymmetry in MVT (~15%) than healthy young controls (~10%). However, similarity in asymmetry values between all ages in the latter study may be due to the calculation of asymmetry as the difference between left and right sides (Perry et al. 2007), as opposed to between the strong and weak (Skelton, Kennedy & Rutherford 2002) or dominant and non-dominant limb (Schimidt et al. 2014). In support of this argument, Skelton, Kennedy & Rutherford (2002) reported that the asymmetries found did not follow the dominance pattern of the limbs, and were not consistent to one side of the body.

2.2.2.3 Explosive Strength Asymmetry

However, as previous research has suggested that strength asymmetries are task- and variable-specific (Maloney et al. 2019), we cannot extrapolate these findings to inform us of typical asymmetry in explosive strength. To the author’s knowledge, asymmetry in peak voluntary RTD has only been assessed in elderly and pathological populations (Table 2.1), with conflicting results. In a study investigating changes in RTD asymmetry following an anterior cruciate ligament (ACL) reconstruction, Knezevic et al. (2014) found that explosive strength asymmetry was significantly greater than that of maximum strength both pre- and post-surgery. Other studies have not identified significantly greater explosive when compared to maximum strength asymmetries

(Maffiuletti et al. 2010, Suetta et al. 2007). These differences may be related to the different populations tested, who were a mix of genders and ages, and who had differing pathologies. Additionally, as maximum strength asymmetry appears to be greater in pathological conditions (15 – 32%; Knezevik et al. 2014, Maffiuletti et al. 2010, Suetta et al. 2007) when compared to healthy, young subjects (1 – 18%; e.g. Kobayashi et al. 2013, Graham-Smith, Al-Dukhail & Jones 2015, Table 2.1), it is difficult to comprehend how, in the latter population, asymmetries in the two forms of strength might differ. Although perhaps not as large as those in pathological populations, we may hypothetically expect to see larger asymmetries in explosive strength, owing to the greater inter-individual variability present in explosive strength (CV = 23 – 48%,), when compared to MVT (CV = 21%; Folland, Buckthorpe & Hannah 2014). Furthermore, there is greater between-session variability in RTD (ICC = 0.90, CV = 7.2%; Buckthorpe et al. 2012) compared to MVT (ICC ≥ 0.90, CV <4%; Buckthorpe et al. 2012, de Ruiter et al. 2004, Place et al. 2007), which suggests that explosive strength asymmetry may be more variable, and potentially therefore larger, than maximum strength asymmetry.

In summary, current literature has focussed mainly on strength asymmetries in special populations such as athletes, the elderly, and those who are suffering from various pathological conditions. There is little research that has explored strength asymmetry in young, healthy, recreationally active individuals with no specific training bias that may predispose the individuals to greater magnitudes of strength asymmetry. Furthermore, the extent of quadriceps explosive strength asymmetry has not been investigated in detail in such a group. Current literature is therefore unclear on the variability within MVT and RTD asymmetry, and how asymmetries in the two forms of strength might differ. An in-depth investigation of the magnitude and variability of strength asymmetry in such a sample would allow comparison to other patient groups, e.g. ITTA, among others. Finally, while the presence of asymmetry may indicate pathology, the global nature of strength assessment alone is not particularly helpful in guiding the rehabilitation process. To this end, it may be useful to quantify the determinants of strength asymmetry. However, before we can consider the determinants of strength asymmetry, it is necessary to first consider how strength is produced and what factors determine the level of force the system is capable of producing at the single-limb level.

2.3 Determinants of Muscular Strength

2.3.1 Muscle Organisation and Mechanism of Contraction

Muscles are innervated by motor neurons. A single α-motor neuron in the anterior horn of the spinal cord can innervate multiple muscle fibres via its peripheral axon – the neuron and all the muscle fibres it innervates is known as a motor unit (MacIntosh, Gardiner & McComas 2006). A muscle is made up of groups of fibres (the elongated, multinucleated muscle cells) collectively known as fascicles. Each single fibre consists of bundles of myofibrils (the contractile elements of muscle) in parallel. The myofibrils are surrounded by cytoplasm (known in the muscle as sarcoplasm), which contains mitochondria, the internal membrane systems of the sarcoplasmic reticulum (SR), the T-system, and glycogen (Keynes & Aidley 2001). A single myofibril is composed of multiple sarcomeres arranged end-to-end. Sarcomeres consist of two types of contractile proteins – actin (known as the thin filament) and myosin (thick filament). Muscle contraction is a result of the relative movement of one type of filament over the other, without a change in the length of the filament themselves (McCubble & Kay 1980). This movement is due to the connection of the two filaments to form cross bridges when stimulated by action potentials from nerves, which allows the production of force (McArdle, Katch & Katch 2010).

The motor unit is the fundamental component of the motor system responsible for all voluntary and involuntary contractions (Doherty et al. 2002). The motor neuron is activated only when a stimulus equal to or greater than the threshold potential (the critical level to which a membrane potential must be depolarised to initiate an action potential) is received. If the stimulus is beneath this threshold, the neuron will not respond, while the response to any stimulus greater than the threshold is the same (i.e. all fibres of the motor unit will be activated): this is known as the all-or-none law (Lucas 1909). Once the threshold has been met, an action potential is transmitted along the length of the axon to the neuromuscular junction between the neuron and activates all of the muscle fibres that motor neuron innervates. This is the beginning of the process that leads to a muscle contraction.

The process of converting an electrical signal to a mechanical response is known as excitation-contraction coupling (Bertorini 2008). Once an action potential has crossed the neuromuscular junction, a wave of depolarisation spreads across the cell membrane (sarcolemma) of the muscle fibre. The sarcolemma is electrically excitable to enable it to transmit action potentials (Keynes & Aidley 2001). This depolarisation is then propagated along the t-tubules (inward foldings of the sarcolemma that runs transversely across the fibre adjacent to the sarcoplasmic reticulum) to the dihydropyridine (DHP) voltage receptors. The DHP receptors, which are coupled with ryanodine receptors on the sarcoplasmic reticulum, undergo voltage-induced conformational change in the presence of an action potential. The shape change of the DHP is transmitted to the ryanodine receptors on the sarcoplasmic reticulum, causing them to be activated allosterically. This allows the release of a large quantity of calcium ions (Ca^{2+}) into the sarcoplasm from stores in the sarcoplasmic reticulum (Keynes & Aidley 2001).

The presence of Ca^{2+} in the sarcoplasm causes changes to the troponin-tropomyosin complex at the sarcomere. Tropomyosin is a long, thin protein that extends between, and binds to, troponin, which associates with actin. The troponin-tropomyosin complex consists of tropomyosin and troponins I (inhibitory), C (calcium binding) and T (tropomyosin binding). In a resting muscle, the complex blocks the myosin binding site on actin. When Ca^{2+} binds to troponin C, however, it undergoes a conformational change which causes the complex to move slightly, dislocating troponin I and tropomyosin (McCubbin & Kay 1980, Lehman, Craig & Bibertt 1994). This acts to free the binding sites for myosin on the actin. Myosin heads carrying adenosine triphosphate (ATP – the molecule involved in providing energy for biological processes) attach to the actin filaments to form cross-bridges. During the formation of the cross-bridge, ATP is hydrolysed in a reaction catalysed by myosin ATPase, to form adenosine diphosphate (ADP) and an inorganic phosphate. Initially, the association is relatively weak; however, the release of the Pi from the myosin head catalyses the transition to a strongly bound state (Holmes 1995). Stronger binding triggers what is known as the powerstroke, during which the myosin head flexes strongly to pull the actin filament over the myosin and the ADP is released from the cross-bridge. It is the powerstroke that generates force, and subsequently returns the actin-myosin complex to its strongly bound state of ‘rigour’ (i.e., no nucleotides are bound). In order for the cross-bridge cycle to continue, the myosin head must be released from the actin filament. This is achieved by the attachment of another ATP molecule to the myosin head. The cross-bridge cycle can therefore begin again. Continued coupling of the actin and myosin occurs until electrical stimulation is no longer present at the muscle fibre (Keynes & Aidley 2001).

When the electrical stimulation from the action potential ceases, so does the depolarisation of the sarcolemma. At this point, Ca^{2+} is no longer released into the sarcoplasm, and the Ca^{2+} bound to the troponin molecules is released and actively transported back into the sarcoplasmic reticulum. The troponin then moves back to its original position, causing the conformational change on the tropomyosin that revealed the binding site on the actin to reverse. This consequently prevents any further cross-bridges from forming, and tension falls (Keynes & Aidley 2001).

Both central and peripheral factors are involved in the production of muscular force (summarised in Figure 2.2). Centrally, agonist neural drive (sections *2.3.2.1* and *2.3.3.1*) is a product of motor unit discharge rate and recruitment. Peripheral factors include the intrinsic contractile properties of a muscle (defined as the contractile response of the muscle to known stimuli, which dictates contractile time; section *2.3.3.2*) and muscle architecture (including muscle size; section *2.3.2.2*). The following sections of the review will therefore discuss the determinants of both maximum and explosive strength and how they differ.

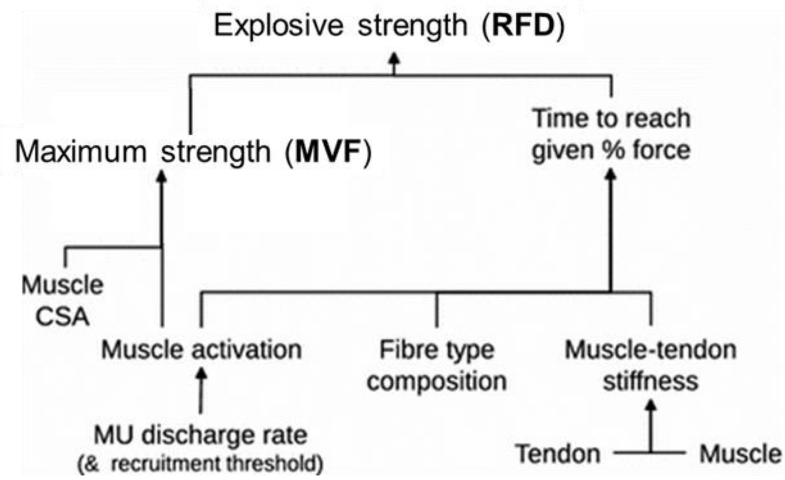


Figure 2.2. Muscular force production in the form of maximum voluntary force (MVF) and rate of force development (RFD) are influenced by multiple factors within the neuromuscular system. Furthermore, factors that contribute to MVF will also improve the mean RFD. Reproduced with permission from Maffiuletti et al. (2016). *CSA, cross-sectional area; MU, motor unit.*

2.3.2 Determinants of Maximum Strength

2.3.2.1 Agonist Neural Drive

The level of force produced during voluntary muscular contractions is largely a result of descending neural drive to the muscle from the motor cortex. Neural determinants of force production include motor unit recruitment and firing frequency (or discharge rate), which together define the muscle activation (Figure 2.2; de Ruiter et al. 2004, 2006). Increasing the number of motor units recruited will increase the number of muscle fibres contributing to force production, therefore increasing force output (MacIntosh, Gardiner & McComas 2006). Meanwhile, the firing frequency of the motoneuron influences both the magnitude of the force produced and the RTD of the muscle (Miller, Mirka & Maxfield 1981, Nelson 1996), although evidence suggests that it is particularly important for the latter (del Vecchio 2019a).

Neural drive during contractions can be measured using electromyography (EMG), or the interpolated twitch technique (ITT). EMG measures the voltage generated across the sarcolemma of the muscle fibres in response to neural activation. The amplitude of EMG signals increases with increased motor unit recruitment and firing frequency, and is therefore useful for measuring neural drive to a muscle (de Luca 1997). Surface EMG, which is used for analysing large muscle groups, is a summation of all the voltage potentials detected by electrodes on the skin’s surface. The signal is therefore affected by factors such as electrode placement, muscle cross-talk, subcutaneous adipose tissue, muscle biochemistry and equipment noise (de Luca 1997, Lanza et al. 2018). As such, a normalisation procedure is usually undertaken to facilitate both between- and within-individual comparisons. The two most commonly utilised methods of normalisation are reporting EMG (1) relative to peak EMG during an isometric maximum voluntary contraction (MVC) and (2) as a ratio of a maximal M-wave (peak-to-peak amplitude, M_{max}) for the same muscle. The

M-wave is a compound muscle action potential recorded in response to electrical stimulation of the motoneurons. M_{max} is achieved when all motoneurons in a motor pool are activated maximally, and therefore time-aligns with the maximum force response for a single electrical impulse (Maffiuletti et al. 2001). The latter method of normalisation is preferable as it allows comparison between muscle, tasks and individuals (Halaki & Ginn 2012); removes the influence of electrode location, and reduces the influence of subcutaneous adipose tissue thickness (which is problematic due to its high electrical resistance; Lanza et al. 2018).

The ITT, meanwhile, assesses the capacity of the central nervous system to activate muscles by calculating the difference in the amplitude of the force produced by twitch contractions evoked at rest and superimposed on an MVC. Theoretically, the superimposed stimulus will recruit the motor units not already active, and/ or increase the firing frequency of active motor units that are firing at submaximal frequencies. In other words, if not all motor units are recruited at MVF, the effect of the superimposed stimulus would to be further increase force output. The force increments from different motor units are summed across the entire muscle to produce an additional twitch. The greater the number of motor units recruited during a voluntary contraction, and the greater the firing frequency, therefore, the smaller the superimposed twitch will be. During a contraction that produces maximal possible muscle force, all motor units in the muscle should be recruited and firing at a sufficient frequency that additional stimulation will not produce an evoked twitch (Merton 1954). Voluntary activation (VA, the outcome measure for ITT, given as a percentage of maximum possible force) has therefore been defined as the level of voluntary drive to a muscle during a contractile effort (Gandevia 2001).

There are several methodological issues that should be considered to improve the reliability and validity of ITT measures. Firstly, as previous research has shown a curvilinear relationship between voluntary and superimposed force, the stimulus should be superimposed when the participant is producing force at or above 80% of their MVF, whereby changes in superimposed force are minimal (Kooistra, de Ruiter & de Haan 2007). Additionally, twitch contractions can potentiate by around 70% immediately following an MVC, before force declines exponentially. However, twitch force remains significantly above the unpotentiated level (~12%) for at least five minutes (Hamada et al., 2000). A potentiated twitch elicited after the contraction (as opposed to an unpotentiated twitch elicited before) theoretically allows more valid comparison to the superimposed stimulus, which appears to be potentiated (Folland 2009).

Some authors have suggested that a single superimposed stimulus may not be adequate to assess the absolute force capacity of a muscle. The force produced by paired stimuli is greater and less variable than for a single twitch, which may not be clearly noticeable at the plateau of an MVC due to signal noise. The second impulse is likely to depolarise any motor units that were both not firing maximally and in the refractory period at the time of the first impulse (Duchateau 2009). However, trains of stimuli are both uncomfortable for the participant, and are likely to be reduced by both antidromic collisions and spinal reflexes (Herbert & Gandeiva 1999).

2.3.2.2 Muscle Architecture and Size

Skeletal muscle architecture can be defined as ‘the arrangement of muscle fibres within a muscle relative to the axis of force generation’ (Lieber & Frieden 2001: 141). The size and architectural properties of a muscle (pennation angle, i.e. the angle at which the fascicle inserts into the aponeurosis, and fascicle length; Figure 2.3) strongly influence its functional capabilities. While the maximum force generating potential of a muscle is dependent architecturally on the maximum number of sarcomeres in parallel (in other words, the physiological cross-sectional area (PCSA) of the muscle, which in itself depends on the pennation angle of the fibres), the maximum shortening velocity is dependent on the number of sarcomeres in series (Narici et al. 2003).

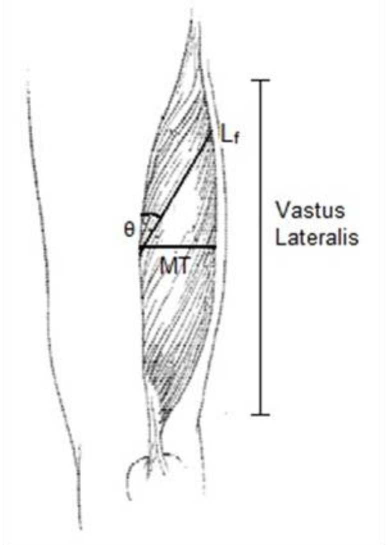


Figure 2.3. Pennate muscles such as VL have fibres that run at an oblique angle to their tendon. Angle of pennation (Θ) and muscle thickness (MT) measured using ultrasonography can be used to calculate L_f (fascicle length). Adapted from Lieber & Friden (2000)

The architecture of a muscle influences its functional properties through the length-tension and force-velocity relationships. Sarcomere length is critical to a muscle's ability to produce force, as it relies on actin-myosin overlap during the formation of cross-bridges (Figure 2.4). On the activation of the process of cross-bridge cycling during muscle contraction, the greatest force is produced at an optimal sarcomere length, with an overlap of actin and myosin that provides maximal cross-bridge interaction. When sarcomere length is outside this optimal length, the force production capability of a muscle decreases**.** At lengths below the optimum, force production decreases due to the overlap of actin from opposite ends of the sarcomere, combined with the compression of myosin filaments colliding with the Z-disk. In contrast, when stretched beyond the optimal length, cross-bridge interaction is decreased as there is less overlap between actin and myosin, which also serves to decrease force production at the sarcomere (Gordon et al. 1966, Lieber et al. 1994).

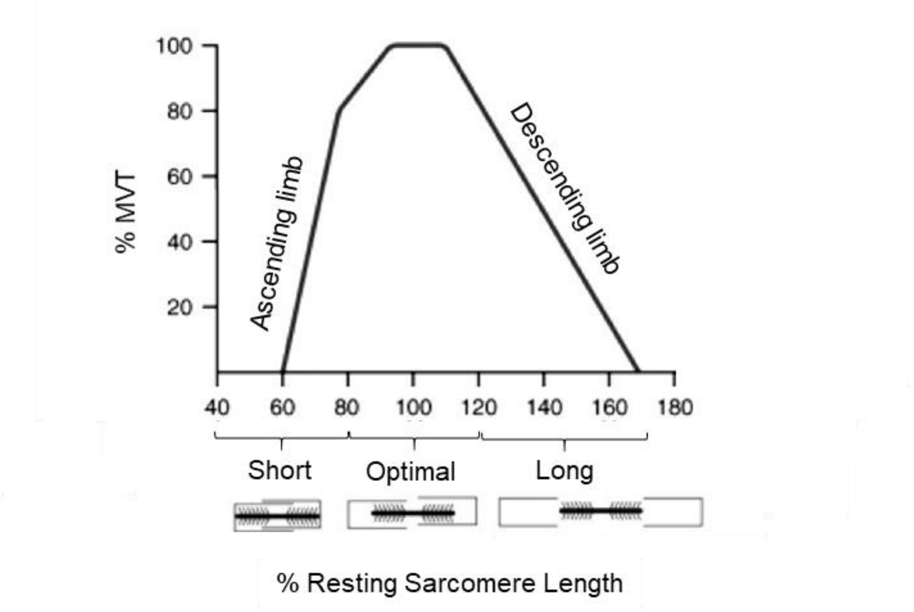


Figure 2.4. The length-tension relationship of a muscle describes the relationship between sarcomere length and its force production capability. It represents the force a sarcomere is capable of generating while held at a series of discrete lengths, and states that isometric tension generation in skeletal muscle is a function of the magnitude of overlap between actin and myosin. Along the ascending limb, adjacent actin filaments overlap, interfering with the myosin-actin cross-bridge mechanism, therefore reducing the potential for force production. At very short sarcomere lengths, the myosin filament collides with the Z-discs, causing further disruption to the cross-bridge mechanism. At the plateau of the length-tension relationship, optimal overlap of the contractile filaments exists, which in turn maximises the number of potential cross-bridges. Along the descending limb, the overlap between actin and myosin filaments is minimised, which reduces the number of potential cross-bridges and, by extension, force production. Adapted from Gordon, Huxley & Julian (1966).

The velocity of muscle contraction dictates the force production capability of a muscle due to the limiting effect of actin-myosin cross-bridge cycling. The force-velocity relationship therefore describes the inverse relationship between muscle force production and contractile velocity (Gordon, Huxley & Juilan 1966; Figure 2.5). The amount of force generated by a muscle is determined by the number of cross-bridges attached at any one time. As it takes a fixed period of time for cross-bridges to attach, the total number of cross-bridges attached at any given point decreases as the velocity of muscle shortening increases. A muscle is therefore capable of producing less force as the velocity of a concentric or isometric muscle contraction is increased. During eccentric contractions, the tension produced by the muscle increases as the velocity of lengthening increases (Hill 1938).

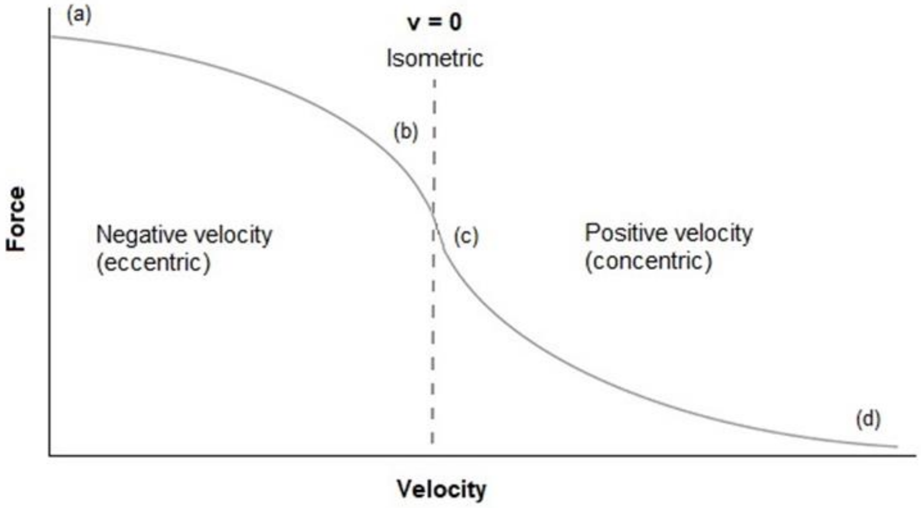


Figure 2.5. The force-velocity relationship describes the behaviour of single muscle fibres (Hill, 1938); however, it also seems to hold true for single joint movements (Hauraix et al., 2017). It is a hyperbolic relationship, i.e. the rate of change of force alters with changing velocity. During eccentric muscle action, where contraction velocity is high, muscle force output can also be high (a). Where muscle force is low, contraction velocity must be low (b). The opposite is true in concentric muscle action: where contraction velocity is high, muscle force must be low (c). Where muscle force is low, contraction velocity must be high (d).

An increased number of sarcomeres in series in a muscle fibre allows the generation of force over greater ranges of motion by increasing the length of the fibre; a property that tends to be very uniform within a muscle (Wickiewicz et al. 1983). Maximum contraction velocity (V_{max}) also increases with a greater number of sarcomeres arranged in series (Blazevitch & Sharp 2006). This is supported by the work of Bodine et al. (1982) who investigated the anatomical and mechanical properties of the cat semitendinosus muscle. This muscle provides a unique model, as it is comprised of two discrete heads (proximal and distal) separated by connective tissue and each with distinct neural innervation. Both heads of the muscle were electronically stimulated separately and then simultaneously. The V_{max} of the shorter proximal and longer distal head was 224 mm/s and 424 mm/s respectively. When the two heads were stimulated simultaneously, the whole muscle V_{max} was 624 mm/s (the sum of the two velocities), indicating a positive proportional relationship between V_{max} and fibre lengths (Figure 2.6).

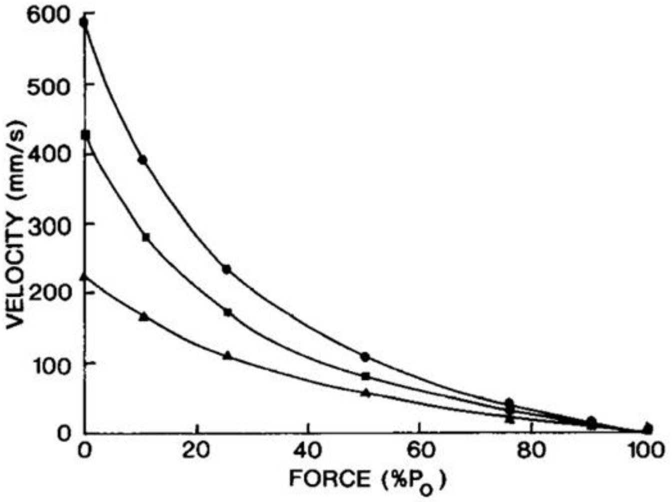


Figure 2.6. Force-velocity relationships for three stimulation conditions of the cat semitendinosus muscle: proximal portion (▲), distal portion (■), and entire muscle (●). Illustrate5s the direct relationship between Vmax and muscle fibre length. Reproduced with permission from Bodine et al. (1982).

In terms of maximal strength production, large pennation angles are biomechanically important as they allow increased attachment of contractile tissue to the tendon and aponeurosis, increasing the muscle PCSA. The PCSA of a muscle represents the sum of the cross-sectional areas of all the muscle fibres within the muscle (see Figure 2.7). It is therefore directly proportional to the maximum tetanic force generated by the muscle (Lieber & Friden 2000). It is calculated using equation 2.1:

$$PCSA\ (mm^2) = \frac{muscle\ mass\ (g) \cdot cos\ \theta}{\rho(g/cm^3) \cdot fibre\ length\ (mm)} \qquad (2.1)$$

where ρ represents muscle density (1.056 g/cm^3 for mammalian muscle) and θ represents surface pennation angle (Powell et al. 1984). This equation divides the volume of a muscle by the length of the fibres and their area. The inclusion of the cosine (*cos*) factor considers the force that is not transmitted directly to the tendon as a result of muscle fibres that are not oriented relative to the axis of force generation (i.e., a pennation angle greater than 0°). The term $x \cdot \cos\theta$ therefore represents the proportion of the muscle fibre force that is transmitted along the muscle axis. Large pennation angles can therefore lead to mechanical disadvantage as a result of reduced transfer of force to the tendon (Fukanaga et al. 1997). Conversely, increased rotation of fibres at greater pennation angles increases tendon displacement for a given shortening of fibres during contraction, which makes it more likely that they can operate closer to their optimum length (Figure 2.4). Larger pennation angles are therefore likely to result in increased force generation (Blazevich & Sharp 2006). However, the positive features of muscles with large pennation angles (i.e. increased PCSA resulting from a greater number of fibres in parallel) offset the negatives, so that increases in pennation angle up to 45° are beneficial. Above this value, however, the advantages are outweighed, and maximal force production is compromised (Kawakami 2005).

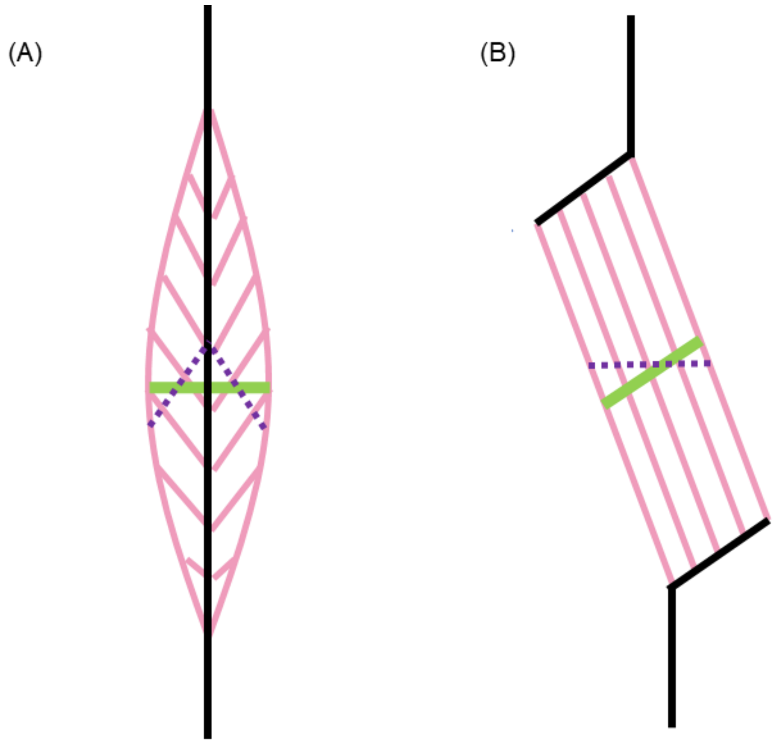


Figure 2.7 Two muscles with the same anatomical cross-sectional area (ACSA – the maximum cross-sectional area of a muscle perpendicular to its long axis) can nevertheless demonstrate different physiological cross-sectional area (PCSA – the maximum cross-sectional area of a muscle perpendicular to its fibres). Pennate muscles such as (A) will have a larger PCSA when compared to fusiform muscles (B).

Anatomical cross-sectional area (ACSA; the cross-sectional area of a muscle perpendicular to its line of pull) and muscle volume are both measures of muscle size, and consequently infer the number of muscle fibres in series and contractile force. It is therefore perhaps unsurprising that strong relationships have been reported between the size of a muscle and its strength. At the quadriceps, similar relationships have been found between different measures of muscle size and isometric strength: ACSA r = 0.73 – 0.87 (Blazevich et al. 2009, Chapman et al. 1984); muscle volume r = 0.77 – 0.84 (Blazevich et al. 2009, Evangelidis et al. 2016); PCSA r = 0.72 (Blazevich et al. 2009). A strong relationship with isometric strength and muscle thickness of the quadriceps, measured using ultrasound, has also been shown: r = 0.76 – 0.88 in old, and r = 0.84 – 0.92 in young men (Strasser et al. 2013).

The muscles that comprise quadriceps femoris (rectus femoris [RF], vastus lateralis [VL], vastus medialis [VM] and vastus intermedius [VMO]) are all pennate muscles, with fascicles that attach obliquely to its tendon. Muscle pennation angles are typically less than 30º (Ward et al. 2009); angles of pennation of the VL muscle, for example, have been reported to be 6 – 27° in healthy subjects (Blazevich et al. 2006, Henriksson-Larsen et al. 1992, Rutherford & Jones 1992). This compares to pennation angles of 10 – 29°, 10 – 14°, and 8 – 9° at VM, RF and VMO, respectively (Blazevich et al. 2006, Strasser et al. 2013). These large pennation angles found at the quadriceps result in large PCSAs (as increases in pennation angle leads to an increase in the number of sarcomeres in parallel) and result therefore in large force production capability (MacIntosh, Gardiner & McComas 2006). In addition, the fascicles of the quadriceps muscles are relatively short (i.e. there are few sarcomeres in series) resulting in low muscular V_{max}. This, however, is also beneficial for force production, as decreased contractile velocity results in greater force production. The quadriceps are therefore well suited to the generation of large forces (Lieber & Friden, 2000).

2.3.3 Determinants of Explosive Strength

2.3.3.1 Agonist Neural Drive

During muscular contractions of large muscle such as the quadriceps, full tetanic fusion is achieved with a firing frequency of ~ 25 – 40 Hz (Masakado 1994, Roos et al. 1999) at maximal force generation, which may be up to > 300 ms from the onset of an isometric contraction (Thorstensson et al. 1976, Tillin, Pain & Folland 2012a). At the onset of maximal voluntary contractions, motor unit firing rates have been recorded at much higher frequencies (100 – 200 Hz) than those required to achieve maximal tetanic fusion (van Cutsem et al. 1998). This high initial neural discharge frequency has been shown to increase with training in association with increases in contractile RTD and decreases in ½ relaxation time (van Cutsem et al. 1998, Aagaard et al. 2002). In addition, Grimby, Hannerz & Hedman (1981) found that an innervation rate (and therefore firing frequency) of 100 Hz produced greater RTD, but not peak isometric force, of the toe extensors when compared to an innervation rate of 50 Hz. This suggests that supramaximal firing frequencies act to increase maximal RTD (Figure 2.8), rather than purely increasing maximal contraction force, as maximal RTD can only be produced when more action potentials per unit time reach the muscle fibres than are necessary for the production of maximal isometric force (Behm 1995, de Ruiter et al. 2006). This is supported by work that has found the contribution of neural drive to be a major influence on explosive force production throughout the force time curve (de Ruiter et al. 2004, 2006, Folland, Buckthorpe & Hannah 2014, Klass et al. 2008). Several studies have found associations between agonist muscle activation measured using EMG and initial force output in explosive contractions, suggesting that increased muscular activation is a key determinant of force production during the early phase (0 – 75 ms) of these contractions (Aagaard et al. 2002, de Ruiter 2004, 2006, Folland, Buckthorpe & Hannah 2014, van Cutsem et al. 1998). This demonstrates the importance of agonist muscle activation for the early stages of explosive muscle contraction.

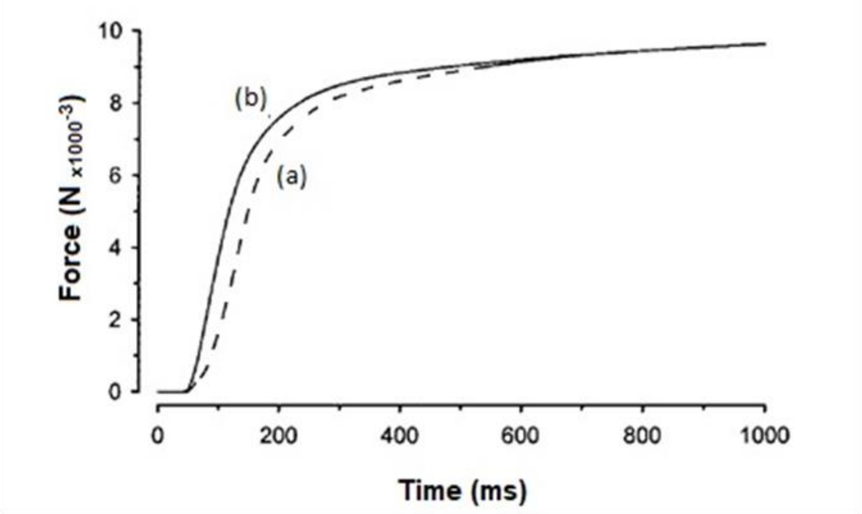


Figure 2.8. Force-time curves recorded from isolated motor units in the soleus muscle of rats when activated at (a) the minimum frequency required to produce maximal tetanic fusion, and (b) a supramaximal frequency that also produced maximal tetanic fusion. It is clear that RFD is greater when firing frequency is supramaximal. Adapted from Nelson (1996).

Evidence of changes in EMG as a result of strength training, and the greater explosive force capability of explosive power athletes compared to controls (ascribed to differences in neural drive to the muscle assessed using EMG; Tillin et al. 2010), underline this relationship still further. Studies consistently report increases in neural activation (increased myoelectrical activity) associated with concurrent increases in RTD in the early phase of explosive contractions following periods of strength training (Aagaard et al. 2002, del Vecchio 2019b, Gruber et al. 2007, Hakkinen, Komi & Alen 1985, Tillin, Pain & Folland 2012b, van Cutsem et al. 1998).

2.3.3.2 Intrinsic Contractile Properties

The contractile characteristics of a muscle are measured during involuntary contractions in response to a controlled stimulus in order to bypass the central nervous system and therefore remove the influence of voluntary neural drive (Figure 2.9). Electrically induced stimuli can be, for example, a supramaximal twitch (evoked from a single electrical impulse), or a tetanic response evoked as a result of a train of impulses. Evoked octets, for example, involve eight electrical stimuli delivered at 300 Hz, used to determine the maximum capacity of the muscle-tendon unit for explosive force production (de Ruiter et al. 2006). Contractile properties can be assessed for a single motor unit (often done *in situ*) or skinned muscle fibres, used to investigate the contractile performance of single muscle fibres in vitro (Bottinelli et al. 1999); or a whole muscle or muscle group *in situ* or *in vivo* by eliciting impulses via electrical stimulation over the muscle belly, or directly to the nerve supplying the muscle of interest (de Ruiter et al. 2004).The intrinsic contractile properties of a muscle (the contractile response of the muscle to known stimuli) are likely determined by fibre type, although this relationship is not completely clear. In support of this hypothesis, Harridge et al. (1996) found that muscle fibres of the same type (determined by histochemical analysis) had identical contractile properties, regardless of the muscle of origin.

Several studies have focused on how intrinsic contractile properties influence explosive strength of a muscle, as they are thought to be a key determinant of the rate of voluntary muscle contraction. Early work by Viltasalo and Komi (1978), building on the assumption that contractile properties are linked to fibre type**,** investigated the composition of the Vastus Lateralis muscle (determined by histochemical analysis) and the relationship of fibre types to RFD during explosive voluntary isometric leg press. There was a low-moderate correlation (r = 0.34 – 0.48) between fibre type and RFD, with participants with greater numbers of fast twitch fibres demonstrating increased RFD normalised to MVF. While histochemical properties of the muscle only explained 12 – 23% of the variance in normalised explosive force, this study provides solid evidence of a link between the two *in vivo*.

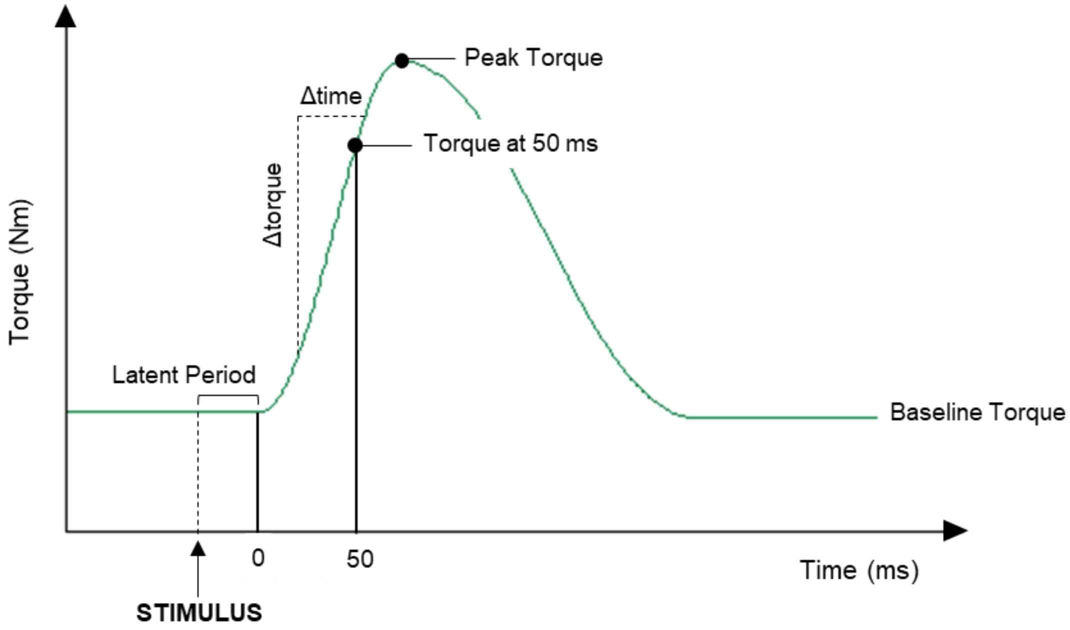


Figure 2.9. Twitch parameters used to assess the intrinsic contractile properties of a muscle. A twitch is a muscle contraction that occurs in response to a single, isolated action potential in a muscle fibre. The latent period is the time between stimulation and force onset during which excitation-contraction coupling takes place. Variables measured in this thesis are peak torque; peak RTD (calculated as the first derivative of the torque-time curve using a 15 ms time constant); and torque at 50 ms from onset (T_{50}). In evoked octets, which display the muscle's response to a train of eight stimuli at 300 Hz, torque at 100 ms from onset (T_{100}) is additionally measured.

More recent work has compared voluntary and involuntary force measures *in vivo* to better understand the association between force development and the intrinsic properties of the muscle. Their relative influence appears to change over the time-course of a contraction. Alongside agonist neural drive (Section *2.3.3.1*), twitch RFD (thought to be dependent upon the rate of cross-bridge cycling within muscle fibres [Clark, Fernhall & Ploutz-Snyder 2006]) seems to be an important determinant of early-phase voluntary explosive force and RFD. Andersen & Aagaard (2006) found a moderate relationship (r = 0.45 – 0.60) between peak twitch RFD and voluntary RFD of the knee extensors. This relationship became weaker at time points greater than 50 ms from the onset of the contraction; and was echoed in the results of a later study.

Folland, Buckthorpe & Hannah (2014) found that twitch response made a substantial contribution to the explained variance during the early phase of contraction (force at 25 – 50 ms, 34 – 40%; RFD from 0 – 50ms, 40%) but not at later phases (i.e. > 75 ms from onset). The importance of twitch response was hypothesised to be particularly important in the early phase due to the low levels of Ca^{2+} present in the sarcoplasm during the initial phases of voluntary contraction.

However, twitch peak RFD has been shown to only produce 25 – 30% of the maximum RFD of an evoked octet (de Ruiter et al. 2006). Combined with the greater reliability of octet when compared to twitch RFD (CV = 7.3% vs. 11.3%, respectively; Buckthorpe et al. 2012), it may therefore be useful to also measure the muscular response to octet stimuli, as twitch response may not be a good indicator of the full explosive capacity of the muscle. Folland, Buckthorpe & Hannah (2014) found that octet RFD was the primary determinant of the steepest phase of RFD (50 – 100 ms, the time window in which peak RFD typically occurs), accounting for 68% of the variance in RFD. This is in contrast to the findings of de Ruiter et al. (2004, 2007) who found that the response of the knee extensors to octet contractions was unrelated to voluntary explosive force production. However, de Ruiter et al. (2004, 2007) did not measure explosive force beyond 40 ms, while Folland et al. (2014) found the relationship did not strengthen until at least 50 ms from onset. It therefore seems that octet RFD is an important determinant of voluntary RFD once agonist neural activation is typically maximal after the first 50 ms.

When the relationships between twitch and octet peak force and relative (normalised to MVF) rather than absolute explosive force were considered, however, the moderate to high bivariate correlations waned (twitch: $r \leq 0.27$, octet: $r \leq 0.25$ across all time windows). Additionally, contractile properties only explained a modest proportion of the total variance in relative explosive force (≤ 15% at 25, 50, and 150 ms from force onset). The lack of relationships between relative evoked responses and relative explosive force indicated a shared variance of absolute twitch and octet force with MVF. This suggests that the increases in explosive force in the mid- to late-stage of contractions are related to the influence of maximum muscle strength rather than the intrinsic contractile properties of the muscle per se.

2.3.3.3 Contractile Capacity

As MVF/ MVT defines the ultimate plateau of voluntary force/ torque production, it seems logical that, all else being equal, a muscle with a greater maximum strength capacity will have greater RFD/ RTD. However, its relative contribution changes throughout the torque-time curve. Andersen & Aagaard (2006) measured the relationship between knee extensor RFD and MVF at multiple points from the onset of an explosive contraction. They found that, while MVF accounted for 18 – 25% of the variance in initial voluntary RFD (0 – 50 ms), RFD became increasingly dependent on MVF as contraction time increased (Figure 2.10). From the period between 100 – 200 ms, MVF accounted for 52 – 81% of the variance in RFD. Similar results were found by Folland, Buckthorpe & Hannah (2014), who found that MVF accounted for the majority of the variation in the later phase of explosive contractions (100 ms, 75%; 150 ms, 90%). Therefore, maximum strength appears to be the primary determinant of explosive strength during the late, but not early, phases of an explosive contraction.

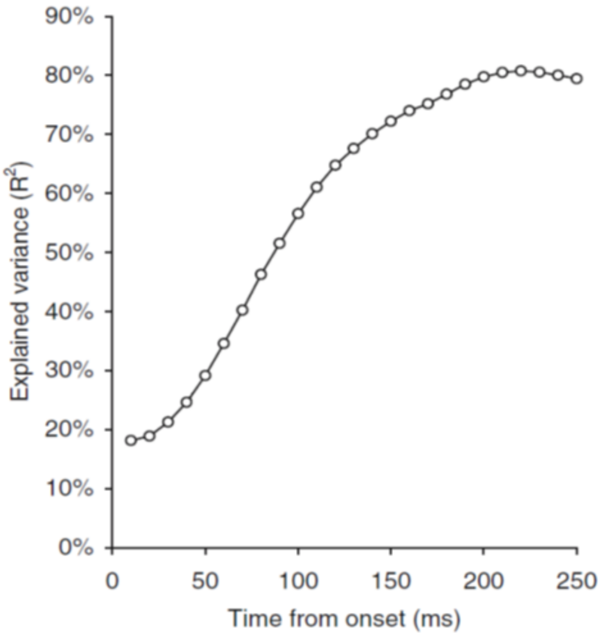


Figure 2.10. Explained variance between MVF and voluntary RFD during an isometric contraction of the knee extensors. RFD was measured at 10 ms time intervals of up to 250 ms from the onset of contraction. Adapted from Andersen & Aagaard (2006).

2.3.4 Determinants of Strength Asymmetry

A potential association between strength asymmetry and a typical determinant of strength may be characterised in one or both of the following ways. Firstly, between-limb differences in a neuromuscular determinant of strength may exist between the strongest and weakest limbs, suggesting asymmetry in strength and the neuromuscular determinant share the same direction. Secondly, the asymmetry in strength and the asymmetry in a neuromuscular determinant of strength may be correlated, suggesting they are proportional to each other. Both approaches are utilised in this thesis. To the author's knowledge, however, no research has directly investigated the underlying determinants of asymmetry in strength utilising the second method. It is therefore unclear whether the neuromuscular underpinnings of strength asymmetry are similar to those that determine force production of a single muscle or limb (as described in Sections 2.3.2 and 2.3.3). One possible approach to this problem may be to make inferences from studies who have determined associations between asymmetries in these factors through the assessment of neuromuscular differences between limbs grouped for strength; for example, in unilateral training or injury studies. In this case, a significant difference in the determinant when split by the strong and weak (or trained/ untrained, involved/ uninvolved) limbs would suggest that asymmetry is in the same direction for the both variables.

For example, let us consider neural drive, which is a key determinant of single limb maximum and explosive strength. As neural drive to muscles within a person comes from the same central nervous system, regardless of limb, we may reasonably expect symmetrical neural drive to muscles. Research into the effects of unilateral injury and training supports this assumption, providing evidence of a cross-over effect of neural function. Specifically, neural drive adaptations occur at the contralateral, as well as the injured/ trained limb (Hart et al. 2010, Bogdanis et al. 2019, Tillin, Pain & Folland 2011). In either case, a stimulus to one limb would not, therefore, have the effect of increasing inter-limb asymmetry in neural drive. Instead, asymmetries in neural drive are modulated by the synchronous reduction/ improvement in neural activation that occurs at both limbs, despite changes in strength larger in one limb relative to the other. In contrast, no changes in peripheral factors (e.g. muscle size) of the contralateral limb have been demonstrated following unilateral stimuli (e.g. Narici et al. 1989). Asymmetry in such factors would therefore increase if a stimulus was applied to only one limb. It is possible, therefore, that there will be distinct adaptations in each limb depending on its use, which may result in inter-limb asymmetries in the neuromuscular determinants of strength being very different between individuals. This may go some way to explain the variation in strength asymmetry commonly reported. For example, in professional Australian football players, Hart et al. (2014) reported significantly greater strength asymmetries (calculated using Limb Symmetry

Index-3; Appendix A) between the kicking and support limb in accurate (~1%), compared to inaccurate (8%), kickers. Accurate kickers also demonstrated significantly smaller lean mass (a key determinant of maximum strength) asymmetry than inaccurate kickers. This study also provides evidence that strength asymmetry may be reflected in altered or asymmetrical movement. Investigation into the neuromuscular mechanisms of strength asymmetry is therefore warranted.

2.4 Associations between Strength and Movement Asymmetry

As adequate muscle strength is necessary for various movements (Bassey et al. 1992, Buchner & Lateur 1991, Chang et al. 2015, de Ruiter et al. 2007, Maffiuletti 2010), and explosive strength seems to be important when time to develop force is limited, e.g. sprinting and jumping (Tillin, Pain & Folland 2013a) it seems logical to hypothesise that asymmetries in strength and movement may be related. In an able-bodied population, gait and bilateral movements such as jumping are frequently assumed to be symmetrical, however, research has shown that this is not necessarily the case (e.g. Benjanuvatra et al. 2013, Lathrop-Lambach et al. 2014). In ITTAs, asymmetries in loading (resulting from asymmetrical movement; Morgenroth et al. 2011, Nolan & Lees 2000) seem to result in reduced use of the muscles of the amputated limb, which may experience disuse-related neuromuscular changes as a result. This section of the review will therefore explore our current understanding of the relationships between strength and movement asymmetries in both populations.

2.4.1 Able-Bodied Populations

In addition to the theorised higher injury risk associated with muscular imbalances (e.g. Knapik et al. 1991), athletic performance may also be impaired in the presence of asymmetry. Hart et al. (2014) measured lower-limb strength asymmetries in

Australian football players using unilateral and bilateral isometric squats. When assessing the relationship of these asymmetries with 20m kicking accuracy, they found that the more accurate group of kickers demonstrated an average of just −1% difference between limbs (i.e. the support limb was 1% stronger). In contrast, the kicking limb of the less accurate group was 8% stronger. This appears to indicate that strength symmetry is important for kicking performance. Furthermore, in a group of competitive cyclists, peak torque asymmetries of the knee extensors were negatively correlated ($r = -0.50$, $p < 0.05$) with power output during a 5 second maximal effort cycling test (Rannama et al. 2015). Further studies have quantified associations between strength asymmetry and jump performance, an important skill in many sporting and recreational activities. At the single-limb level, some authors have reported moderate associations between bilateral jump height and explosive ($r = 0.53$ – 0.68; Chang et al. 2015, de Ruiter et al. 2007) and maximal strength of the knee extensors ($r = 0.56$, Chang et al. 2015). It seems logical to hypothesise therefore that strength asymmetry of the quadriceps may play a role in jump height; however, to the author’s knowledge, no research has currently investigated relationships between knee extensor maximal or explosive strength asymmetry and jump performance. Despite this, two previous studies have examined the relationship between jump height and maximum strength asymmetry of the entire lower limb. Comparing computer simulated musculoskeletal models that were symmetrical and 10% asymmetrical for lower limb strength, Yoshioka et al. (2011) found a non-significant difference of only 0.5% in jump height. They concluded that possibly the stronger leg compensated for the weaker; however, *in vivo* this compensation may not be adequate to fully make up for the weaker side. In support of this hypothesis, Bailey et al. (2013) reported mean between-limb asymmetries of 6.6 ± 5.1% and found weak to moderate negative correlations between bilateral strength asymmetries and jump height in loaded and unloaded jumps ($r = -0.39$ to -0.52, $p < 0.01$). While a large amount of variance remains unexplained, these data provide an indication that asymmetries of a greater magnitude may contribute to reduced jump performance.

Jump take-off, as a planar movement with a clearly defined objective (to jump as high as possible) and large contribution from the quadriceps to jump performance (Chang et al. 2015, de Ruiter 2006, 2007) may provide a useful model for the investigation of asymmetries in quadriceps muscle strength. In the rising phase of a jump take-off (i.e. from the point at which centre of mass [CoM] velocity becomes positive, until toe-off) asymmetries of 0.8 – 6.4% have been found in peak vertical ground reaction force (vGRF; Bačič et al. 2010, Bell et al. 2014, Impellizzeri et al. 2007, Lawson et al. 2006, Newton et al. 2006, Stephens et al. 2007). As vGRF represents the combined actions of primarily the lower extremity triple extensors (hip and knee extensors and ankle plantarflexors) during the force production portion of a jump, asymmetries in vGRF may stem from the way the lower limbs, pelvis, and trunk are coordinated to perform the jump (Benjanuvatra et al. 2013, Chang et al. 2015). The level of strength asymmetry at the quadriceps may be important for this coordination: Impellizzeri et al. (2007) found that correlations between maximum quadriceps strength asymmetry (assessed via unilateral isokinetic leg extension test) and vGRF in vertical jumps were moderate, but significant, for both 60°·s^{-1} ($r = 0.48$) and 240°·s^{-1} ($r = 0.48$). However, no research has developed this further to investigate associations with knee joint loading asymmetries (e.g. asymmetries in peak knee extension moments, 3.23 – 5.26%; and peak knee powers, 0.90 – 2.73%; Lawson et al. 2006, Stephens et al. 2007). Given the positive relationships apparent between knee extensor strength and vGRF asymmetries described above, it is logical to hypothesise that greater maximum strength asymmetry at the quadriceps may also be related to asymmetry in loading at the knee joint. Specifically, we may expect to see greater loading occurring at the knee of the maximally strong limb.

As the take-off portion of a jump is rapid, high-demand movement, the ability to quickly produce force is therefore important. This is substantiated by the strong relationships between single-limb explosive quadriceps strength and jump height (de Ruiter 2006, 2007). Furthermore, Chang et al. (2015) reported 47.6 and 31.4% of the variability in vertical jump height was explained by knee extensor early phase RTD and MVT, respectively, suggesting that the former is more important for jump performance. It may be that asymmetry in explosive strength is a better predictor of movement asymmetry than that of maximum strength; however, to the author's knowledge, this has not been investigated.

Landing from a jump is another useful model for investigating the associations between strength and movement asymmetries due to the importance of the quadriceps in force dissipation: proportionally, the knee performs most of the eccentric joint work involved in decelerating the CoM from a jump landing (39% compared to 34% and 27% at the hip and ankle, respectively; Decker et al. 2003, McNitt-Gray et al. 1993, Yeow et al. 2011, Zhang et al. 2000). Previous research has found evidence of between-limb differences in vGRF of 3.53 – 27.4%, and more substantial asymmetries in knee extension moments of 17.4% – 38.3% (Bates et al. 2013, Schot, Bates & Dufek 1994) in landing tasks. It may therefore be the case that strength asymmetries of the quadriceps are related to asymmetries in landing.

Furthermore, landing mechanics have been associated with increased injury risk at the knee. Specifically, it is the rapid development of force that occurs during landing that has been associated with various knee injuries, including osteoarthritis and non-specific knee pain (Murphy et al. 2003). In limbs that have undergone ACL reconstruction, previous research has demonstrated decreased dynamic force absorption at the knee in landing tasks (i.e. a reduction in knee extensor eccentric muscle action to attenuate load) to be associated with large strength asymmetries.

While the uninvolved limb was consistently loaded more on landing, possibly in an attempt to protect the involved knee, this lack of shock absorption in the involved knee was hypothesised to explain the high prevalence of re-injury (Kuenze et al. 2015). However, no research has investigated the relationship of strength and landing asymmetries in a healthy population. It may be that explosive strength is more important than maximum strength for the rapid energy absorption required in the lower limb on landing. If we consider that injuries such as ACL ruptures occur within 50 ms of ground contact (Krosshaug et al. 2007) it is likely that the ability to produce force rapidly is of more importance than maximum strength (as maximum force is generally not produced until > 300 ms from onset in isometric and eccentric muscle contractions [Thorstensson et al. 1976, Tillin, Pain & Folland 2012a]). Larger asymmetries in explosive strength may therefore result in one limb attempting to dissipate greater loads more rapidly, in which case we may expect to see greater (1) differences in peak loads and loading rate with the limbs split by explosive strength, or (2) correlation coefficients when asymmetry in loading variables are compared to explosive, rather than maximum, strength asymmetry. If such associations were apparent, this may have implications for injury

2.4.2 ITTAs

A deeper understanding of the relationships between strength and movement asymmetries may be gained by investigating their association in a population who are known to demonstrate large asymmetries. ITTAs (a population with anatomically imposed musculoskeletal asymmetry due to the loss of the ankle joint and surrounding musculature on one limb, which has major implications on their functional capabilities) display knee extensor maximum strength asymmetries of 41 – 57% between the amputated and intact limb (section *2.5.1.1*; Isakov et al 1996a, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Renstrom, Grimsby & Larsson

1983). This compares to 1 – 18% between limbs in healthy, young, non-injured controls (Table 2.1; Graham-Smith, Al-Dukhail & Jones 2015, Kobayashi et al. 2013, Menzel et al. 2013, Ruas, Brown & Pinto 2015). Additionally, compared to able-bodied controls, ITTAs walk more slowly (Bohannan 1997, Oberg et al. 1993, Sanderson & Martin 1997); spend more time in double stance (Gailey et al. 2008); have decreased balance (Vanicek et al. 2009); can't jump as high (Schoeman, Diss & Strike 2012) and fall more often (Kulkarni et al. 1996). Adequate gait function is fundamental for daily living and exercise, and as a result, health and quality of life. This may be particularly true in ITTAs who are not necessarily able to perform more intense exercises (e.g. running) due to the greater musculoskeletal demand on the weaker amputated limb.

2.4.2.1 Gait Asymmetry and Functional Performance

Gait analyses have shown that ITTAs walk asymmetrically, with the intact limb playing a dominant role. This is evidenced by reduced amputated limb stance and single-limb support time (Isakov et al. 2000, Kovač et al. 2010, Mattes et al. 2000, Nolan et al. 2003) and intact limb step length (Barnett et al. 2009, Sanderson & Martin 1997). These between-limb differences result in asymmetries of 4.3 – 7.2% in step length and ~7% in single support time (Barnett et al. 2009, Howard et al. 2013, Isakov et al. 2000). Kinetically, the amputated limb exhibits decreased peak vertical ground reaction force, resulting in asymmetries in whole limb loading of 4 – 10% (Menard et al. 1992, Powers 1994, Sanderson & Martin 1997).

The ankle and surrounding musculature provide most of the propulsive power in able-bodied gait (Winter & Sienko 1988). In ITTAs, who are missing this joint on one side, other muscle groups must attempt to compensate for this loss of forward progression. Greater muscular strength of both the amputated and intact limb knee and hip flexors and extensors, as well as of the hip abductors, have been associated with improved gait function and symmetry in ITTAs assessed via temporospatial (TS) gait characteristics such as cadence, and step and stride length, and single support time (Renstrom et al. 1983, Powers et al. 1996). Furthermore, walking speed, which is an important indicator of functional gait capacity (van Velzen et al. 2006), tends to be lower in ITTAs than able-bodied populations (1.25 m/s vs. ~1.4 m/s; Bohannan 1997, Oberg et al. 1993, Sanderson & Martin 1997). As walking speed has been shown to be predictive of a range of outcomes in multiple populations including functional dependence (Purser et al. 2005, Shimada et al. 2013, Shinkai et al. 2000), institutionalisation (Woo et al. 1999) and falls (Montero-Odasso et al. 2005), it is important that ITTAs are able to maintain adequate speed while walking. Quadriceps strength is thought to be important for walking speed: for example, in patients who had undergone total hip arthroplasty, maximum walking speed was related to knee extensor RTD (but not MVT; Suetta et al. 2004). However, to the author's knowledge, no previous studies have investigated the association between knee extensor explosive strength asymmetry and walking speed in ITTAs.

Adequate, symmetrical muscle strength is thought to play an important role in the maintenance of ITTA gait function, with large inter-limb differences in strength potentially related to asymmetrical limb loading. In support of this hypothesis, Lloyd et al. (2010) found that knee extensor strength asymmetry was correlated with frontal plane knee moment load rate asymmetry (rho = 0.71, $p < 0.05$), while knee flexion strength asymmetry moderately correlated with the intact side ground reaction force load rate (rho = 0.50). The decreased loading on the amputated side is suggested to be the result of a strategy adopted to protect the residual limb (Engsberg et al. 1991, Nolan & Lees 2000, Nolan et al. 2003, Royer & Koenig 2005). Specifically, Lloyd et al. (2010) proposed that it is due to attempts to avoid generating large moments at the knee and the residual limb-socket interface, which create large areas of pressure and discomfort. Muscle action at the knee is important in the coordination of ankle and hip mechanics throughout gait, and to help regulate joint movement through the eccentric control of knee flexion (Neptune et al. 2008). When the quadriceps are activated during gait, they produce an internal knee extension moment in response to the external knee flexion moment; however, previous research has found that knee extension moments in walking and jumping are reduced in the amputated compared to intact limb (Fey & Neptune 2012, Powers, Rao & Perry 1998, Schoeman, Diss & Strike 2012, Silverman et al. 2008, Winter & Sienko 1988). The reduced extension moments apparent when compared to the intact limb therefore suggest that the demand of the amputated side quadriceps is reduced.

If we consider gait in two roughly equal halves defined by antero-posterior ground reaction force (hGRF), we can break the main tasks down into braking (0 – ~50%, hGRF < 0 N/kg; whereby the goal is to decelerate the CoM while maintaining forward progression) and propulsion (~50 – 100%, hGRF > 0 N/kg). In ITTAs, evidence suggests that limb load is substantially less on the amputated limb when compared to the intact in both phases. At the knee, Sanderson & Martin (1997) reported that in braking, amputated limb knee moment was reduced to the point that it remained flexor during the entirety of early stance. Furthermore, in dynamic simulations of amputee gait, the work undertaken by RF and the vastus muscles was decreased in the residual limb relative to both the intact and control limbs, but particularly in braking (Silverman & Neptune 2012). Schmalz et al. (2001) found decreased EMG activity of VL, particularly during the early stance phase of gait in the amputated vs. intact limb of ITTAs. These findings are consistent with previous work showing atrophy of the quadriceps muscle in ITTAs (Moirenfeld et al. 2000, Renstrom et al. 1983), which may be one contributor to smaller force output from these muscles. However, no further work has attempted to clarify the relationship of maximum or explosive quadriceps strength asymmetry with the asymmetry that evident in both TS and loading parameters of gait in this population. If symmetry in quadriceps strength is associated with gait symmetry, it may have important implications for exercise prescription and functional capacity in ITTAs.

In summary, evidence of associations has been found between performance deficits and greater strength asymmetries, but the underpinning mechanisms of these alterations in movement have not been explored. Both the take-off and landing phases of jumps are rapid, planar movements with large contributions from the quadriceps, and asymmetries evident in both whole-limb and single joint load (e.g. Bates et al. 2013, Lawson et al. 2006, Newton et al. 2006). As such, these movements may prove useful for the investigation of the association between quadriceps strength and movement asymmetries in a healthy control population. Quantifying the associations between such asymmetries in individuals with no specific loading/training bias may allow comparison to specialist populations with strength or neuromuscular adaptations which may predispose them to overloading or injury (Murphy et al. 2003, Lloyd et al. 2010). Building on previous research in injured groups, we hypothesise that an able-bodied population will also demonstrate associations between strength and movement asymmetries (i.e. where strength asymmetry is present, the stronger limb will bear increased load to compensate for the weaker limb). Furthermore, investigating these relationships in established ITTAs (who are known to be asymmetrical in both strength and gait) may allow the opportunity to establish the relationships between asymmetries in strength and movement in further detail, as it is likely that relationships may be more observable. As ITTAs are increasingly encouraged to exercise for health and quality of life, understanding if strength asymmetry is associated with changes in movement patterns (specifically, performance and asymmetry in limb/ joint loading) may be important to inform healthy movement prescription.

2.5 ITTA Asymmetry as a Model of Disuse

Disuse of the residual limb (discussed below) may contribute to the functional changes seen in ITTAs due to alterations in neuromuscular factors affecting the way that the muscles produce voluntary force. This section of the review will therefore explore the evidence for disuse of the amputated limb of ITTAs, changes in neuromuscular function resulting from disuse, and how these changes may be expressed in, or effect, the quadriceps muscles in ITTAs.

Initially following amputation, the amputated limb of ITTAs is fully unloaded, causing disuse of the muscles of this limb. During rehabilitation, and then onward, it undergoes repetitive, but limited loading (Esquenazi & DiGiacomo 2001). The disuse of the amputated limb resulting from gait asymmetry (as discussed in section *2.4.2.1*) may predispose ITTAs to maladaptations in neuromuscular function. Significant decreases in muscle strength and size, and in bone mineral density, of the amputated compared to the intact and control limbs support this theory of disuse (Royer & Koenig 2005, Tugcu et al. 2009). These changes may be associated with a number of comorbidities that ITTAs are prone to, such as joint pain and osteoporosis on the amputated side (Norvell et al. 2005, Vignon et al. 2006). Several determinants of strength are likely to be affected; however, current understanding of what causes weakness in the quadriceps the amputated limb of ITTAs is poor. Determining the underlying causes of this weakness may allow better targeting of rehabilitation interventions. Additionally, current knowledge of the effects of muscle disuse on neuromuscular function is based on short-term (typically <120 days) studies utilising models of unloading that include microgravity, unilateral lower-limb suspension (ULLS), limb immobilisation and bed rest. When undergoing bed rest, participants are confined to their bed, free to move but without any load on the lower limbs. In ULLS, one leg is kept flexed and suspended above the ground by the use of a shoulder harness; and in limb immobilisation a cast blocks the movement and loading of a limb. Each of these protocols are effective and sufficient to induce the expected adaptations of muscles to disuse (i.e. reductions in strength, neural drive, and altered muscle architectural and contractile properties, as discussed in section 2.5.1); however, the short-term nature of these studies makes it difficult to draw conclusions about the long-term effect of disuse on skeletal muscle. The decreased loading habitually experienced by the quadriceps of the residual limb of ITTAs means that this population may provide a useful model for investigating the long-term effects of muscle disuse, by using the intact limb as an internal control.

In order to understand the muscular changes that may occur in ITTAs as a result of long-term disuse of the quadriceps in the amputated limb, it is necessary first to understand the strength changes and underlying neuromuscular determinants that occur in muscles as a result of short-term disuse in a healthy population.

2.5.1 Neuromuscular Adaptations to Disuse

Muscles adapt to disuse in multiple ways, for example through atrophy, myosin heavy chain [MHC] shifts and decreases in neural drive, all of which can impact muscular strength, as discussed below.

2.5.1.1 Muscle Strength

The quadriceps muscles, as part of their roles in acting against gravity and contributing to locomotion, are particularly susceptible to degenerative changes resulting from disuse. Studies utilising bedrest, limb suspension, limb immobilisation and microgravity as models of unloading (Figure 2.11) have reported reductions in quadriceps maximum strength of approximately 2% per day for the first ten days (Berg & Tesch 1996, Gamrin et al. 1998, Rozier et al. 1979, Thom et al. 2001), slowing to ~1% per week up to 30 days, with an eventual plateau resulting in average strength losses of around 23% after 120 days of disuse (Dirks et al. 2013, 2016, Horstman et al. 2012, Narici & de Boer 2011, Suetta et al. 2012, Wall et al. 2013). Furthermore, decreases in RTD of between 15 – 54% have been found, although compared to maximum strength, explosive force production capacity has been understudied (Bamman et al. 1998).

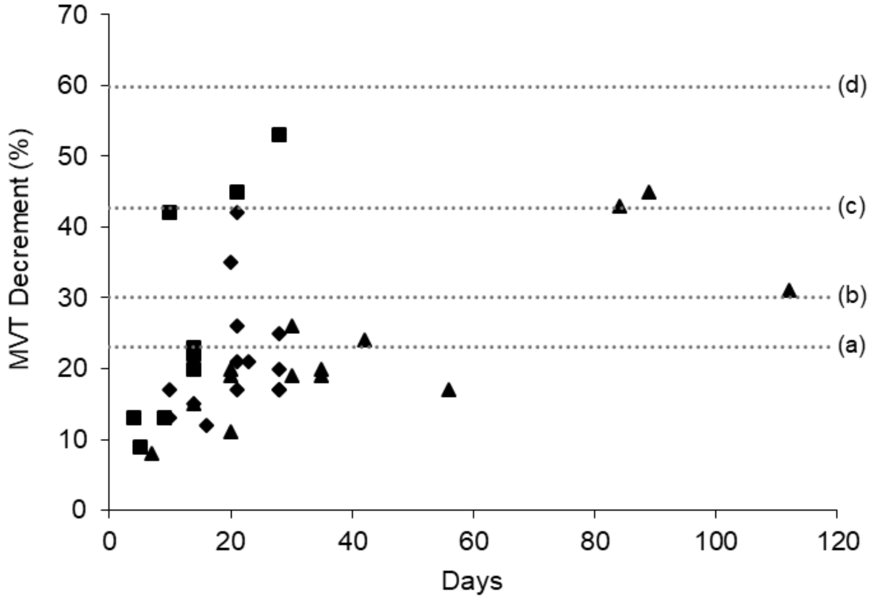


Figure 2.11. Percentage decrease in maximum voluntary torque (MVT) at the knee extensors following differing periods of ground-based unloading (▲ bed rest; ■ limb immobilisation; ◆ unilateral lower-limb suspension [ULLS]), reported relative to MVT pre-intervention. Dashed lines show mean knee extensor MVT decrease for (a) all unloading studies; (b) the intact vs. a control limb; (c) amputated vs. intact limb; and (d) the amputated vs. a control limb (Isakov et al 1996a, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Renstrom, Grimsby & Larsson 1983). Adapted from Narici & de Boer (2011). See Appendix B for full data table.

In ITTAs, strength decrements have been found in both the amputated (-64%; Figure 2.11d) and intact (-28%; Figure 2.11b) limb when compared to non-injured controls; however, the loss of strength in the amputated limb is always greater, resulting in maximum strength asymmetries ~42% (Figure 2.11c; Isakov et al 1996a, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Renstrom, Grimsby & Larsson

1983). The decrements in maximum strength experienced in ITTAs are therefore, on average, much greater than those resulting from short-term disuse. This is interesting, as current disuse studies suggest that strength decreases exponentially, with limited change after the first few days (Figure 2.11). Long-term disuse may therefore result in more dramatic decreases in strength than predicted from short-term studies. In contrast, explosive strength in ITTAs has not been investigated.

Multiple previous studies have reported significant deficits of between 19 – 34% in maximum strength when the intact limb is compared to the control (Isakov et al. 1996a, Lloyd et al. 2010, Pedrinelli et al. 2002). However, the magnitude of these differences may be attributable to the inclusion criteria applied to the participants in these studies. Thus, it is unclear if the intact limb of the ITTA participants was representative of a normal, healthy limb from which to compare the effects of disuse on the amputated limb. For example, all three studies who included ITTAs with amputations resulting from vascular conditions and infection, which means that the results may be affected by the influence of disease (Sacchetti et al. 2013). Inactivity is known to have a detrimental effect on muscle function (Narici & de Boer 2011); however, none of these studies controlled for activity level, so it is unclear what affect this may have had on the results. Indeed, it is likely that the participants may have been inactive: 20% of the TTAs tested by Pedrinelli et al. (2002) relied on walking aids, which is likely to severely to have limited their activity levels. Additionally, there were no exclusion criteria for age, and the latter studied assessed a mixture of males and females (Pedrinelli et al. 2002), so the results may have been affected by the confounding effects of gender (Hannah et al. 2012) and ageing (Pincivero et al. 2004) on neuromuscular function. It may be that excluding ITTAs with amputations resulting from disease, and controlling for age, gender and activity, would minimise the negative effect of disuse on the intact limb, and provide an internal control with neuromuscular characteristics similar to that of an able-bodied limb.

2.5.1.2 Muscle Size and Architecture

Skeletal muscle is highly plastic, rapidly gaining or losing contractile tissue in response to changes in loading. Strength changes due to short-term disuse (periods of time from 10-120 days) are accompanied by decreases in muscle volume of 5-18% (Alkner & Tesch 2004, Campbell et al. 2013, de Boer et al. 2007, Funato et al. 1997). Muscle atrophy in disuse is caused by abnormal skeletal muscle protein turnover. This is a dynamic process that, in healthy individuals, balances synthesis and breakdown, allowing the body to maximise the use of a limited supply of amino acids, regulate enzymatic systems and metabolic processes, and remove defective proteins (Stein & Wade 2005). However, during periods of unloading, such as in disuse, this balance is disturbed. This causes increased protein breakdown compared to protein synthesis so that the net balance of protein turnover is negative (Ferrando et al. 2002), resulting in muscle atrophy.

At the quadriceps, Gibson et al. (1987) found a decrease in muscle protein turnover after 37 days of lower limb immobilisation, and a corresponding decrease in quadriceps muscle mass due mainly to a considerable (25%) depression of muscle protein synthesis. Other work has shown that a loss of muscle mass is evident within seven days of immobilisation through either bed rest (Ferrando et al. 1995) or limb suspension (Dudley et al. 1992), and this decrease continues throughout the period of disuse (Alkner & Tesch, 2004, Clark et al. 2007). The loss of strength combined with decrease in muscle size is also seen in ITTAs: Schmalz et al. (2001) reported that the quadriceps muscles of the amputated limb were significantly thinner than those of the intact limb (side-to-side differences measured using ultrasonography were 84.3%, 80.2% and 76.2% in RF, VL and VM, respectively; $p < 0.001$). Similar decreases were observed by Renstrom et al. (1983), who found atrophy of both type I and II fibres in the amputated limb contributed to a decrease in the size of the quadriceps in ITTAs. Additionally, changes in muscle size were correlated to the side-to-side differences in strength that have been reported in ITTAs (Renstrom, Grimsby & Larsson 1983). Together, this supports the hypothesis that muscle atrophy is directly related to decreases in strength. However, greater decreases in strength relative to the loss of muscle mass following unweighting (Berg et al. 1997), suggests that other aspects of the force-generating capacity of the neuromuscular system are compromised. In support, a study by Kawakami et al. (2001) found no relationship between change in muscle size and strength following 20 days of bed rest in a control group. This suggests that the atrophy of the quadriceps that has previously been found in ITTAs is not the only factor contributing to the decrease in strength in that population.

Two studies that examined the effects of ULSS in humans found a decrease in muscle fascicle length at the quadriceps from 6 – 9% after three weeks (Campbell et al. 2013, de Boer et al. 2007). This represents a loss of sarcomeres in series resulting in a potential shift on the length-tension curve. In addition, decreases in pennation angle, and a decrease in number of fascicles in parallel of ~8 – 13% in VL are evident from 23 – 35 days of ULLS (de Boer et al. 2007, 2008, Campbell et al. 2013). Similarly, when examining gastrocnemius muscle atrophy resulting from unilateral disuse due to injury to one leg, Narici et al. (1998) found that ACSA of the injured limb was highly correlated with a decrease in pennation angle (-16%) and fibre length (-13% compared to the control limb). Disuse seems therefore to involve a loss of sarcomeres in parallel and in-series (Figure 2.12). These changes have the potential to negatively impact maximum torque production, as fascicles in parallel indicate the PCSA of the muscle, which is strongly correlated with maximum strength (see Section *2.3.2.2*). The detrimental effects of the loss of sarcomeres in series could therefore be moderated by a more efficient force transmission to the tendon arising from the decrease in pennation angle (Fukanaga et al. 1997).

Figure 2.12. Schematic diagram illustrating the changes in fibre length and pennation angle occurring with muscle atrophy resulting from disuse. Muscle thickness, pennation angle and fibre length before and after atrophy are indicated by t_1 and t_2, $θ_1$ and $θ_2$, L_{f1} and L_{f2}, respectively. Reproduced with permission from Narici (1999).

The above research indicates that muscle architectural remodelling resulting from disuse is very rapid, with major changes occurring within weeks, and some detectable from just days after the initialisation of the immobilisation period (Magnusson et al. 2008). However, these are all short-term studies (the maximum time studied is 35 days) which is not necessarily indicative of the changes that occur with long term disuse due to sedentary behaviours or injury, such as in amputees. Additionally, as muscle turnover in skeletal muscles is relatively slow (Johnson et al. 2000), the decreases in strength that have been found after just nine days of immobilisation (Rozier et al. 1979) indicate that other factors such as neural drive and the intrinsic contractile properties contribute to strength deficits resulting from muscle disuse (LeBlanc et al. 1992, de Boer et al. 2007).

2.5.1.3 Neural Drive

On a global level, previous research demonstrates a strong relationship between changes in neural drive measured using EMG, and muscular strength loss per day with disuse (r = 0.76; Campbell et al. 2019). Several studies have investigated alterations in neural drive following short-term disuse by exploring the response of the quadriceps. During maximal isometric contractions, reductions in RMS EMG of 19% after 42 days of BR (Berg et al. 1997) have been recorded, while Deschenes et al. (2002) reported a 16% reduction in integrated EMG after 14 days of the same model of unloading. Following 89 days of bed rest, RMS EMG at MVC decreased by 38% (Alkner & Tesch 2004). These results seem to demonstrate consistent reduction in neural drive to the quadriceps with disuse. In contrast, other studies have found no change in maximum EMG in VL (de Boer et al. 2007, Campbell et al. 2013) following immobilisation by ULLS. The disparity in these results may be a result of the different models of unloading used, or the treatment of the data. Berg et al. (1997), Deschennes et al. (2002) and Alkner & Tesch (2004) examined the entire quadriceps muscle group EMG as opposed to VL alone, while de Boer et al. (2007) and Campbell et al. (2013) only analysed EMG of VL. Additionally, the latter two studies normalised the data to the maximal M-wave (de Boer et al. 2007, Campbell et al. 2013); while Berg et al. (1997) normalised EMG data to pre-unloading by using the mean of the three baseline measurements for each muscle, and Alkner & Tesch (2004) did not normalise their EMG data at all. Raw EMG data can be used only for limited comparisons, so normalisation is preferable (see Section *2.3.1.1*). Of the two methods given, normalising EMG to maximal M-wave (the signal recorded at the muscle following the supramaximal stimulation of its peripheral motor nerve) is favourable as it allows comparison between muscle, tasks and individuals (Halaki & Ginn 2012). The differences in the methodologies and treatment of the data between these studies therefore makes it difficult to generalise these results.

Further studies have attempted to quantify neural drive to the quadriceps muscles through voluntary activation measured using ITT. While studies measuring neural drive at MVT using VA (also measured as Central Activation Ratio) have found non-significant reductions of 0.5 – 5% between 14 – 30 days of ULLS (Cook et al. 2014, de Boer et al. 2007, Campbell et al. 2013, Horstman et al. 2012), Kawakami et al. (2001) reported a significant ($p < 0.05$) central activation deficit of 7% following 20 days of bed rest. The difference in the results of these studies may be due to the different models of disuse employed, as there are subtle but relevant differences are present in the response to each specific protocol (Reggiani 2015). For example, it could be that in ULLS there is still some action from RF throughout gait in its role as a hip flexor.

2.5.1.4 Intrinsic Contractile Properties

In vivo, the response of muscle fibres to disuse is contested. After 6 – 8 weeks of immobilisation, Ducheteau et al. (1990) found a decreased twitch peak force (38 – 42%) in human adductor pollicis and first dorsal interosseous muscles. This was accompanied by shorter contraction (13 – 16%) and decreased half relaxation times (12 – 13%; indicative of the rate of Ca^{2+} re-uptake at the sarcoplasmic reticulum). In the plantarflexors, 28 days of ULLS resulted in significant reductions in absolute early (-15%), but not late phase (-7%) evoked RFD (Clark, Fernhall & Ploutz-Snyder 2006). These responses are all likely direct consequences of the decline in the force production capacity of the muscle evidenced by decreased twitch force. However, the latter study also observed that declines in relative peak RFD were not as great as those expressed in absolute terms, suggesting that the contractile speed of the muscle increased with disuse (Clark, Fernhall & Ploutz-Snyder 2006).

As research investigating the changes in intrinsic contractile properties following disuse at a whole-muscle level is limited, and there is a hypothetical link between evoked contractile properties and fibre type (Harridge et al. 1996), we can attempt to draw further conclusions based on single-fibre and cellular changes. In rodents, spaceflight induced muscular disuse leads to an increased distribution of fast MHC isoforms (Caiozzo et al. 1994), which results in increased V_{max}, fatiguability, relative RFD and contraction time. This is reflected in the response of human quadriceps muscles to short term spaceflight (11 days), which also demonstrate a slow to fast MHC shift and increased fatiguability (Day et al. 1994).

Ground based models of quadriceps unloading have reported either no MHC shifts, or a shift to a faster profile. After two weeks of ULLS, Deschenes et al. (2002) found no significant changes in muscle fibre type or distribution in the knee extensors. In contrast, Bamman et al. (1998) reported a small, non-significant shift in MHC expression at VL (6.5% increase in type IIb compare to IIa) following 14 days bed rest. Furthermore, Trappe et al. (2004) found a significant increase in hybrid fibres (i.e. those that co-express two or more MHC isoforms), with shift toward faster MHC isoforms in VL after 84 days of bedrest. This was accompanied by a significant decrease (-29%) in Type I MHC. The difference between the two studies is likely to be a result of the longer period of disuse utilised by the latter researchers. In ITTAs, a single study found a similar shift of muscle fibre types: a decrease in the percentage of type I fibres in the amputated (33%) compared to non-amputated leg (38%; $p > 0.05$) was evident, together with significantly fewer type IIa ($p < 0.01$) and more type IIb ($p < 0.05$) fibres (Renstrom et al. 1983). A slow to fast MHC profile shift of an entire muscle would, in theory, lead to faster contractions, which would be reflected in increased RTD and decreased contraction time. However, we still have no clear picture of what happens in terms of MHC distribution, or, by extension, the intrinsic contractile properties of the quadriceps when subjected to long-term disuse.

Another adaptation to disuse is muscle fibre size changes. Limb immobilisation causes slight preferential atrophy of Type I and IIa fibres compared to Type IIb fibres in both humans and rodents (Bamman et al. 1998, Maier et al. 1976). This is supported by evidence that single muscle fibres experience significant decrease in myosin content (35%) following 3 weeks ULLS (Campbell et al. 2013). Trappe et al. (2004) found reductions in CSA of MHC I (-15%) and IIa fibres (-8%) after bed rest, which was accompanied by significant decreases in peak force in both fibre types (-47%, MHC I; -25%, MHC IIa). Although, when corrected for cell size, the type I fibres still showed a decline in peak force ($p < 0.05$), suggesting that changes in fibre size could not entirely explain this loss, decreased myosin content may be one factor contributing to the overall loss of strength in the quadriceps.

2.5.2 Strength and Neuromuscular Adaptations to Long-Term Disuse

In summary, short-term (<120 days) studies suggests reductions occur in both maximal and explosive strength. There is also evidence for decreased muscle size, agonist neural drive, fascicle length and pennation angle, and increased speed of contractile properties. However, the response of muscle strength and its underpinning neuromuscular factors to long-term disuse is not known. It may be possible to advance our understanding of the above by investigating the neuromuscular characteristics of established ITTAs, a population with known movement asymmetries, which results in disuse of the amputated limb compared to the intact.

2.6 Summary and Thesis Aims

It is clear that some level of strength asymmetry is present at the quadriceps in multiple populations. However, there is debate surrounding the normal level of strength asymmetries at the quadriceps, primarily due to the multiple ways in which asymmetry has been determined, using a range of limb classifications (left/ right, dominant/ non-dominant, strong/ weak) and multiple calculations (Appendix A). The typical magnitude and variability of strength asymmetry – and particularly that of explosive strength, which is theoretically important but understudied - in a healthy, non-specialist population needs documenting for future comparison to specialised groups. Furthermore, while strength is determined primarily by a muscle’s intrinsic contractile properties and its architecture, in addition to the neural drive to the muscle, no research has currently investigated how these factors interact to produce strength asymmetry, which may be useful for the targeting of interventions. **Chapter 4 therefore aimed to compare the magnitude and variability in maximum and explosive strength asymmetries, and to determine the underpinning**

neuromuscular mechanisms of these strength asymmetries in a population of young, healthy, recreationally active males with no specific training bias.

The association of strength asymmetry at the quadriceps with movement asymmetry is also unclear. As strength asymmetry is implicated in ageing, injury and other special populations, it is important to understand its relationship to movement performance and symmetry in a healthy, young population with no training bias for comparison to other groups. Take-off from a jump, and drop landings, being planar movements with substantial quadriceps contributions, are useful for the assessment of functional capacity. For this reason, **Chapter 5 aimed to investigate the associations between maximum and explosive quadriceps strength asymmetry and the (1) performance of a maximal countermovement jump, assessed via jump height, and (2) the underpinning kinetic asymmetries in jump take-off and landing in an able-bodied population.** However, given the postulated difficulty in identifying consistent asymmetries within a group of individuals unless they are highly specialised to an asymmetric task (Lawson et al. 2006), studying an inherently asymmetrical population may allow relationships between strength and movement asymmetries, if present, to be more observable. ITTAs display substantial asymmetries in strength and movement. Furthermore, their reduced functional gait capacity, evidenced by slower walking speeds when compared to able-bodied populations, may affect their participation in sport and thus health and quality of life. **Chapter 6 therefore aimed to assess the relationship between quadriceps strength asymmetries and (1) walking performance, assessed via walking speed, and (2) temporospatial and kinetic asymmetries during gait at two different walking speeds in ITTAs.**

It may be possible to manipulate asymmetry to help us to study broader physiological questions such as the mechanisms underpinning chronic disuse. Studies utilising enforced unloading of the lower limb have found losses of strength of the knee extensors with disuse, potentially caused by decreases in muscle size and neural drive, along with alterations in the architectural and contractile properties of the disused muscles. However, the evidence for these changes are from short-term studies only, so we do not know how these factors change over extended periods of time. ITTAs, who exhibit habitual, long-term disuse of the amputated limb and the knee extensors on this side, provide a unique model to assess the long-term effect of disuse on both maximum and explosive strength, and in addition, an understanding of the mechanisms underlying weaknesses in ITTAs may help to target interventions to improve movement and health in this population. **Chapter 7 therefore aimed to determine the effects of long-term muscle disuse in ITTAs on strength and its neuromuscular determinants.**

Chapter 3

Methods

This chapter presents the methods completed in preparation for data collection, data collection itself, and the data processing conducted for the entire thesis. Specific information pertaining to variable extraction and analysis of the data will be detailed in each experimental chapter.

3.1 Ethical Approval and Participant Recruitment

This project was approved under the procedures of the University of Roehampton's Ethics Committee on 11/07/16 (reference LSC/ 16/176; Appendix C1). Both ITTAs and able-bodied individuals were recruited. An additional ethical application was also approved by the NHS Health Research Authority (reference number 17/NW/0566; Appendix C2). Two NHS limb-fitting centres (Bowley Close and Queen Mary's Hospital, Roehampton) agreed to aid with recruitment through identification of potential ITTA participants. Both sites were supplied with advertisement materials (fliers and posters) to distribute accordingly. Additionally, Consent to Contact Forms (Appendix D1) were provided to allow the researchers to contact potential participants who wished to receive further information about the project.

Once contact had been established with a potential participant, they were provided with an information sheet informing them of the purpose of the study, the protocols to be followed (including details of risks involved), and their right to withdraw from all or parts of the study at any time (Appendix D2). All participants provided informed written consent prior to commencing the study (Appendices D3 and 4). Participants were offered £10 in cash per session (£30 in total) in compensation for their time and effort. As the ITTAs were more difficult to recruit, and generally travelled further than the control cohort, they were offered up to an additional £20 per session to cover travel expenses upon provision of a ticket receipt or details of distance driven (reimbursed at £0.45/mile; Appendices D5 and 6).

3.2 Participant Populations

All recruited participants were male, with moderate to high levels of habitual physical activity. While females were not precluded from participation, the inability to recruit female ITTAs dictated a male recruitment in able-bodied controls to facilitate comparisons. Physical activity was assessed using the International Physical Activity Questionnaire (Short Format, http://ipaq.ki.se/downloads.htm). Participants with an MET-mins/week score of 600-3000 were described as moderately active, and those with a score of >3000 as highly active (Craig et al. 2003).

Amputees were included if they had a unilateral transtibial amputation performed >6 months prior to involvement in the study, to ensure established ambulation. Additionally, ITTAs were required to have achieved a K-rating of K3 (basic ambulation with the ability to traverse most environmental barriers) or K4 (capability for high level ambulation exhibiting large stresses and impacts; Orendurff et al. 2016). This was deemed necessary to ensure that ITTAs participating in the study would be able to safely perform higher demand movements such as fast walking. These factors were assessed using an amputation history questionnaire (Appendix D7). ITTAs were excluded if they experienced any pain or discomfort in the residual limb whilst using their prosthesis, or if their amputation occurred due to any reason other than trauma.

While amputations following complications arising from metabolic conditions (i.e. diabetes) are the largest demographic of ITTAs in the UK (British Society of Rehabilitation Medicine 2018), this restriction was imposed to control for health status. This is because disease is known to have negative effects on neuromuscular function (Sacchetti et al. 2013) and would likely confound the results.

Exclusion criteria for both groups included cardiovascular disease risk factors or neuro-musculoskeletal injuries (other than a transtibial amputation in the case of the ITTAs), assessed using a questionnaire modified from the AHA/ACSM Position Stand (1998, Appendix D8). Participants over the age of 45 years were excluded due to the independent effect of ageing on neuromuscular function (Pincivero et al. 2004) and strength asymmetry (Skelton, Kennedy & Rutherford 2002).

3.3 Study Design

This thesis was part of a larger project in conjunction with another PhD researcher. Participants visited the laboratory for 2-3 hours on three separate occasions, 3-7 days apart, to complete a familiarisation session, and two measurement sessions. The familiarisation session and first measurement session were identical and involved assessment of neuromuscular function of the quadriceps muscle group on both limbs. The second measurement session involved motion capture to allow analysis of various movements. All three sessions commenced at a consistent time (± 2 hours) of the day for each participant, following at least 36 hours without strenuous exercise, and 24 hours with no alcohol.

During the neuromuscular function assessment sessions, participants completed a series of voluntary and electrically evoked involuntary (twitch and 300 Hz octet) isometric contractions of the knee extensors. Maximum voluntary torque (MVT; measures maximum strength) was assessed during maximal voluntary contractions (MVCs) whilst rate of torque development (RTD; measures explosive strength) were assessed during explosive voluntary contractions. Torque responses measured during the involuntary evoked contractions were used to assess the intrinsic contractile properties of the muscle. Neural activation at MVT was assessed by (i) Voluntary Activation (VA) measured using the interpolated twitch technique with doublets stimulation; and (ii) the mean quadriceps root mean square (RMS) EMG (normalised to maximal M-wave) at MVT. Explosive agonist neural drive was assessed by quadriceps RMS EMG (normalised to maximal M-wave) at 100 ms from the onset of explosive voluntary contractions. Muscle architecture variables (muscle thickness, pennation angle and fascicle length) of the Vastus Lateralis were assessed using a static ultrasound image.

The movement analysis session involved collecting kinetic and kinematic data during several different activities: step ascent and descent; walking at a habitual and fast pace; jogging; running; and unilateral and bilateral jumping and drop-landing. The movements that will be analysed as part of this thesis are ITTA walking (at both speeds), and control group bilateral jumps and landings. All other movements performed part of the other PhD project (Moudy 2019).

Eight participants returned for a fourth session upon completion of their involvement in the main study for investigation of the reliability of the neuromuscular measures. This additional session was identical to the two previous neuromuscular sessions, other than that data were collected for one limb (the right) only. Test-retest reliability data for all neuromuscular measures are presented in Appendix F.

3.4 Strength and Neuromuscular Function

Maximum and explosive strength measures (sections *3.4.2.3* and *3.4.2.5*) are reported in all four experimental chapters (Chapters 4 – 7). All other neuromuscular function variables are relevant for Chapters 4 (able-bodied neuromuscular asymmetry) and 7 (the effect of chronic disuse on neuromuscular function) only.

3.4.1 Experimental Setup

3.4.1.1 Knee Extension Torque

Participants were firmly secured in an isokinetic dynamometer (Humac Norm, Computer Sports Medicine Inc., Massachusetts, USA) using adjustable straps fastened tightly across the pelvis and shoulders, to prevent extraneous upper-body movement. Some basic modifications were made to minimise knee joint angle changes, including the use of a dense foam padding on the seat and leg attachment, and a custom-made lower limb knee pad which could be tightly clamped down to remove unnecessary rotation (usually present in dynamometers to assist dynamic movement) around the knee adaptor (Appendix E1). In all participants, the adaptor was placed at the lowest point of the limb permitted by anatomy and participant comfort, remaining proximal to the ankle and flush with the tibia. Amputees did not wear their prosthesis in the chair. As a result, for the amputated limb in the ITTA group, the crank arm was flipped by 180° to account for the shorter residual tibia, and thus enable a higher placement of the adaptor relative to the intact and control limbs (Appendix E2). The adaptor was placed lower on the shank in the intact and control limbs for several reasons. Firstly, if the adaptor is placed too high on a healthy limb, greater external compression forces are experienced at the shank (as a result of the smaller moment arm for the same level of torque production). This can be painful, and leads to sub-maximal contraction efforts. Furthermore, the high placement of the adaptor on a healthy limb causes considerable occlusion to the shank tissues, which causes significant discomfort to the participant. The same is not true of ITTAs due to the nature of their residual limb following the amputation. However, the placement of the attachment should not, in theory, affect torque measurements, as the isokinetic dynamometer measures external torque.

Participants were seated with a hip angle of 100° (80° of flexion from anatomical zero). The axis of rotation of the crank arm was aligned with the lateral knee joint space whilst participant produced an MVC at a knee angle of ~110° (full extension = 180°). Knee joint angle was set manually using a handheld goniometer centred at the knee joint space and aligned with lines drawn on the skin over the thigh and shank, between the greater trochanter and knee joint space, and between the knee joint space and the lateral malleolus, respectively. In ITTAs, the shank line was drawn from the lateral knee joint space and extended along the line of the tibia to the end of the residual limb.

The analogue torque signal was sampled at 2000 Hz using an external A/D converter (16-bit signal recording resolution; Micro 1401, CED, Cambridge, UK) and interfaced with a PC using Spike 2 software (version 8; CED).

3.4.1.2 EMG

Electromyographic signals were recorded from the superficial knee extensors (rectus femoris [RF], vastus medialis [VM], vastus lateralis [VL]) using a Noraxon TeleMyo Desktop DTS System (Noraxon, Arizona, USA). The skin was prepared by shaving, abrading and cleansing with 70% alcohol. Dual Ag/Ag/Cl surface electrodes (two contacts with a 2 cm inter-electrode distance, Noraxon) were attached over the belly of each muscle at SENIAM recommended recording sites (Stegeman & Hermans, 2007), parallel to the presumed orientation of the muscle fibres (Figure 3.1). The raw EMG signals were wirelessly transmitted from sensors connected to the electrodes (Wireless Research EMG Probes, Part 542, Noraxon) to a receiver (Desktop DTS, Part 586, Noraxon), with a total gain of 500. Signals were sampled at 2000 Hz in synch with torque, via the same A/D converter and PC software.

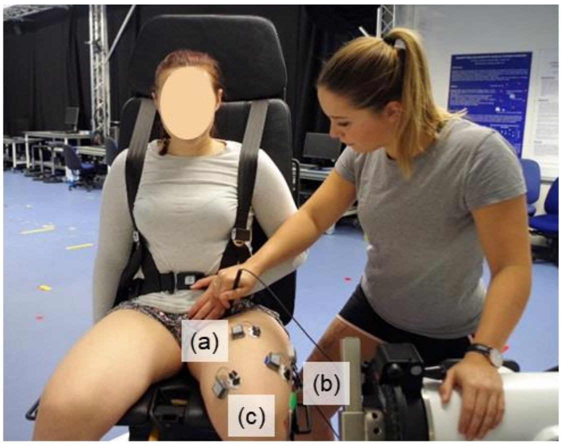


Figure 3.1. Setup of the isokinetic dynamometer for percutaneous electrical stimulation of the femoral nerve (achieved via a cathode stimulation probe firmly pressed into the femoral triangle) and EMG. Locations of EMG electrodes for (a) Rectus Femoris, (b) Vastus Lateralis and (c) Vastus Medialis are visible.

3.4.2 Data Collection

Muscle architecture of the vastus lateralis at rest was assessed using static ultrasound images. Participants then completed a series of voluntary and electrically evoked involuntary isometric contractions in each limb separately in the following order: evoked twitch and 300 Hz octet contractions to assess contractile properties, MVCs to assess MVT, and explosive voluntary contractions to assess RTD. To assess neural activation, MVCs were superimposed with electrically evoked doublet contractions, and surface EMG was collected from the three superficial heads of the quadriceps throughout. All measurements of neuromuscular function were taken in the first measurement session, apart from the octet contractions, which were taken in the familiarisation session only, due to discomfort caused by the octet stimulation. Neuromuscular measures were taken on both legs and the first leg tested was randomised for each person but remained consistent between sessions.

3.4.2.1 Muscle Architecture

A static ultrasound image (Hitachi Noblus, Hitachi Medical Systems, UK) of the VL was taken using a linear array probe with a 94 mm scan width (HI VISION L53L, Hitachi Medical Systems, UK), to assess muscle thickness, fascicle length and pennation angle. The image was taken at the start of the session, prior to any measurements whilst the participant was seated in the dynamometer at rest, with a knee joint angle of 100°, to prevent measures being confounded by increased blood flow to the muscles with exercise (Joyner 2015). The probe was placed perpendicular to skin surface, over the belly of the VL, at 50% of the line between the greater trochanter and the knee joint centre, and aligned so that the muscle fascicles of the VL and their insertion into the deep aponeurosis were clearly visible.

3.4.2.2 Electrical Stimulation

Evoked contractions were performed before voluntary muscle contractions to prevent post-activation potentiation of the twitch response (Sale 2004). Electrical stimuli were delivered via percutaneous stimulation of the femoral nerve to evoke contractions of the knee extensors (Figure 3.1). Measures included supramaximal twitch, doublet (two stimuli evoked at 100 Hz as part of the ITT; see Section *3.4.2.4*) and octet (eight pulses at 300 Hz, thought to elicit the maximum capacity of the muscle-tendon unit for explosive force production; de Ruiter et al. 2006).

The cathode stimulation probe (1 cm diameter, protruding 2 cm from a plastic base, Electro Medical Supplies, Wantage UK) was firmly pressed into the femoral triangle and maintained in place by adhesive tape. The surface of the anode, a 10 x 7 cm carbon rubber electrode, was held in place over the greater trochanter with adhesive tape. Square wave pulses, 0.2 ms in duration, were delivered via a constant current variable voltage stimulator (Model DS7AH, Digitimer, Ltd, Welwyn Garden City, UK). The precise location of the cathode was determined as the position that evoked the greatest twitch response for a particular submaximal electrical current (typically 30 – 60 mA). Single impulses were delivered with step-wise increments in the current, separated by 15 s, until a plateau in the amplitude of twitch torque and compound muscle action potentials (M-waves) were reached. The stimulus intensity was then increased by 20% to ensure supramaximal stimulation, and three supramaximal twitch contractions, separated by 20 s, were delivered. The current was reduced prior to commencing the octet contractions, and stepwise increments in the current were delivered 15 s apart until the supramaximal current used for twitch contractions was attained. Subsequently, three supramaximal octet contractions were evoked.

3.4.2.3 Knee Extension MVCs

Participants performed a series of ~20 warm-up contractions of 3-s duration at progressively higher torque levels over ~2 min before completing six MVCs. Each MVC lasted 3 – 5 s and was followed by 30 – 60 s rest. Participants were instructed to push ‘as hard as possible’ and strong verbal encouragement was given throughout the contractions. Participants received real-time biofeedback regarding the torque response during and after each contraction.

3.4.2.4 Neural Drive at MVT

The 2nd, 4th, and 6th MVCs had a single doublet (two impulses at 100 Hz at the same supra-maximal stimulation intensities as the twitch and octet) superimposed at the plateau of the torque-time curve. Doublets were evoked only provided voluntary torque was ≥ 90% of MVT, to ensure that participants were pushing as close to maximal as possible to allow reliable between-participant comparison. Doublets were chosen as the superimposed stimulus, as they elicit less discomfort than a train of more than two impulses (e.g., an evoked octet), and the force produced by paired stimuli is greater and less variable than for a single twitch. This is because the second impulse is likely to depolarise any motor units that were both not firing maximally and in the refractory period at the time of the first impulse (Duchateau 2009).

As the superimposed doublet appears to be potentiated (Folland 2009), two further doublets were evoked at rest immediately after the MVC (see Section *2.3.1.1* for further explanation of the methodical issues surrounding the ITT).

3.4.2.5 Explosive Voluntary Contractions

To measure explosive strength, participants completed 10 – 15 explosive isometric contractions of the knee extensors, each separated by 20 s rest. Participants were instructed to extend their knee ‘as fast and hard as possible’ for ~1 s, with the emphasis on ‘fast’, whilst aiming to achieve > 80% MVT as quickly as possible. Real-time biofeedback on their RTD was provided via the slope of the torque-time curve (15 ms time constant), which was displayed on the computer monitor throughout, with participants being encouraged to beat their best peak slope (peak RTD) with each attempt. The baseline torque level was displayed on a sensitive scale to provide biofeedback on whether any countermovement or pretension occurred before the contraction.

3.4.3 Data Processing

All strength and neuromuscular data were processed using Spike 2 software (version 8, CED, Cambridge, UK).

3.4.3.1 Knee Extension Torque

Off-line, torque data was filtered using a fourth-order low pass Butterworth filter with a cut-off frequency of 10 Hz, and baseline resting torque was subtracted from all active torque recordings to correct for the weight of the limb. Torque signal onsets (voluntary and evoked) were detected using a threshold method of identification (Andersen & Aagaard 2006, Schmidt et al. 2014), and defined as the point at which torque exceeded 5 Nm, at which point baseline noise was cleared.

3.4.3.2 EMG

Prior to analysis, raw EMG signals were band-pass filtered off-line between 6 and 500 Hz using a fourth-order zero-lag Butterworth filter.

3.4.4 Variable Extraction

3.4.4.1 Muscle Architecture

Muscle thickness, pennation angle and fascicle length (Figure 3.2) were determined offline using Tracker software (an open source Video Analysis Tool, available from http://physlets.org/tracker/). Muscle thickness was defined as the mean distance between the deep and superficial aponeuroses at three points: at the middle and either end of the image. Pennation angle was defined as the mean of the angle between three separate muscle fascicles and their insertion on the deep aponeurosis. Previous studies assessing the reliability of muscle thickness and pennation angle measures using ultrasound have reported errors of ~2% and 5-10%, respectively (Franchi et al. 2018, Kwah et al. 2013), while test-retest reliability of these methods evidenced CVs of 4 – 11% (Appendix F). Fascicle length was extrapolated from the pennation angle and muscle thickness using trigonometry (de Brito Fontana, Roesler & Herzog 2014, Franchi et al. 2014, Tillin, Pain & Folland 2012b), as the entire length of the fascicle was not visible in the image. This is a commonly used method, although it is associated with some errors due to the curvature of the muscle fascicle and aponeurosis. However, in this study fascicle length was estimated at the mid-section of the muscle where fascicle and aponeurosis curvature is minimal, and muscle architecture relatively uniform, compared to more proximal or distal portions of the muscle (Blazevich, Gill & Zhou 2006). The errors associated with linearly extrapolated fascicle length are reported to be in the region of 2-7% when compared to image fitting (Finni & Komi 2002, Reeves & Narici 2003) or ~12% when compared to extended field of view ultrasonography (Noorkoiv et al. 2010). Between-day reliability of the architectural features assessed as part of this thesis are reported in Table F2 (Appendix F).

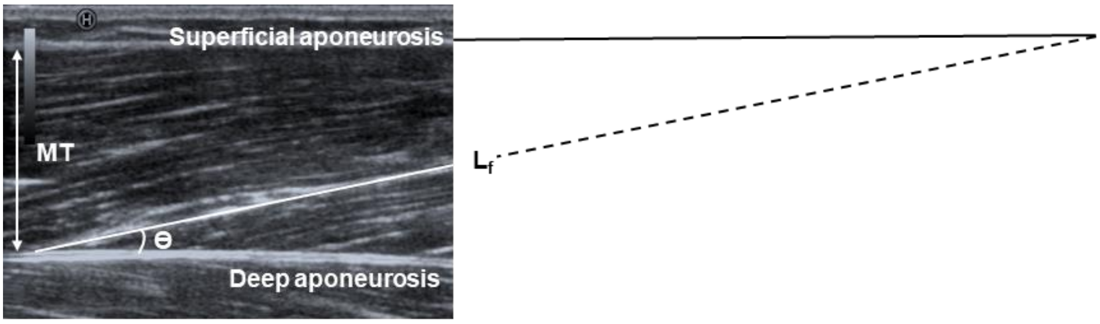


Figure 3.2. Muscle architecture variables. Muscle thickness (MT) is the distance between the superficial and deep aponeuroses, while pennation angle (Θ) is the angle between the fascicle and the deep aponeurosis. The fascicle length ($L_{f)}$ is the length that the fascicle extends between the aponeuroses. Fascicles typically extended off the image, so the non-visible portion of the fascicle was estimated via linear extrapolation.

3.4.4.2 Twitch and Octet Parameters

The mean M-wave peak-to-peak amplitude of the three supramaximal twitch contractions was defined as the maximal M-wave (M_{max}). Torque measurements extracted from the evoked contractions were twitch and octet peak torque (PT) and peak RTD (calculated using a 15 ms moving time constant), presented as absolute and relative to PT. Each of these variables were averaged across the three supra-maximal twitch and octet contractions recorded.

3.4.4.3 Maximum and Explosive Strength

MVT was defined as the greatest instantaneous peak voluntary torque (not due to superimposed stimulation) recorded during any of the MVCs or explosive contractions. Any contractions with a visible countermovement (an initial antagonist torque production) or pre-tension (active tension in the muscle prior to contraction onset), quantified as change of baseline torque < 0.5 Nm during the 100 ms prior to visible torque onset, were discarded. These criteria were applied as the level of pretension and countermovements have been shown to affect the subsequent levels of force production (Buckthorpe & Roi 2017). Furthermore, as RTD has been found to have a strong positive relationship with the peak torque achieved in the contraction in which it was measured (Maffiuletti et al. 2016), contractions with peak torque > 80% MVT were discarded. Peak voluntary RTD (Figure 3.3) was extracted from the three explosive voluntary contractions with the highest peak RTD that conformed to these criteria.

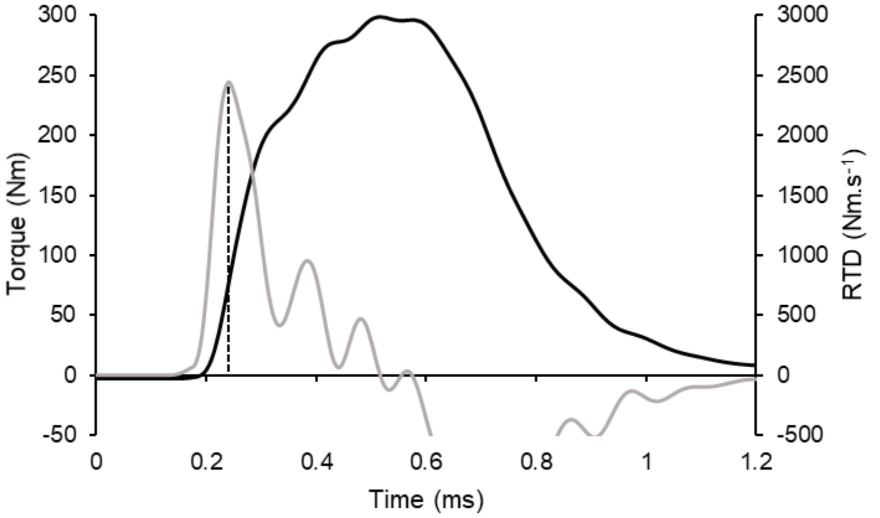


Figure 3.3. Identification of peak rate of torque development (RTD; denoted by dashed black line) from explosive torque (black line) using the slope of the torque-time curve (grey line). Slope calculated using a 15 ms time constant.

3.4.4.4 Neural Drive at MVT

The difference between superimposed and mean resting potentiated doublet torque was used to calculate voluntary activation for each contraction (VA; a measure of neural drive at MVT), using the equation:

$$VA(\%) = 100 \times (1 - (D_s/D_c)) \tag{3.1}$$

where D_s and D_c are the superimposed and control doublets, respectively. The mean

VA for all three contractions was reported.

The RMS of the EMG signal for each quadriceps muscle (RF, VM, VL) was calculated over a 500 ms window which wasn't influenced by stimulation artefact, centred on- or nearest to MVT (EMG_{MVT}). EMG_{MVT} was normalised to M_{max} of the same muscle (Halaki & Ginn 2012, Lanza et al. 2018) and averaged across the three quadriceps muscles.

3.4.4.5 Explosive Neural Drive

To assess neural drive during the explosive contractions, the RMS amplitude of the EMG signal for each quadriceps muscle (RF, VM and VL) was calculated for the time-period most closely aligned to RTD (i.e. 0-100 ms) and normalised to M_{max} at the same muscle. EMG onset, defined as the onset of the first muscle to be activated, were identified with a standardised systematic protocol of visual identification (Tillin et al. 2010, Tillin, Pain & Folland 2013b). Visual signal inspection is recognised as a gold standard method for event detection (Staude & Wolf 1999). Briefly, EMG recordings were initially viewed with a consistent *y*-axis scale of 0.05 mV, and an *x*-axis scale of 500 ms. This scale provided sufficient resolution for the accurate detection of signal onset, defined as the last peak or trough before the signal deflected away from baseline noise. A vertical cursor was then placed on the signal onset before the signals were viewed at a higher resolution (*y*-axis scale of 0.02 mV, and an *x*-axis of 25 ms) to verify the accurate placement of the cursor (Figure 3.4). EMG values were expressed as a percentage of their respective M_{max}, and averaged across the three quadriceps muscles within the time period (EMG_{0-100}). EMG measurements were averaged across the three explosive contractions chosen for analysis.

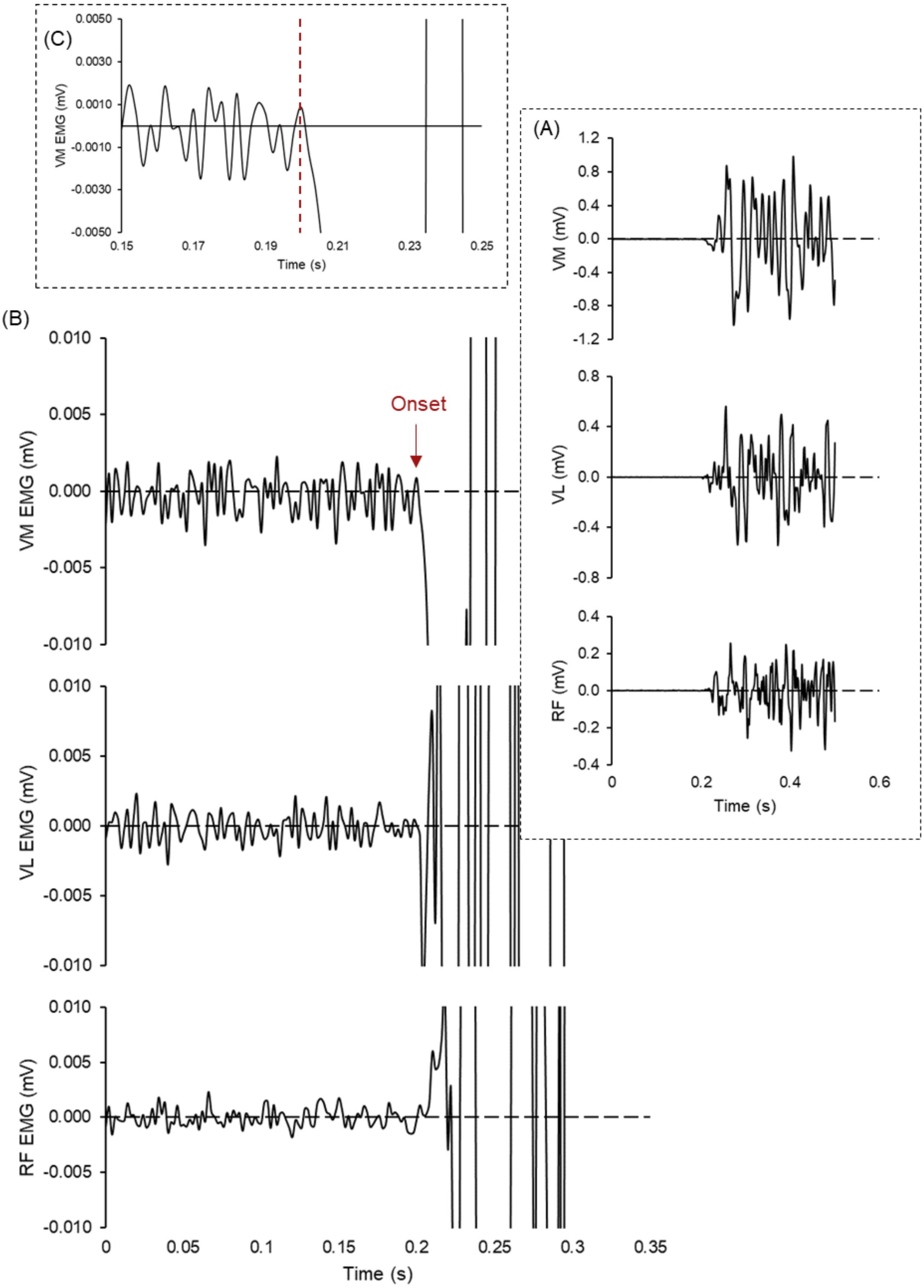


Figure 3.4. Typical Vastus Medialis (VM), Vastus Lateralis (VL) and Rectus Femoris (RF) electromyograph (EMG) signals during an isometric voluntary explosive contraction (A). EMG onset was defined as the first instance in which the signal deflected away from the baseline noise in any muscle. In this example, onset was detected in the VM signal (B). The signal in (C) shows the scale at which the investigator determined onset (red dashed line), which was standardised for every contraction analysed.

3.5 Movement Analysis

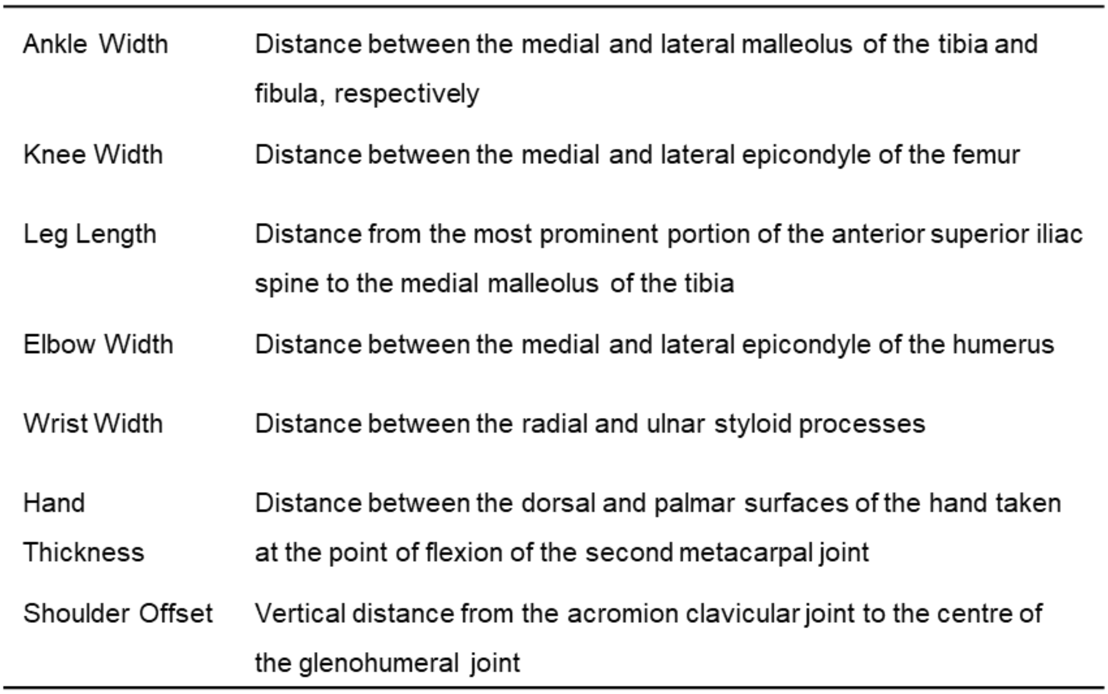
3.5.1 Experimental Setup

Kinematic data were collected using twelve Vicon Vantage V5 (Vicon Motion Systems Ltd.; Oxford, UK) motion capture cameras sampling at 200 Hz. Three in-series Kistler force plates (Type 9281c; Kistler Instruments Ltd., Hampshire, UK) sampling at 1000 Hz were in the middle of a 15 m walkway. Two sets of Brower TC timing gates (Brower Timing, Utah, USA) placed 2 m either side of the force plates were used to capture average walking pace.

3.5.2 Anthropometric Measures

Body mass was taken alongside height, leg length and various joint width measures (Table 3.1) for input into Vicon Nexus. Anthropometric measures were taken twice, except in the instance that the difference between the two measures was > 2 mm, in which case the measure was taken a third time. The mean of two measurements that were within 2 mm of each other were used as the data input for the Plug-in Gait subject model.

Table 3.1. Anthropometric measures collected for input into Vicon Nexus to create the subject model. All were measured using sliding calipers, except leg length (measured using a metal tape measure).



Ankle Width	Distance between the medial and lateral malleolus of the tibia and fibula, respectively
Knee Width	Distance between the medial and lateral epicondyle of the femur
Leg Length	Distance from the most prominent portion of the anterior superior iliac spine to the medial malleolus of the tibia
Elbow Width	Distance between the medial and lateral epicondyle of the humerus
Wrist Width	Distance between the radial and ulnar styloid processes
Hand Thickness	Distance between the dorsal and palmar surfaces of the hand taken at the point of flexion of the second metacarpal joint
Shoulder Offset	Vertical distance from the acromion clavicular joint to the centre of the glenohumeral joint

3.5.3 Marker Placement

Thirty-nine retroreflective markers (14 mm diameter) were attached directly to the skin in accordance with the full-body marker set (Davis et al. 1991). In the case of ITTAs, markers for the shank, ankle and foot were placed in positions on the prosthetic corresponding as closely as possible to those on the intact limb (Rusaw & Ramstrand 2010, 2011, Kent & Franklyn-Miller 2011).

3.5.4 Data Collection

As the data collection for this thesis was run in conjunction with another PhD student, data were collected for more movements than analysed within the scope of this thesis. The movement analysis data collection was performed in the following order: step descent/ ascent, habitual and fast walking, jogging, running, bilateral and unilateral countermovement jumps, and bilateral and unilateral drop landings. Task demand increased throughout the testing protocol, and participants were provided rest between each change in task and, when appropriate, demand (i.e. walking at two different speeds, jogging and running) to avoid fatiguing effects on subsequent activities. Kinetic and kinematic data were collected for all movements.

The take-off phase of bilateral maximal vertical CMJs and landing from a drop were assessed to determine if asymmetries in strength and loading during movement were associated in a control population (Section 2.4.1). Control CMJ and drop landing variables (sections *3.5.4.1* and *3.5.4.2*) are reported in Chapter 5.

Walking variables (section *3.5.4.3*) were assessed for two reasons: firstly, to determine if there were associations in strength and kinetic asymmetries in a movement commonly performed by a pathological population (Chapter 6; ITTAs only), and secondly, to quantify the relative disuse of the amputated and intact limbs of ITTAs in comparison to a control limb (Chapter 7).

3.5.4.1 Counter-Movement Jump

The jumps were performed with a low mass plastic bar resting on participants' shoulders to remove the effect of arm swing (Hara et al. 2006). Each foot started on a separate force plate. Participants were instructed to do a countermovement to a comfortable depth before jumping as high as they possibly could and to land with each foot on a separate force plate. To familiarise the participant to the movement, three to five practice attempts were made before data collection began, which involved participants completing maximal effort jumps until three successful jumps had been captured. The jump was considered successful if the participant landed each limb fully on their respective force plates and was able to stabilise without moving their feet.

3.5.4.2 Drop Landing

Bilateral drop landings, as distinct from the landing of a CMJ, were performed to remove the influence of jump height on landing techniques – i.e. drop height was standardised to ensure similarity of touchdown velocity. Vertical momentum was therefore reduced by the same amount across participants, making comparisons equivalent. While it is usual for drop landings to be performed by rolling off a box of a standardised height (e.g. Orishimo et al. 2009), the requirements for participants to land in front of the box introduces a horizontal velocity component to the landing. Additionally, if the roll-off is performed incorrectly, participants may jump slightly, raising the centre of mass, and therefore changing the vertical velocity at touch-down. To combat these limitations, a specially designed, adjustable metal drop landing frame (Figure 3.5) was used. The frame was placed around the force plates to gather data from both limbs individually. The grab rail of the frame was adjusted so that the participants were 30 cm off the ground (Durall et al. 2011, Doherty et al. 2014, Orishimo et al. 2009) as measured from the heel while hanging. Following a demonstration, participants lowered themselves to a hanging position from the bar. Once still, participants were instructed to hold for one second before dropping to land in a natural manner (i.e. they could land with straight legs or bent legs depending on their preference). Participants completed drop landings until three successful trials were captured. A successful landing was defined when the participant: (i) did not visibly pull up before dropping; (ii) landed with each foot on a separate force plate; and (iii) stabilised upon landing within 2-3 s without changing their foot position or using the frame for stabilisation.

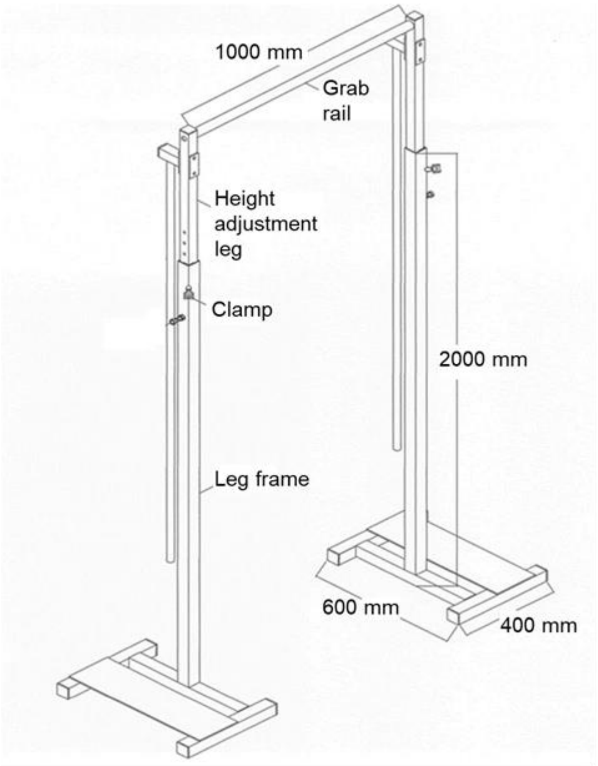


Figure 3.5. Schematic of the drop landing frame.

3.5.4.3 Walking

Participants were instructed to walk along the walkway (with ITTAs wearing their usual prosthetic limb) at their self-selected everyday walking pace, determined from three preliminary practice trials. They were then prompted to maintain this speed for the remainder of the passes until five good trials (defined as a single pass within ± 5% of the average walking speed and a successful force plate strike) were collected. The same protocol was repeated at a second (fast) speed, with participants instructed to walk at a pace they would choose if they were late for an appointment. Three good trials, defined as a single pass with at least one successful force plate strike and no gaps in marker data > 40 frames, were selected for analysis for each walking speed.

3.5.5 Data Processing

Data processing was completed using Nexus (version 2.7.1, Vicon, Oxford), software that interfaces marker and force-plate data. A low-pass zero-lag fourth-order Butterworth filter was used to filter raw marker trajectories (8 Hz) and analogue force data (200 Hz cut-off). Hip joint centres were determined using the Newington-Gage model, while knee and ankle joint centres were calculated using a modified chord function, as per Vicon's Plug-in Gait Model (Vicon 2016), based on the Newington-Helen Hayes marker sets. Plug-in Gait is a model used by Nexus to calculate kinematic and kinetic data such as joint angles, velocity, moments, powers, etc. Inverse dynamics equations were used to calculate net joint moments (reported as internal moments), which were reported both as absolute values (Chapter 6) and normalised to each participant's body mass (Chapters 5 – 7). Impulse was calculated as the cumulative time-integral of the waveform of interest (knee extension moment or vGRF as appropriate).

The anthropometric characteristics of all participants were based on Dempster's values (Dempster, 1955). In ITTAs, the inverse dynamic analysis assumes that the prosthetic components were anatomically equivalent to the intact limb. While this may overestimate the inertia of the prosthetic limb, variables of interest occurred during the stance phase of gait only. As lower-extremity accelerations in stance are relatively small, errors in calculating inertia of the prosthetic limb have been shown to have only a minor effect on joint kinetics (Miller, 1987). This modelling method is frequently used in the literature assessing joint moments across a range of tasks in for ITTA (e.g. Baum et a. 2019, Sanderson & Martin 1997, Schoemann, Diss & Strike 2012, 2013, Strike, Arcone & Orendurff 2018, Vanicek et al. 2010).

3.5.6 Variable Extraction

Extraction of movement variables was performed in Matlab (R2016a, The Mathworks Inc, Natick, MA) using custom-written programs. Unlike the strength and neuromuscular measures, the movement variables analysed were specific to the experimental chapter in question. Therefore, data extraction for CMJ and drop landing will be described in Chapter 5. Walking variable extraction will be described in Chapter 6 (ITTAs only, for the investigation into the relationships between ITTA strength and gait asymmetries) and Chapter 7 (both groups, for the quantification of the level of disuse at the amputated and intact limb compared to a control).

3.6 Data and Statistical Analysis

3.6.1 Asymmetry Calculations

In this thesis, limbs were split between strong/ weak due to the problems associated with right/ left and dominant/ non-dominant limb classification. Furthermore, in Chapter 6, ITTA intact limbs are stronger, and, as a result of the amputation, the dominant limb. However, this does not necessarily reflect which limb was dominant before their amputation.

Bilateral Strength Asymmetry (BSA, equation 3.2; Nunn & Mayhew 1988) was chosen for analysis in Chapter 4 because a scale with perfect symmetry represented by 0% is more comparable to the majority of asymmetry literature.

$$BSAx(\%) = \frac{STRONGx - WEAKx}{STRONGx} \times 100 \tag{3.2}$$

For Chapter 5, Bilateral Asymmetry Index (BAI, equation 3.3; Bishop et al. 2018) was chosen due to the bilateral nature of the movement tasks (CMJ and drop landing) that were assessed.

$$BAIx(\%) = \frac{STRONGx - WEAKx}{STRONGx + WEAKx} \times 100$$

(3.3)

BAI is preferable for the quantification of asymmetry in a bilateral task, as the differences in force between limbs are always relative to the sum force value (see Appendix A, Section A.3 for further discussion of the interpretation of asymmetry indices). Modified forms of the BAI and BSA indices were used to study asymmetry in ITTAs. Further discussion of these can be found in Chapter 6, Section *6.2.3.1* and Chapter 7, Section 7.2.4.

In Chapters 4 and 5, asymmetry values were considered in two ways: absolute, and directional. The following sections discuss the difference in the calculation for these.

3.6.1.1 Absolute Asymmetry

Absolute asymmetry for each variable of interest was calculated using the equations given in Table 3.2, where x was the strength, neuromuscular or biomechanical variable of interest, and *STRONG* and *WEAK* are the legs with the highest and lowest values respectively, for x. This equation quantifies the magnitude of asymmetry without specifying its direction i.e., which leg was demonstrated the greater value for the given variable.

Consider, for example, that the variable of interest is VA. An individual may present with the following data (N.B. the greater value for the variable of interest is presented in bold for ease of interpretation):

	Left	Right
VA (%)	94	**97**

For this individual, then, absolute asymmetry indices would be calculated thus:

$$BSA_{VA}(\%) = \frac{97 - 94}{97} \times 100 = 3.09\%$$

$$BAI_{VA}(\%) = \frac{97 - 94}{97 + 94} \times 100 = 1.57\%$$

These values will always be positive.

3.6.1.2 Directional Asymmetry

The calculation of directional asymmetry is a novel method to describe both the magnitude and direction of the asymmetry in a given variable. When correlating the asymmetry between two variables, it was important to denote the direction of the predictor variable asymmetry relative to that of the dependent variable (i.e. directional asymmetry), accounting for the fact that the asymmetry may be in opposite directions for the two variables. The equations for calculating directional asymmetry are therefore:

$$\mathrm{BSA\_DIR}x\ (\%) = \frac{STRONG\_DIRx - WEAK\_DIRx}{STRONGx} \times 100$$

(3.4)

$$BAI\_DIRx(\%) = \frac{STRONG\_DIRx - WEAK\_DIRx}{STRONGx + WEAKx} \times 100$$

(3.5)

where x is again the variable of interest, and *STRONG_DIR* and *WEAK_DIR* are the limbs with the highest and lowest values, respectively, for the variable that x is being assessed relative to (i.e. asymmetry in MVT or RTD). Continuing the earlier example then, let us study the same individual. We now need to consider the maximum strength of each limb (predictor variable) as well as the dependent variable (in this case, VA). The data for this individual may be:

	Left	Right
MVT (Nm)	324	**356**
VA (%)	94	**97**

As the maximally strong limb is the same as the limb with the greatest VA (i.e. the right limb), directional asymmetry is calculated as follows:

$$\mathrm{BSA\_DIR}_{VA}\,(\%) = \frac{97-94}{97} \times 100 = 3.09\%$$

$$BAI\_DIR_{VA}(\%) = \frac{97-94}{97+94} \times 100 = 1.57\%$$

In this instance, directional asymmetry is identical to the absolute asymmetry values as calculated previously in Section 3.6.1.1. However, there may be situations in which the strong leg does not present with the greatest value for the variable of interest. Let us change our example data so that the maximally strong limb becomes the left leg:

	Left	Right
MVT (Nm)	**356**	324
VA (%)	94	**97**

In this case, directional asymmetry is calculated as follows:

$$\mathrm{BSA\_DIR}_{VA}\,(\%) = \frac{94-97}{97} \times 100 = -3.09\%$$

$$BAI\_DIR_{VA}(\%) = \frac{94-97}{97+94} \times 100 = -1.57\%$$

The calculated asymmetry index is now negative, indicating that the asymmetry in the variable of interest is in the opposite direction to that of the strength (predictor) variable. Notice, however, that the magnitude of the asymmetry index is the identical as the denominator is the same (the larger value for the variable of interest, i.e. *STRONG*x rather than *STRONG_DIR*x).

3.6.2 Interpreting Asymmetry Indices

For all asymmetry indices, calculations were done first at the individual level before averaging across the group for the mean value. The group means for each asymmetry index are presented in the results of each chapter. However, as directional asymmetry for a given variable is not necessarily in the same direction for all individuals, the interpretation of the r values from correlational analysis becomes challenging. Scatterplots should be investigated for each analysis. Figure 3.6 shows some basic examples of how asymmetry index values may be distributed for given r values.

Figures 3.6A and C both show a scatterplot where $r = 1$. In contrast, Figures 3.6B and D both show a scatterplot where $r = -1$; however, the interpretation is different between these two sets of graphs.

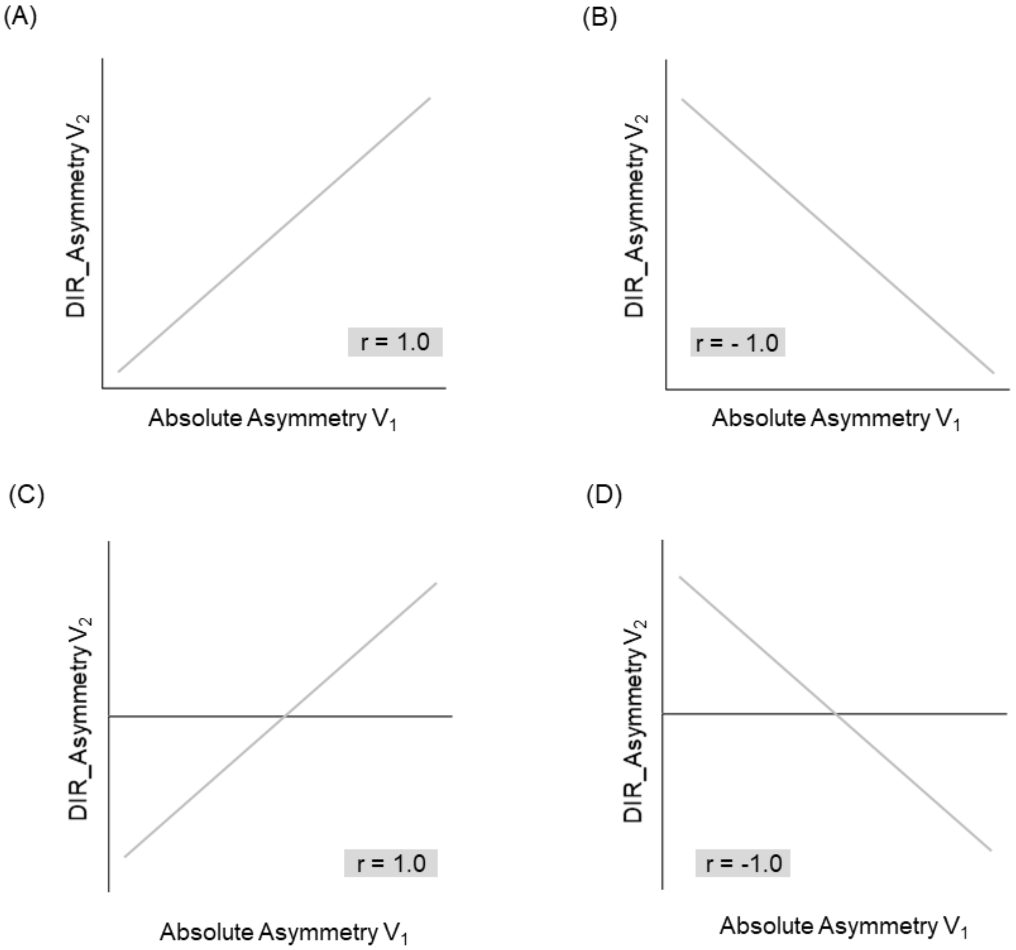


Figure 3.6. Schematic representation of possible scatterplot distributions when correlating absolute and directional asymmetry values for a hypothetical variable 1 (predictor; V_1) and 2 (dependent; V_2).

For Figure 3.6A, the asymmetry of both V_1 and V_2 increase proportionally; while Figure 3.6B shows that as asymmetry in V_1 decreases, asymmetry in V_2 increases. However, it is possible to have a situation where some individuals within the group experience a different relationship between the asymmetry of the predictor and dependent variables. Consider Figures 3.6C and D, for which the interpretation is more complex. For both figures, the relationship between asymmetry in V_1 and V_2 is different in half the group, dependent on where the data lies. In the top half of Figure 3.6C, asymmetry in V_1 increases as asymmetry in V_2 increases, while in the bottom half, asymmetry in V_1 increases as asymmetry in V_2 decreases. The opposite is true for Figure 3.6D.

Where the interpretation of the r value is complex, such as in Figures 3.6C and D, scatterplots of data are presented in this thesis as appropriate to aid the interpretation of the r values presented.

3.6.2 Statistical Analysis

Specific information pertaining statistical analysis of the variables extracted will be detailed in each experimental chapter.

Chapter 4

Associations between Asymmetry in Quadriceps Strength and its Neuromuscular Determinants in an Able-Bodied Population

4.1 Introduction

Typical human movements are bi-pedal, whereby effective movement relies on the co-ordinated contribution of two limbs for absorbing and generating momentum. On this basis, it is often assumed that strength discrepancies between the limbs, indicating bilateral strength asymmetry, may limit effective movement (Bishop, Turner & Read 2017). Strength asymmetry of the quadriceps muscles, which are large contributors to triple extension actions (walking, running, jumping, landing; e.g. Chang et al. 2015, Decker et al. 2003, Neptune et al. 2008, Yeow et al. 2011), has been implicated in a variety of special populations. For example, greater quadriceps strength asymmetry has been observed in: old vs young adults (Skelton, Kennedy & Rutherford 2002); older adult fallers vs non-fallers (Perry et al. 2007); ITTAs vs. able-bodied (Lloyd et al. 2010); patients with multiple sclerosis vs controls (Sandroff, Sosnof

& Motl 2013) and patients with knee osteoarthritis vs controls (Suetta et al. 2007). Furthermore, authors have suggested that quadriceps strength asymmetries of >10 – 15% place athletes at a greater risk of injury (Kiesel, Plisky & Voight 2007, Knapik et al. 1991, Nadler et al. 2001).

Studies that have investigated quadriceps inter-limb strength asymmetries have typically assessed dynamic maximum strength using isokinetic dynamometry (measured as peak torque), and reported it to range from on average 1 – 18% in athletic populations, 15 – 32% in clinical populations, 10 – 20% in those aged > 65 years, 10 – 14% in children (Table 2.1), and 1 – 5% in control populations (Kobayashi et al. 2013, Lanshammar & Ribom 2010, Schiltz et al. 2009). However, the latter studies on control groups all split limbs by dominant vs. non-dominant for the calculation of asymmetry, and therefore may underestimate the magnitude of these asymmetries (due to cancellation from negative values; Appendix A). There remains therefore limited literature on the typical magnitude and variability of maximum strength asymmetries present in a young, healthy recreationally active population, which may be useful for comparing to special populations that demonstrate bilateral, asymmetrical loading patterns that may predispose them to strength asymmetries (e.g., ITTAs, osteoarthritis patients, injured athletes). Furthermore, there has been no investigation of the magnitude and variability in quadriceps explosive strength asymmetry, which may be more functionally relevant than maximum strength asymmetry in certain situations, given the functional importance of explosive strength to rapid human actions such as joint stabilisation, restabilising the body following a loss of balance, and certain sports activities (Behan, Pain & Folland 2018, Maffiuletti et al. 2010, Pijnappels et al. 2008, Tillin, Pain & Folland 2013a).

Given that MVT is one of the primary determinants of explosive strength (Andersen & Aagaard 2006, Folland, Buckthorpe & Hannah 2014), it is logical to posit that the direction of asymmetry will be the same for both (i.e., the maximally strong leg will also be explosively stronger). However, the results of previous research suggest large inter-individual variability is present in explosive strength when compared to MVT (Folland, Buckthorpe & Hannah 2014), together with greater between-session variability in RTD compared to MVT (see Section 2.2.2; Buckthorpe et al. 2012, de Ruiter et al. 2004, Place et al. 2007). These reliability values are similar to those reported in this thesis (Appendix F). Together, this suggests that explosive strength asymmetry may be more variable, and potentially not in the same direction, as maximum strength asymmetry.

To the best of our knowledge, no research has sought to investigate the underpinning neuromuscular mechanisms of strength asymmetry. At the single-limb level, a muscle’s capacity for force production is typically dependent upon both neural and muscular factors (Fig 2.2). Maximum strength is determined primarily by neural drive, muscle size, and architecture (Blazevich et al. 2009, Fitts, McDonald & Schluter 1991, Lieber & Friden 2000, MacIntosh, Gardiner & McComas 2006), whilst explosive strength is largely determined by maximum strength, neural drive, measured using EMG, and the intrinsic contractile properties of muscle, such as evoked twitch and octet RTD (Andersen & Aagaard 2006, Folland, Buckthorpe & Hannah 2014, Grimby, Hannerz & Hedman 1981). It is unclear if any of these determinants of single-limb maximum and explosive strength may underpin maximum and explosive strength asymmetries. A cross-over effect has been shown in neural drive, for example, whereby unilateral training or injury has influenced neural function on the opposite limb (Hart et al. 2010, Bogdanis et al. 2019, Tillin, Pain & Folland 2011), suggesting there are common mechanisms underpinning the neural drive to contralateral limbs that would limit asymmetries in neural drive and thus strength. In contrast, changes in peripheral factors (e.g. muscle size and the intrinsic contractile properties) are limb specific and do not cross-over to the contralateral limb (e.g. Narici et al. 1989). Thus, it may be that asymmetry in peripheral determinants of strength are more likely to determine asymmetry in strength. A potential association between strength asymmetry and a typical determinant of strength may be characterised in one or both of the following ways: (1) between-limb differences in a neuromuscular determinant of strength may exist between the strongest and weakest limbs, suggesting asymmetry in strength and the neuromuscular determinant share the same direction; or (2) the asymmetry in strength and the asymmetry in a neuromuscular determinant of strength may be correlated, suggesting they are proportional to each other.

The aims of this study were therefore two-fold. Firstly, we aimed to compare the magnitude and variability of quadriceps maximum and explosive strength asymmetries for a population of young, healthy, recreationally active males with no specific training bias. The second aim was to determine the underpinning neuromuscular mechanisms of these strength asymmetries. It was hypothesised that, compared to maximum strength asymmetry, asymmetry in explosive strength would be in the same direction, but larger and more variable. Additionally, we hypothesised that inter-limb asymmetries in strength would be explained by neuromuscular mechanisms similar to those that determine single-limb strength, but more likely by peripheral determinants (e.g. muscle architecture, intrinsic contractile properties) than central factors such as neural drive.

4.2 Methods

A comprehensive description of participant recruitment, inclusion criteria and the methods followed for data collection and processing are given in Chapter 3, sections 3.3 – 3.5.

4.2.1 Participant Information

Twenty-one physically active male participants (age 34.2 ± 6.52 years, height 180 ± 6.07 cm, mass 82.7 ± 11.2 kg, activity 4775 ± 2677 MET-mins/week) took part in this study. Participant inclusion and exclusion criteria, and detail about assessment of physical activity, can be found in Chapter 3, Section 3.2.

4.2.2 Data Collection

Briefly, participants visited the laboratory for 2-3 hours on two separate occasions, 3-7 days apart, to complete a familiarisation and a measurement session. The sessions were identical and involved assessment of voluntary quadriceps maximal and explosive muscle strength of both limbs, as well as the intrinsic contractile properties and neural drive to the knee extensors, and architectural properties of VL. Full detail on experimental setup, protocol, data processing and extraction is given in section 3.4.

4.2.3 Data Analysis

Strength data analysed were MVT and peak voluntary RTD. Neuromuscular data analysed included knee extensor evoked twitch and octet peak torques and RTD; VA, EMG at MVT, and explosive EMG_{0-100}; and muscle thickness, pennation angle and fascicle length of VL.

4.2.2.1 Asymmetry Calculation

Bilateral Strength Asymmetry (BSA; Nunn & Mayhew, 1988) was calculated for MVT and RTD using equation 3.2 (Chapter 3, Section 3.6.1). This equation quantifies the magnitude of BSA without specifying the direction of that BSA i.e., which leg was stronger.

However, when correlating the BSA between two variables it was important to denote the direction of the predictor variable BSA relative to that of the dependent variable (BSA_DIR, i.e. directional BSA; equation 3.4), accounting for the fact that the asymmetry may be in opposite directions for the two variables. This equation may provide a negative magnitude if the direction of asymmetry for the predictor variable is opposite to that of the dependent variable. For correlating asymmetry in MVT with that in RTD, the former was considered the predictor and the latter the dependent variable. For correlating the BSA for a strength variable (i.e. MVT or RTD) with the BSA of a determinant variable of strength (i.e. VA, EMG_{MVT}, $EMG_{0\text{-}100}$, muscle thickness, etc.), the strength variable was considered the dependent, and the determinant variable considered the predictor variable.

4.2.2.2 Statistical Analysis

Data are reported as mean ± standard deviation (SD).

Statistical analysis was completed using SPSS version 24, with the significance level set at $p < 0.05$. Levene's test was used to check for equality of variances prior to running all analyses, and Shapiro-Wilkes assessed normality of the data. Paired t-tests were used to compare the size of BSA in MVT with RTD. To assess if there were relationships between maximum and explosive strength asymmetries, Pearson's product moment bivariate correlations were calculated between BSA RTD and BSA_DIR for MVT to demonstrate if there was an association between both the magnitude and direction of asymmetry in MVT relative to that in RTD.

Associations between asymmetries in the two measures of strength and their determinants were assessed in two ways. Firstly, paired t-tests were used to detect any between-limb differences in each determinant of a given strength variable (performed between the strong and weak limb as established for that specific variable of strength). Effect size (specifically Cohen's *d*) was calculated for between-group comparisons and interpreted as small (d = 0.2 – 0.5), medium (d = 0.5 – 0.8) and large effects ($d > 0.8$, Lakens 2013). Secondly, to determine if there were relationships between strength asymmetries and those of their determinants, bivariate correlations were performed between BSA for each strength variable (MVT and RTD) and BSA_DIRs of the corresponding predictor variables. All relationships were interpreted as strong (r > 0.7), moderate (r = 0.5 – 0.7), weak (r = 0.3 – 0.5) or non-existent (r < 0.3; Mukaka 2012).

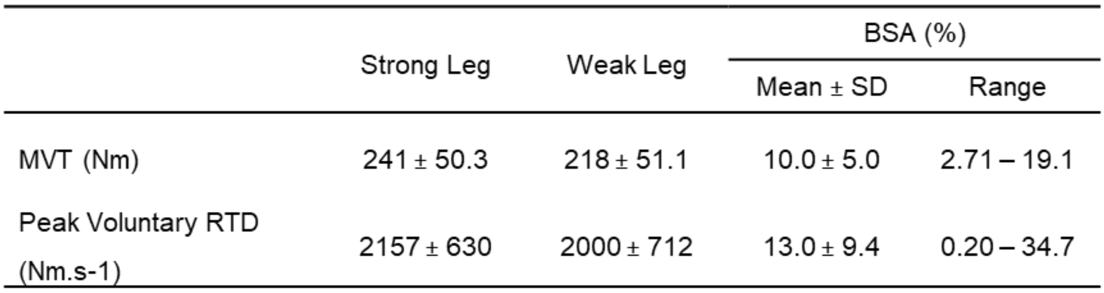
To examine the combined influence of asymmetry of their determinants, separate stepwise multiple linear regressions were run in the event that more than one moderate and/or significant correlation was present between BSA for the strength variable of interest and BSA_DIR of its determinants.

4.3 Results

4.3.1 Asymmetries in Maximal and Explosive Strength

There was no significant difference in the magnitude of BSA between MVT and absolute peak voluntary RTD (*p* = 0.173, *d* = 0.36; Table 4.1). The magnitude of BSAs in MVT were <20% for all participants, with 11/21 participants displaying BSAs <10%. In contrast, BSAs were >20% for 4/21 participants for RTD (Figure 4.1). Accordingly, the variability in RTD BSA was considered greater than MVT BSA, as evidenced by an almost two-fold greater range and standard deviation (Table 4.1).

Table 4.1. Bilateral asymmetries (BSA) in isometric knee-extensor maximal voluntary torque (MVT), peak rate of torque development (RTD). Data are presented as n = 21.



	Strong Leg	Weak Leg	BSA (%)	
			Mean ± SD	Range
MVT (Nm)	241 ± 50.3	218 ± 51.1	10.0 ± 5.0	2.71 – 19.1
Peak Voluntary RTD (Nm.s-1)	2157 ± 630	2000 ± 712	13.0 ± 9.4	0.20 – 34.7

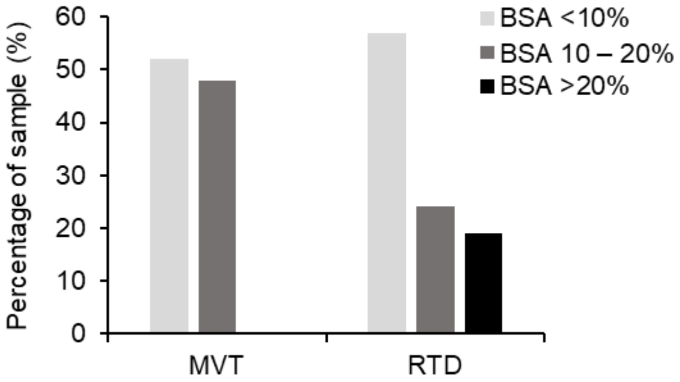


Figure 4.1. Percentage of the sample within each asymmetry band for bilateral asymmetries (BSA) in maximum voluntary torque (MVT) and peak rate of torque development (RTD).

BSA RTD was not always in the same direction (i.e. leg with the highest value) as the BSA_DIR MVT, as illustrated by the negative values in Figure 4.2 for 8/21 of the participants. A moderate, significant relationship was apparent between BSA RTD and BSA_DIR MVT (i.e. when the asymmetry in MVT was considered relative to that of RTD; r = 0.56; *p* = 0.008; Figure 4.2). This indicates that those with greater asymmetries in maximum strength displayed greater asymmetries in explosive strength.

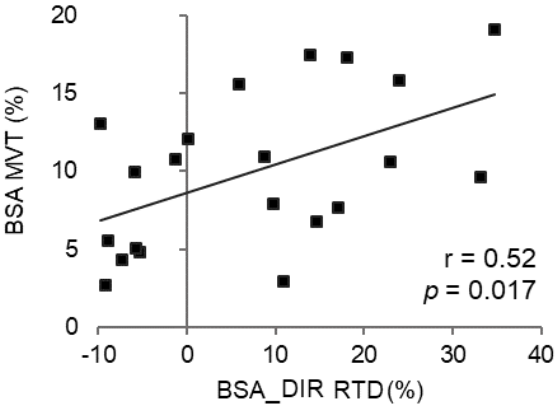


Figure 4.2. Pearson's product-moment correlation between bilateral asymmetries (BSA) in isometric knee-extensor maximum voluntary torque (MVT) and relative BSA (BSA_DIR) for: peak voluntary rate torque development (RTD). BSA_DIR provides a measure of BSA, relative to the direction of BSA in MVT.

4.3.2 Determinants of Asymmetries in Maximal Strength

BSA for each determinant of MVT BSA was on average <10% for twitch PT, octet PT, muscle thickness, and VA, but ~14 – 18% for EMG_{MVT}, fascicle length, and pennation angle (Table 4.2). BSA MVT was not always in the same direction as the BSA for each determinant of MVT, as reflected by some negative values of BSA_DIR (i.e., BSA of each determinant relative to MVT BSA), and mean BSA_DIRs close to zero for all variables (-0.95 – 2.57%; Table 4.2). Accordingly, there were no statistically significant differences between the maximally strong and weak limb in neural drive (VA: p = 0.808, d = 0.05 and EMG_{MVT}: p = 0.992, d < 0.01), intrinsic contractile properties (twitch PT: p = 0.710, d = -0.04; and octet PT: p = 0.223, d = 0.13) or architectural properties (fascicle length: p = 0.612, d = 0.15; pennation angle: p = 0.991, d = 0.03; muscle thickness: p = 0.278, d = 0.16; Table 4.3) of the muscle.

All relationships between BSA in MVT and the BSA_DIR in each determinant of maximum strength were non-existent or weak (r = -0.18 – 0.21, p = 0.354 – 0.955; Table 4.2), although there was a trend toward significance when BSA was correlated with BSA_DIR VA (r = 0.38, p = 0.095). Owing to the lack of moderate, significant correlations between MVT BSA and BSA_DIR of any of its determinants (see Section *4.2.2.2*), multiple regression analyses were not performed.

4.3.3 Determinants of Asymmetries in Explosive Strength

BSA in most determinants of RTD was ~10 –15%, the exceptions being BSA twitch PT and muscle thickness (~7 – 8%) and BSA RMS $EMG_{0\text{-}100}$ (19%; Table 4.2). As with asymmetries in the determinants of MVT, BSA RTD was not always in the same direction as the BSA for each determinant of RTD, which again resulted in some negative values of BSA_DIR. As a result, BSA_DIR was <5% (range -4.50 – 3.65%) for all determinants of BSA RTD other than pennation angle (BSA_DIR 6.83%; Table

4.2). Correspondingly, there were no statistically significant differences between the explosively strong and weak limb in explosive neural drive (RMS EMG_{100}: p = 0.652, d = 0.10), intrinsic contractile properties (p = 0.302 – 0.985, d = -0.04 – 0.18) or architectural properties (p = 0.102 – 0.887, d = -0.37 – 0.41; Table 4.3) of the muscle.

Table 4.2. Bilateral asymmetries (BSA) in the determinants of maximum and explosive strength in the knee extensors of an active able-bodied population (measured as maximum voluntary torque [MVT] and rate of torque development [RTD], respectively). BSA calculated as the absolute difference between the two limbs. BSA_DIR calculated between the strong and weak limb for the strength variable in question. Bivariate correlation coefficients (r) correspond to the relationships between BSA MVT and BSA RTD with BSA_DIR in the neural, contractile and architectural determinants of each strength variable. Data are presented as mean ± SD for n = 20 (evoked octet) and n = 21 (all other variables). * indicates a significant ($p < 0.05$) relationship.

		BSA (%)	MVT		Peak RTD	
			BSA_DIR (%)	r	BSA_DIR (%)	r
Contractile Capacity						
MVT		9.99 ± 4.98	-	-	4.64 ± 10.3	0.56*
Neural Drive						
VA		6.23 ± 3.95	0.45 ± 7.50	0.38	-	-
RMS EMG_{MVT}		17.8 ± 10.6	0.29 ± 21.04	-0.18	-	-
Explosive RMS $\text{EMG}_{0\text{-}100}$		19.1 ± 14.7	-	-	0.82 ± 24.4	0.17
Intrinsic Contractile Properties						
Evoked Twitch	PT	8.04 ± 7.17	-0.95 ± 10.78	0.19	-0.13 ± 10.8	-0.12
	RTD	13.8 ± 12.0	-	-	3.38 ± 17.9	-0.41
Evoked Octet	PT	10.5 ± 8.22	2.57 ± 12.94	0.17	3.33 ± 12.8	-0.05
	RTD	10.8 ± 9.66	-	-	3.65 ± 14.3	-0.23
Architectural Variables						
Muscle Thickness		7.46 ± 5.34	2.34 ± 9.01	0.03	-0.06 ± 9.33	-0.09
Pennation Angle		14.9 ± 12.9	0.36 ± 20.0	-0.01	6.83 ± 18.7	-0.04
Fascicle Length		14.7 ± 13.5	1.82 ± 20.1	0.21	-4.50 ± 19.7	-0.26

BSA, bilateral strength asymmetry; BSA_DIR, BSA of a neuromuscular variable relative to the strength variable; MVT, maximum voluntary torque; RTD, rate of torque development; VA, voluntary activation; RMS EMG, root mean square electromyography; PT, peak torque.

Table 4.3. Neural, contractile and architectural determinants of maximum (measured as maximum voluntary torque, MVT) and explosive strength (measured as rate of torque development, RTD) in the knee extensors of an active able-bodied population. Data are grouped by either the maximally or the explosively strongest and weakest legs, and presented as mean ± SD for n = 20 (evoked octet) and n = 21 (all other variables).

		MVT		Peak RTD	
		Strong	Weak	Strong	Weak
Neural Drive					
Voluntary Activation (%)		89.4 ± 6.34	89.0 ± 7.55	-	-
RMS EMG_{MVT} (% M_{max})		8.35 ± 2.17	8.36 ± 2.10	-	-
Explosive RMS $EMG_{0\text{-}100}$ (% M_{max})		-	-	7.70 ± 2.85	7.46 ± 2.11
Intrinsic Contractile Properties					
Evoked Twitch	PT (Nm)	38.5 ± 9.11	38.8 ± 9.12	38.6 ± 9.16	38.6 ± 9.07
	RTD ($Nm.s^{-1}$)	-	-	831 ± 225	792 ± 198
Evoked Octet	PT (Nm)	129 ± 30.8	125 ± 29.4	129 ± 29.0	125 ± 31.1
	RTD ($Nm.s^{-1}$)	-	-	2116 ± 446	2054 ± 532
Architectural Variables					
Muscle Thickness (mm)		26.9 ± 4.03	26.2 ± 4.16	26.5 ± 3.9	26.6 ± 4.31
Pennation Angle (°)		14.0 ± 3.05	13.9 ± 2.63	14.5 ± 2.80	13.4 ± 2.78
Fascicle Length (mm)		114 ± 24.9	111 ± 20.2	108 ± 17.7	118 ± 25.7

BSA, bilateral strength asymmetry; BSA_REL, BSA of a neuromuscular variable relative to the strength variable; MVT, maximum voluntary torque; RTD, rate of torque development; VA, voluntary activation; RMS EMG, root mean square electromyography; PT, peak torque.

As stated above, a significant relationship was apparent between BSA RTD and BSA_DIR MVT (r = 0.56, *p* = 0.008; Figure 4.2B, Table 4.2), and absolute twitch RTD BSA_DIR was weakly correlated with BSA RTD (r = -0.41, *p* = 0.067; Table 4.2). No correlations were apparent between BSA RTD and BSA_DIR of any other determinants of explosive strength asymmetry (r = -0.26 – 0.17, *p* = 0.109 – 0.956; Table 4.2).

As there were no moderate, significant correlations between BSA RTD and BSA_DIR of any predictor variables other than for BSA_DIR MVT, no multiple regression analyses were performed.

4.4 Discussion

This study aimed to compare the magnitude and variability in quadriceps BSA for MVT and RTD, and determine the neuromuscular determinants of BSA, in a group of healthy young males with no specific training background. No difference was determined in the size of asymmetry between maximal (10%) and explosive strength (~13%) between limbs, although the variability of asymmetry for explosive strength was much greater than for maximal strength. BSAs in maximum and explosive strength were not necessarily in the same direction, and the large variability present in these measures were likely a result of very high variability in BSA_DIR of all determinants of strength. It seems unwise therefore to rely on a single metric of strength to inform us of clinically relevant changes in functional capacity, injury risk or presence of pathology. Furthermore, despite large BSAs in neural and architectural determinants of strength, there was no apparent systematic combination of asymmetry variables that would explain the asymmetries present in strength. This is evidenced by the lack of between limb differences in any of the neuromuscular determinants, which shows that asymmetries in strength and their determinants were not always in the same direction, as well as the lack of any correlations in the BSAs of strength and the BSA_DIR of the strength determinants. Thus, it appears that the neuromuscular determinants of strength asymmetry are highly individualised.

4.4.1 Maximum and Explosive Strength Asymmetry

The magnitude of maximum strength asymmetry (mean 10%) found in the participants of this study was substantially greater than that previously reported at the quadriceps in similar populations (1 – 5%; Kobayashi et al. 2013, Lanshammar & Ribom 2010, Schiltz et al. 2009), although the results are not directly comparable, as the above studies assessed dynamic strength measured between 60 – 240°s^{-1} (Table 2.1) and split limbs categorised by limb dominance. BSAs in RTD were more variable (range ~35%) than BSA MVT (range ~17%), and a greater proportion of the sample demonstrated larger BSAs in RTD than MVT (Figure 4.1). Possibly this is due to the greater inter-individual (RTD: CV = 23 – 41% vs. MVT: CV = 21% Folland, Buckthorpe & Hannah 2014) and between-session variability in explosive when compared to maximum strength (RTD: ICC = 0.90, CV = 7.2%; MVT: ICC ≥ 0.90, CV <4%; Buckthorpe et al. 2012, de Ruiter et al. 2004, Place et al. 2007). Between-day reliability for strength measures assessed in this thesis were similarly larger in RTD (ICC 87%, CV 8%) than MVT (ICC 0.95%, CV 6%; Appendix F). Unexpectedly, however, BSA RTD (13%) was of a similar magnitude to that of MVT (Table 4.1), possibly because MVT is an important determinant of peak RTD (Folland, Buckthorpe & Hannah, 2014). This is supported by the relationship apparent between BSA RTD and BSA_DIR MVT (r = 0.56, *p* = 0.008; Figure 4.2B), which suggests that these two aspects of strength asymmetry are positively associated, i.e. as BSA RTD increases, so does BSA_DIR MVT. This is despite the maximally strong limb not always being the explosively strong limb. These directional differences in MVT and RTD asymmetry may be partially due to the task difference: a review by Maloney et al. (2019) suggested that inter-limb asymmetries are task- and variable-specific, which may also hold true for strength. Participant history may also have had an effect on the relative directions of strength asymmetries, as inter-limb strength asymmetries are likely present due to a combination of factors such as training background (Rahnama, Lees

& Bambaecichi 2005, Theoharopoulos et al. 2000), previous injury (Newton et al. 2006), and limb dominance (Sjöström et al. 1991). We attempted to minimise the confounding effects of such issues through careful participant recruitment. Specifically, we attempted to minimise the effects of laterality (i.e. the preferential use of one side of the body when presented with a motor task to perform; Maloney 2018) and training history by ensuring that there was no specific training bias for the participants in this thesis. Further, we categorised limbs by force dominance (i.e. the limb demonstrating superior strength in a given task), rather than the self-selected dominant limb, the latter of which introduces issues with limb classification (discussed further in Appendix A, Section A.3.1).

4.4.2 Neuromuscular Asymmetry

Based on previous literature, which has evidenced maximum strength being determined by muscle size, architecture, and peak contractile torques (Fitts, McDonald & Schluter 1991, Lieber & Friden 2000), and explosive strength by neural drive, intrinsic contractile properties and maximum strength (Andersen & Aagaard 2006, Folland, Buckthorpe & Hannah, 2014) we hypothesised that inter-limb strength asymmetries may be determined by asymmetries in factors that determine single limb strength. However, this was not supported by our data. Indeed, the results of this study suggest that there is no apparent systematic combination of determinants that would cause the maximum strength asymmetry in the quadriceps, i.e. the coordination of neuromuscular function between limbs for force production is different between individuals. Other than the moderate correlation between BSA RTD and BSA_DIR MVT (discussed in Section 4.4.1), asymmetries in strength and their determinants were not correlated (Table 4.2). Furthermore, they were not in the same direction, as evidenced by the lack of between-limb differences in the determinants when grouped for the strong and weak limbs (Table 4.3). Indeed, despite absolute

BSAs of 6.2 – 19.1%, when considered relative to the strength variable of interest, the group mean in the magnitude of determinant BSA_DIR was close to zero (determinants of MVT: 0.3 – 2.6%; determinants of RTD: 0.1 – 4.6; Table 4.2). In other words, when grouped relative to the strength of the limbs suggest asymmetries in strength determinants mostly cancel out. Consequently, determinant asymmetries appear to combine differently across participants, which perhaps explains the lack of explanatory relationships (Table 4.2). This may potentially be a result of factors such as limb dominance (Sjöström et al. 1991), previous injury (Newton et al. 2006) and training background (Rahnama, Lees & Bambaecichi 2005, Theoharopoulos et al. 2000). Each of these factors causes adaptations at the limb undergoing the stimulus (e.g. training causes adaptations such as increased muscle thickness and enhanced neural drive [Narici et al. 1989, Tillin & Folland 2014]) and each participant will have experienced a different combination of these between limbs in their lives.

4.5 Conclusion

This study is the first to compare the magnitude and variability in quadriceps maximum and explosive strength asymmetries, together with asymmetries of their underlying neuromuscular determinants, in a young, healthy, recreationally active adult population. No difference in the size of inter-limb asymmetries between maximal and explosive strength was apparent, possibly due to the key role maximum torque capacity plays in determining explosive strength capabilities at a muscle. Despite this, the relative direction of asymmetry in the two measures of strength was not consistent, i.e. the maximally strong limb was not necessarily the more explosive limb. Additionally, other than the moderate relationship between asymmetries in the two measures of strength, neither maximum nor explosive strength asymmetry was explained by a systematic combination of asymmetries in the neuromuscular factors which are thought to determine strength. As both underpin different functional capabilities of muscle, for a complete profile for diagnosis and monitoring of muscle strength asymmetry, both aspects of strength should be considered. It remains unclear to what extent the variability in maximum and explosive quadriceps strength observed in the current study is relevant to function, i.e., whether strength asymmetries are associated with asymmetrical loading during movements that rely on quadriceps torque contributions.

Chapter 5

The Association between Asymmetries in Strength and Jump Take-off/ Drop Landing Capabilities in an Able-Bodied Population

5.1 Introduction

The previous chapter provided evidence that large inter-limb asymmetries in quadriceps strength and neuromuscular function are prevalent even in a young, healthy control population. Previous research has linked quadriceps strength asymmetries with performance deficits in a range of sport-specific skills, e.g. kicking accuracy (Hart et al. 2014) and sprint cycling power (Rannama et al. 2015). Furthermore, in some populations (e.g. those with ACL injuries and ITTAs) quadriceps strength asymmetries are implicated in movement, and consequently, limb loading asymmetries (Grindem et al. 2016, Lloyd et al. 2010, Schmitt et al. 2015). This suggests there may be a direct association between the magnitude and direction of asymmetries in strength and loading during movement; however, such associations remain unclear.

Jumping is an important skill in many sport and recreational activities, and it is a useful model to assess lower-body function due to the planar nature of movement with its single clear performance outcome (i.e. to jump as high as possible). While it may be expected that both limbs would contribute equally to take-off, and thereby jump height, previous research has indicated that this supposed equality between limbs is not necessarily the case. For example, asymmetries of 0.8 – 6.4% have been found in peak vertical ground reaction force (vGRF; Bačič et al. 2010, Bell et al. 2014, Impellizzeri et al. 2007, Lawson et al. 2006, Newton et al. 2006, Stephens et al. 2007). It is possible therefore that strength asymmetry may contribute to jump height in a bilateral CMJ. This hypothesis is supported by the work of Bailey et al. (2013), who found greater lower-limb strength asymmetries to be indicative of reduced jump height. Evidence of loading asymmetry in jump take-off has also been found to be associated with strength asymmetry. Impellizzeri et al. (2007) found strong correlations between peak vGRF asymmetry (calculated between strong and weak limbs) during a bilateral CMJ and strength asymmetry determined via an isometric leg press. As there is a large quadriceps contribution to the take-off phase of a jump, with the knee contributing approximately 49% of the total work done in a maximal CMJ (Hubley & Wells 1983, Impellizerri et al. 2007), it is possible that quadriceps strength asymmetry may contribute to both jump height and asymmetrical limb load. Specifically, we may expect: (1) the stronger leg to contribute a higher load (e.g., peak vertical, GRFs, rates of loading) during a jump; and/or (2) the asymmetry in strength to be correlated with the asymmetry of loading during the jump. However, the associations between quadriceps strength asymmetry, jump height, and asymmetries in limb loading during the take-off phase of a jump have not been quantified.

Landing from a jump occurs frequently in sport but carries a high risk of injury (Murphy et al. 2003). In a computer modelling study, Sandler and Robinovitch (2001) demonstrated that decreased strength adversely affects energy absorption mechanisms as a result of decreased eccentric torque production at the lower limb joints. The knee extensors have a large contribution to the energy absorption in landing. They contract eccentrically to control knee flexion and decelerate the CoM, performing a substantial proportion of energy dissipation when compared to other lower limb joints (39% compared to 34% and 27% at the hip and ankle, respectively; Decker et al. 2003, McNitt-Gray et al. 1993, Yeow et al. 2011, Zhang et al. 2000). It is possible therefore that quadriceps strength asymmetries may result in asymmetries in loading (both at a whole-limb level, and at the knee specifically) during a landing task. Indeed, in limbs that have undergone ACL reconstruction, previous research has demonstrated that individuals with maximum strength asymmetries of > 15% of the quadriceps displayed reduced involved limb peak knee internal extension moments and vGRFs, and lower peak loading rates in the landing from a drop jump (Schmitt et al. 2015). While these authors did not perform a correlational analysis, investigating differences between limbs in this way is another way of measuring associations – in this case between loading parameters in landing and the involved (weak) and uninvolved (strong) limbs. The differences found suggest that there is an association between the asymmetry in quadriceps strength and knee joint loading in landing; however, these associations have not been explored in a non-injured population, which may respond differently.

It possible that asymmetry in RTD may be more closely associated with asymmetries in jump take-off and landing than that of MVT, as the take-off and landing phases of a bilateral jump are both movements with high accelerations. The ability to quickly produce force is therefore important for both. For example, de Ruiter et al. (2007) reported significant positive linear relationships ($r = 0.76 – 0.86$) between the first 40 ms of unilateral explosive knee extension torque production and bilateral jump height. Moreover, Chang et al. (2015) reported 47.6 and 31.4% of the variability in vertical jump height was explained by unilateral knee extensor early phase RTD and MVT, respectively, suggesting that the former is more important for jump performance. Furthermore, the rapid development of force that occurs during landings has been associated with various knee injuries, including osteoarthritis and non-specific knee pain (Murphy et al. 2003). If we consider that injuries such as ACL ruptures occur within 50 ms of ground contact (Krosshaug et al. 2007), but the time to develop maximum force either isometrically or eccentrically is generally > 300 ms (Thorstensson et al. 1976, Tillin, Pain & Folland 2012a), it may be that asymmetry in explosive strength is more important than that in maximum strength for the rapid energy absorption required in the lower limb on landing. As large loading rates have been linked to injury risk (Gailey et al. 2008, Mundermann et al. 2005), we may expect the stronger and more explosive quadriceps to be able to absorb the momentum more effectively in early loading, evidenced by larger limb loads. However, these associations have not yet been explored.

The purpose of this study was therefore to determine whether (1) jump height and (2) loading asymmetries in jump take-off and landing phases are associated with asymmetries in knee extensor maximum and explosive strength. We hypothesised that: (1) the stronger limb would experience greater loading (whole-limb and at the knee) during both jumping take-off and landing. (2) the bilateral asymmetry in strength would be correlated with the asymmetry in loading during both jumping and landing. (3) asymmetry in quadriceps explosive strength would be more closely associated than asymmetry in maximum strength, with jump performance and loading asymmetry in both jumping and landing.

5.2 Methods

A comprehensive description participant recruitment, inclusion criteria and the methods followed for data collection and processing are given in Chapter 3, sections 3.3 – 3.5.

Briefly, participants visited the laboratory for 2-3 hours on three separate occasions, 3-7 days apart, to complete a familiarisation session, and two measurement sessions. The familiarisation session and first measurement session were identical and involved assessment of voluntary quadriceps muscle strength on both limbs (sections *3.4.2.3* and *3.4.2.5)*. The second measurement session involved motion capture to allow analysis of CMJ and drop landings (sections *3.5.4.1* and *3.5.4.2*).

5.2.1 Participant Information

Twenty-one physically active male participants (age 34.2 ± 6.52 years, height 180 ± 6.07 cm, mass 82.7 ± 11.2 kg, activity 4775 ± 2677 MET-mins/week) took part in this study. Participant inclusion and exclusion criteria, and detail about assessment of physical activity, can be found in Chapter 3, Section 3.2.

5.2.2 Data Extraction and Analysis

Strength data (MVT and RTD) were extracted for both limbs and processed as discussed in section *3.4.4.3.* Details about movement analysis data processing can be found in Sections 3.5.5 and 3.5.6. For both movements, values were recorded for each limb and averaged across the three trials selected for analysis.

5.2.2.1 CMJ

Jump height was calculated using CoM velocity at take-off (TOV) using the equation

$$Jump\ Height = \frac{TOV^2}{2g} \tag{5.1}$$

where g = 9.81 m.s^{-2} (Moir 2008). CoM was calculated as the weighted mean of all body segment CoMs as calculated by Vicon Nexus. The take-off phase was broken into two sub-phases: lowering (eccentric, defined from the start of the countermovement to the point at which CoM velocity = 0) and rising (concentric, defined as the first positive value for CoM velocity until toe-off; Figure 5.1A).

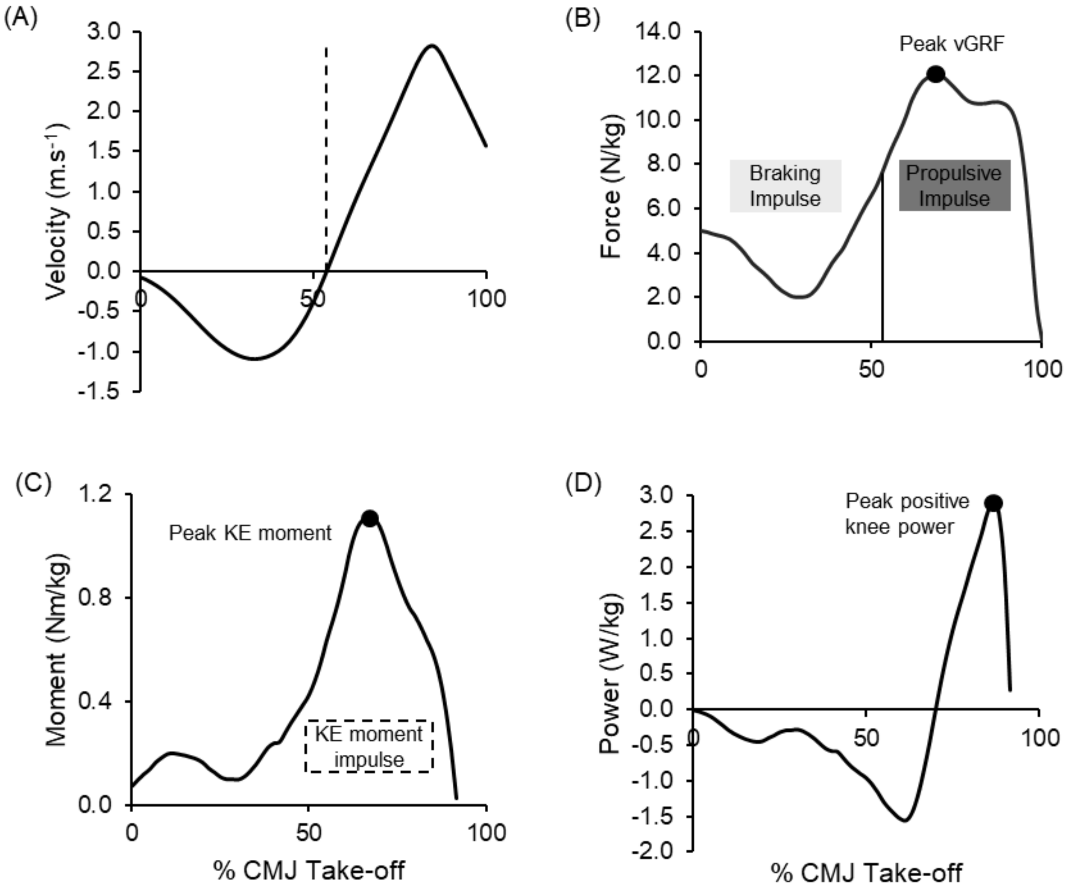


Figure 5.1. Kinetics of the take-off phase of a bilateral countermovement jump (CMJ). Centre of mass (CoM) velocity (A) was used to calculate sub-phases for vGRF variables, determined by the change from eccentric to concentric muscle action, i.e. braking ends when CoM velocity becomes positive. Variables of interest include (B) peak vertical ground reaction force (vGRF), RFD (Δforce/Δtime from beginning of propulsion to peak vGRF), decay rate (Δforce/Δtime from peak vGRF to toe-off), and impulse in both sub-phases; (C) peak knee extension (KE) moment and total KE moment impulse; and (D) peak knee power. The knee joint phase of interest was defined from the beginning of the take-off phase to the last point of positive knee power. Impulse is calculated as the area under the waveform of interest.

The loading variables extracted from the CMJ take-off are represented in Figure 5.1B – D. Peak vGRF, peak positive knee power, peak knee extensor moment and total knee extensor moment impulse throughout the entire CMJ take-off were extracted.

Additional variables considered were mean vGRF RFD, calculated from the end of the lowering phase to peak vGRF; mean vGRF decay rate, calculated from 20-80% of the time from peak vGRF to toe-off (Moudy et al. 2018); and net vGRF impulse for the lowering and rising phases, respectively. RFD and decay rate were both calculated using a 5 ms time constant.

5.2.2.2 Drop Landing

The phase of interest (absorption) was defined as touchdown to maximum knee flexion. The variables extracted from the drop landing are represented in Figure 5.2. Peak vGRF, peak knee extensor moment and peak negative knee power (determined as the minimum value throughout the entire drop landing) were extracted. Total knee extensor moment impulse; mean vGRF loading rate, calculated from touchdown to peak vGRF; and mean knee extensor moment loading rate, calculated as the linear rate between touchdown and peak knee extension moment, were also extracted.

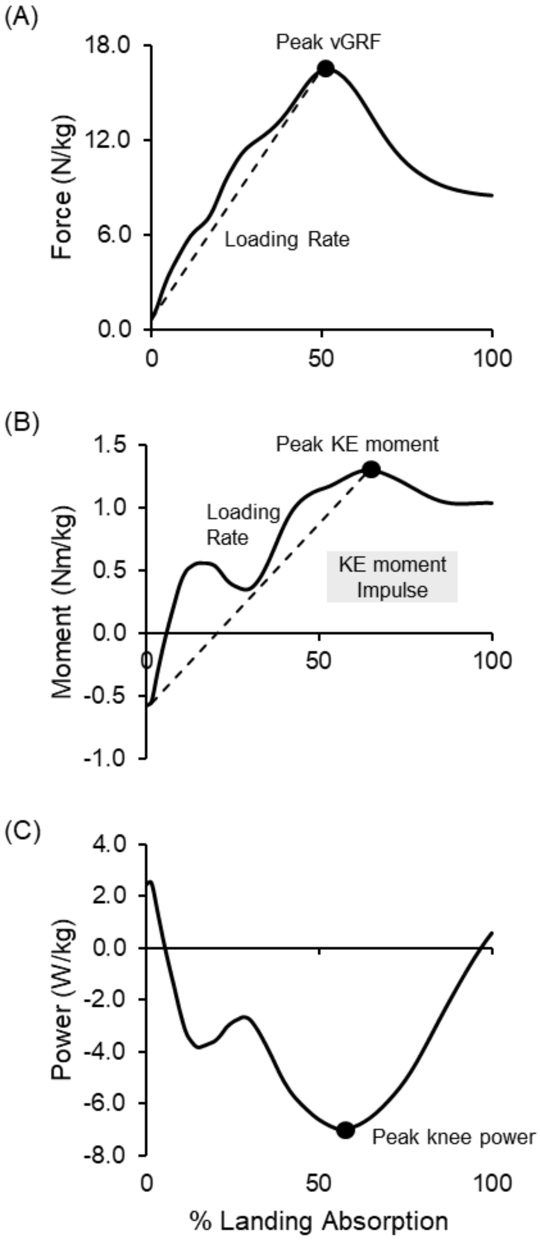


Figure 5.2. Kinetics of the absorption phase of a bilateral drop landing, defined as the time from touchdown to maximum knee flexion. Variables of interest include (A) peak vertical ground reaction force (vGRF) and average vGRF loading rate; (B) peak knee extensor (KE) moment, average KE; loading rate and KE moment impulse; and (C) peak negative knee power. Impulse is calculated as the area under the waveform of interest.

5.2.2.3 Asymmetry Calculation

Chapters 4 and 6 use Bilateral Strength Asymmetry (BSA) as the index of asymmetry. However, for this analysis, Bilateral Asymmetry Index (BAI; Bishop et al. 2018) was used. BAI is preferable for the quantification of asymmetry in a bilateral task, as the differences in force between limbs are always relative to the sum force value (see Appendix A, Section A.3.3 for further discussion of the selection of asymmetry indices).

BAI was calculated for MVT, RTD and all loading parameters of CMJ and drop landings using equation 3.3 (Chapter 3, Section 3.6.1). This equation quantifies the magnitude of BAI without specifying the direction of that BAI, i.e. which leg was stronger.

However, when correlating the BAI between two variables it was important to denote the direction of the BAI of the predictor (i.e. strength) variable relative to the BAI of the dependent (i.e. loading) variable (BAI_DIR, i.e. directional BAI; equation 3.5), accounting for the fact that the BAI may be in opposite directions for the two variables. This equation may provide a negative magnitude if the direction of BAI for the predictor variable is opposite to the BAI of the dependent variable. For correlating the BAI for a strength variable (i.e. MVT or RTD) with the BAI of a movement variable (i.e. peak vGRF, KE moment impulse, etc.) the strength variable was considered the predictor, and the movement variable considered the dependent variable.

5.2.2.2 Statistical Analysis

Data are presented as mean ± standard deviation (SD) unless otherwise stated. Statistical analysis was completed using SPSS version 24, and the significance level was set at $p<0.05$. Levene’s test was used to check for equality of variances prior to running all analyses, while Shapiro-Wilkes assessed normality of the data.

To assess if there was an association in the direction of strength and loading variables during a CMJ and drop landing, all loading variables for each movement were grouped into strong and weak for both MVT and RTD separately, and paired t-tests compared the magnitude difference of each variable between limbs. Effect sizes (specifically Cohen's *d*) were calculated for between-group comparisons and interpreted as small (*d* = 0.2 – 0.5), medium (*d* = 0.5 – 0.8) and large effects (*d* > 0.8, Lakens 2013).

Pearson's product moment bivariate correlations were performed to determine if there was an association between strength BAI and jump height. To determine if the direction and magnitude of strength and loading asymmetries were associated, Pearson's product moment bivariate correlations assessed the relationships of BAI for both maximum and explosive strength with the BAI_DIR for each individual loading variable in both landing and jumping. All relationships were interpreted as strong (r > 0.7), moderate (r = 0.5 – 0.7), weak (r = 0.3 – 0.5) or non-existent (r < 0.3; Mukaka 2012).

5.3 Results

Relatively small asymmetries (calculated using equation 5.1) were present between limbs in both quadriceps maximal (BAI MVT: 5.32 ± 2.78%) and explosive strength (BAI RTD: 7.07 ± 5.69%).

5.3.1 CMJ

Average jump height across all participants was 0.33 ± 0.07 m. Although no correlation was evident between jump height and BAI MVT (r = 0.07, *p* = 0.752) a weak, non-significant positive relationship was present between jump height and BAI RTD (r = 0.36, *p* = 0.113; Figure 5.3).

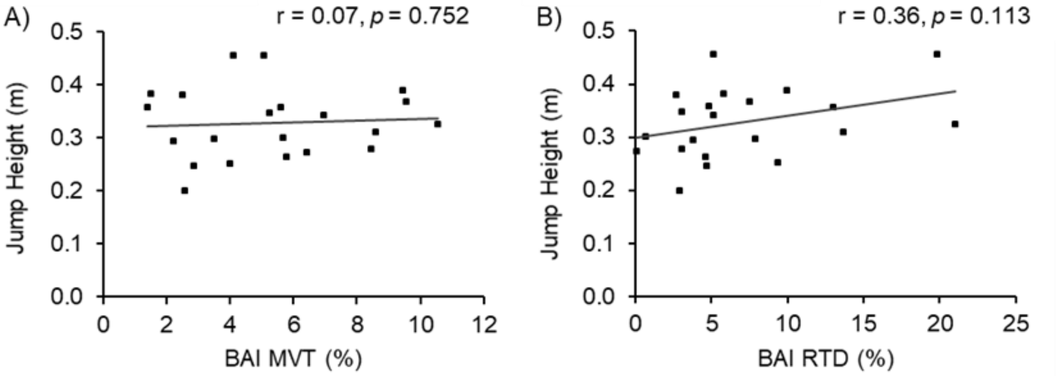


Figure 5.3. Bivariate correlation coefficients (r) between countermovement jump height and Bilateral Asymmetry Index (BAI) in (A) maximum and (B) explosive strength (measured during voluntary isometric contractions of the knee extensors) for n = 21 active, able-bodied males.

BAI was ≤ 10% for peak vGRF, vGRF impulse (in lowering and rising), and peak knee extensor moment, but substantially larger for peak positive knee power (39.1%) and knee extensor moment impulse (23.3%; Table 5.1). Individual strength BAIs were not always in the same direction as the BAI for each loading variable (e.g. BAI RTD and BAI_DIR vGRF lowering impulse; Figure 5.4), which occasioned most mean values of BAI_DIR being close to zero (Table 5.1). The exceptions were BAI_DIR for knee extensor moment impulse (relative to MVT, 8.37%; RTD, -8.63%) and peak positive knee power (relative to MVT, 17.1%; RTD -6.6%; Table 5.1).

Table 5.1. Bilateral asymmetries (BAI) in the kinetic characteristics of the take-off phase a countermovement jump in an active, able-bodied population. BAI calculated as the absolute difference between the two limbs. BAI_DIR calculated between the strong and weak limb for the strength variable in question, i.e. maximum or explosive strength of the knee extensors (measured as maximum voluntary torque [MVT] and rate of torque development [RTD], respectively). Bivariate correlation coefficients (r) correspond to the relationships between BAI MVT and BAI RTD with BAI_DIR in the kinetic parameters of movement. Data are presented as mean ± SD for n = 21. * indicates a significant ($p < 0.05$) relationship.

		BAI (%)	MVT		RTD	
			BAI_REL (%)	r	BAI_REL (%)	r
vGRF	Peak	3.07 ± 2.31	0.47 ± 3.87	-0.08	1.41 ± 3.63	0.27
	RFD	5.82 ± 4.17	0.78 ± 7.24	0.11	-1.79 ± 7.05	-0.10
	Decay Rate	6.69 ± 6.46	4.47 ± 8.23	0.12	-0.68 ± 9.39	0.25
	Lowering Impulse	6.25 ± 4.88	-1.65 ± 7.87	-0.06	-0.06 ± 6.98	-0.45*
	Rising Impulse	3.17 ± 2.14	0.22 ± 3.89	-0.05	1.27 ± 3.67	0.16
KE Moment	Peak	10.2 ± 8.59	2.87 ± 13.2	0.32	-0.53 ± 13.5	-0.09
	Total Impulse	23.3 ± 20.0	8.37 ± 30.0	0.22	-6.61 ± 30.4	-0.19
Peak Positive Knee Power		39.1 ± 27.6	17.1 ± 45.3	0.20	-8.63 ± 47.8	-0.07

BAI, bilateral asymmetry index; vGRF, vertical ground reaction force; RFD, Rate of Force Development; KE, knee extension

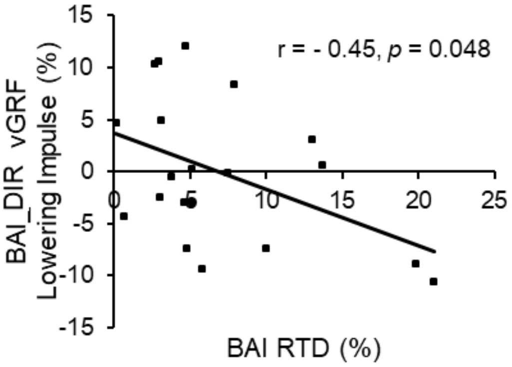


Figure 5.4. Bivariate correlation coefficient (r) between Bilateral Asymmetry Index (BAI) in explosive strength of the knee extensors, (measured as Rate of Torque Development, RTD) and BAI_DIR in vertical ground reaction force (vGRF) lowering phase impulse. BAI_REL calculated between the explosively strong and weak limb. Data are presented for n = 21 active, able-bodied males.

There was a statistically significant difference in vGRF decay rate (p = 0.047, d = 0.27; Table 5.2) between the maximally strong and weak legs, whereby the limb with the greatest quadriceps MVT also displayed the greatest vGRF decay rate. No statistical differences were evident between maximally strong and weak limbs for any other loading variables (p = 0.208 – 0.942, d = 0.02 – 0.44; Table 5.2). There was a trend for the limb with the greatest quadriceps RTD to demonstrate smaller peak vGRF (p = 0.079, d = 0.29), but no other significant differences were evident between the explosively strong and weak limbs (p = 0.095 – 0.490, d = 0.07 – 0.43; Table 5.2).

A weak, significant negative relationship was present between BAI RTD and BAI_DIR braking vGRF impulse (r = -0.45, p = 0.048; Figure 5.4). All other correlations between BAI_DIR of a loading variable and BAI of either MVT or RTD were non-significant and weak or non-existent (Table 5.1).

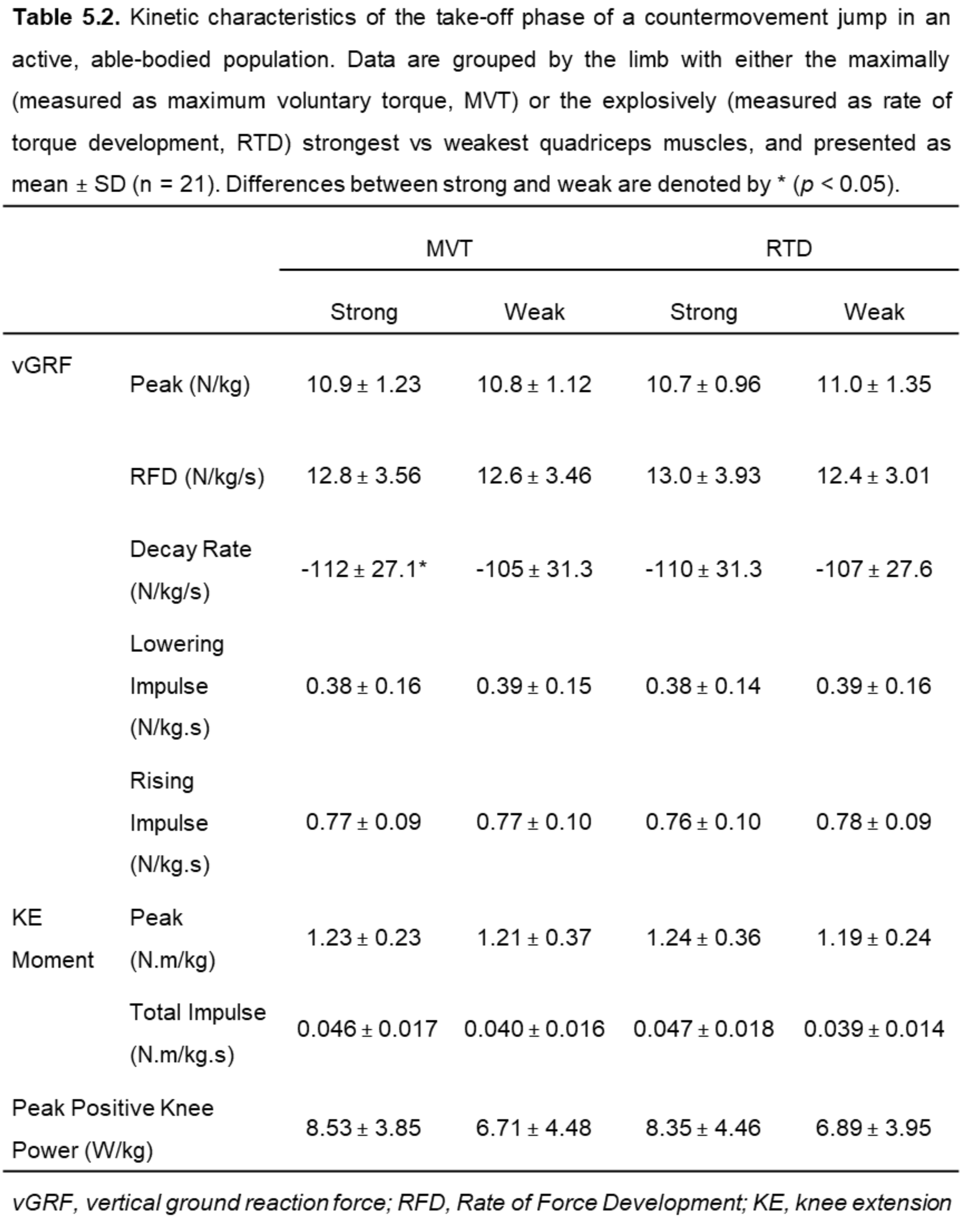


Table 5.2. Kinetic characteristics of the take-off phase of a countermovement jump in an active, able-bodied population. Data are grouped by the limb with either the maximally (measured as maximum voluntary torque, MVT) or the explosively (measured as rate of torque development, RTD) strongest vs weakest quadriceps muscles, and presented as mean ± SD (n = 21). Differences between strong and weak are denoted by * ($p < 0.05$).

		MVT		RTD	
		Strong	Weak	Strong	Weak
vGRF	Peak (N/kg)	10.9 ± 1.23	10.8 ± 1.12	10.7 ± 0.96	11.0 ± 1.35
	RFD (N/kg/s)	12.8 ± 3.56	12.6 ± 3.46	13.0 ± 3.93	12.4 ± 3.01
	Decay Rate (N/kg/s)	-112 ± 27.1*	-105 ± 31.3	-110 ± 31.3	-107 ± 27.6
	Lowering Impulse (N/kg.s)	0.38 ± 0.16	0.39 ± 0.15	0.38 ± 0.14	0.39 ± 0.16
	Rising Impulse (N/kg.s)	0.77 ± 0.09	0.77 ± 0.10	0.76 ± 0.10	0.78 ± 0.09
KE Moment	Peak (N.m/kg)	1.23 ± 0.23	1.21 ± 0.37	1.24 ± 0.36	1.19 ± 0.24
	Total Impulse (N.m/kg.s)	0.046 ± 0.017	0.040 ± 0.016	0.047 ± 0.018	0.039 ± 0.014
Peak Positive Knee Power (W/kg)		8.53 ± 3.85	6.71 ± 4.48	8.35 ± 4.46	6.89 ± 3.95

vGRF, vertical ground reaction force; RFD, Rate of Force Development; KE, knee extension

5.3.2 Drop Landing

BAI was 16 - 26% for all variables other than for peak vGRF and average vGRF loading rate (< 7%; Table 5.3). No statistically significant differences were evident between maximally strong and weak limbs in any loading variables during landing (*p* = 0.520 – 0.921, *d* = 0.02 – 0.17; Table 5.4). However, several significant differences were evident in loading parameters compared between the explosively strong and weak limb. The limb with the most explosive quadriceps displayed lower peak negative knee powers ($p = 0.019$, $d = 0.73$), peak knee extension moments ($p = 0.042$, $d = 0.62$); and greater peak vGRF ($p < 0.001$, $d = 0.43$), and vGRF loading rates ($p = 0.029$, $d = 0.22$; Table 5.4). Additionally, there was a trend toward the explosively strong limb demonstrating lower knee extension moment loading rates ($p = 0.083$, $d = 0.47$), but KE moment impulse was similar between limbs ($p = 0.890$, $d = 0.02$; Table 5.4).

All correlations between BAI of either MVT or RTD and BAI_DIR for any loading variable during landing were non-significant ($p \geq 0.178$) and small at best ($r \leq 0.31$; Table 5.3).

Table 5.3. Bilateral asymmetries (BAI) in the kinetic characteristics of a 30 cm drop landing task in an active, able-bodied population. BAI calculated as the absolute difference between the two limbs. BAI_DIR calculated between the strong and weak limb for maximum or explosive strength of the knee extensors (measured as maximum voluntary torque [MVT] and rate of torque development [RTD], respectively). Bivariate correlation coefficients (r) correspond to the relationships between BAI MVT and BAI RTD with BAI_DIR in the drop landing kinetic parameters. Data are presented as mean ± SD for n = 21.

		BAI (%)	MVT		RTD	
			BAI_DIR (%)	r	BAI_DIR (%)	r
vGRF	Peak	6.78 ± 3.60	-0.17 ± 7.82	0.31	5.07 ± 5.85	0.01
	Loading Rate	6.24 ± 4.21	-0.79 ± 7.61	0.11	3.42 ± 6.81	0.05
KE Moment	Peak	19.3 ± 14.1	-3.72 ± 24.0	0.05	-8.08 ± 22.9	-0.12
	Total Impulse	15.7 ± 14.7	4.73 ± 21.2	0.23	-1.97 ± 21.7	0.05
	Loading Rate	20.8 ± 17.2	-3.01 ± 27.2	-0.06	-9.53 ± 25.6	-0.17
Peak Negative Knee Power		26.4 ± 17.3	0.92 ± 32.1	0.15	-14.6 ± 28.6	-0.22

BAI, bilateral asymmetry index; vGRF, vertical ground reaction force; KE, knee extension

Table 5.4. Kinetic characteristics of a 30 cm drop landing in an active, able-bodied population. Data are grouped by either the maximally (measured as maximum voluntary torque, MVT) or the explosively (measured as rate of torque development, RTD) strongest and weakest legs, and presented as mean ± SD for n = 21. Differences compared to the weak limb of each group are denoted by * ($p < 0.05$) or ** ($p < 0.001$).

		MVT		RTD	
		Strong	Weak	Strong	Weak
vGRF	Peak (N/kg)	23.3 ± 6.33	23.2 ± 5.40	24.5 ± 6.21**	22.0 ± 5.24
	Loading Rate (N/kg/s)	210 ± 63.3	214 ± 65.0	219 ± 65.1*	205 ± 62.3
KE Moment	Peak (N.m/kg)	2.05 ± 0.87	2.19 ± 0.88	1.86 ± 0.45*	2.38 ± 1.10
	Total Impulse (N.m/kg.s)	31.7 ± 17.2	30.4 ± 20.7	30.9 ± 19.9	31.2 ± 18.1
	Loading Rate (N.m/kg/s)	34.1 ± 17.1	37.5 ± 21.8	31.3 ± 15.2	40.3 ± 22.4
Peak Negative Knee Power (W/kg)		-16.0 ± 8.68	-16.1 ± 9.63	-13.0 ± 5.49*	-19.2 ± 10.8

vGRF, vertical ground reaction force; KE, knee extension

5.4 Discussion

This study aimed to determine if quadriceps strength (both maximum and explosive) asymmetry was associated with jump performance and bilateral asymmetry in loading variables during jumping and landing. While jump height was weakly correlated with BAI RTD, there was no relationship with BAI MVT. Furthermore, there were no between limb differences when split for maximally or explosively strong and weak, except in decay rate when split for maximum strength. Additionally, there were no correlations except a weak relationship between asymmetry in explosive strength and vGRF impulse in the lowering phase of jump take-off. However, the significant differences in most loading variables in landing when grouped by explosive strength of the quadriceps suggests that there was systematic agreement between the direction of strength asymmetries and those of some loading variables. In particular, the more explosive limb demonstrated increased loads and loading rates at the whole-limb (i.e. vGRF), but decreased knee joint loading (i.e. knee extension moments), possibly a result of different coordination strategies between the joints between the two limbs.

5.4.1 Associations between Strength and Movement Asymmetries

5.4.1.1 Jump Height

The hypothesis that there would be a negative relationship between strength asymmetry and jump height does not appear to be supported by the results of this study. We found no correlation between jump height and MVT asymmetry. While the positive correlation with RTD asymmetry suggests that greater inter-limb asymmetry in explosive strength may result in a higher jump, care should be taken when drawing conclusions from these data given the weak, non-significant nature of this relationship. The lack of association between strength asymmetry and jump height may be explained by the knee extensors of the stronger leg compensating for the weaker leg, or perhaps it is the overall asymmetry of the limb (i.e., the coordination of each joint with the others) that is important for jump height. The latter hypothesis is supported by the work of Bailey et al. (2013) who found significant moderate negative correlations between lower-limb strength asymmetries and jump height. Alternatively, it may be the homogeneity in the group that limits the correlations, as all participants demonstrated low levels of strength asymmetry (~5 – 7%), and similar jump heights (0.33 ± 0.07 m).

5.4.1.2 Loading in CMJ Take-Off

This study provided limited evidence of a link between strength and loading asymmetries in the take-off phase of a CMJ. Other than a weak relationship between asymmetry in explosive strength and vGRF lowering impulse (Figure 5.4), no correlations were apparent between any strength and loading asymmetries (Table 5.1). Furthermore, the lack of differences found in loading variables when split for either maximum or explosive strength, suggests that the asymmetries in these variables were not necessarily in the same directions between individuals (Table 5.2). The exception is vGRF decay rate (i.e. the acceleration in unloading prior to take-off), which describes the rate at which the body unweights in response to the force generated in the concentric phase of a jump-take off. This is likely related to coordination of the triple extensors causing the limbs to extend and/ or extension velocity, and is thought to be a key predictor of jump height (Moudy et al. 2018). Despite no correlation between maximum strength asymmetry and vGRF decay rate, suggesting that the magnitudes of asymmetry weren’t proportional, a significant difference was apparent between the maximally strong and weak limbs. This suggests that the asymmetries in these two variables are in the same direction i.e., when compared to the weaker, the maximally strong limb systematically produced greater decay rate in jump take-off. It may be that maximum strength asymmetries are a clinically relevant feature when looking to maximise jump performance; however, the mechanisms of this are unclear. As a greater decay rate is indicative of a higher CoM acceleration, and therefore likely a higher velocity at take-off, this result is potentially the consequence of the stronger leg extending with a higher velocity.

The lack of association between asymmetries in strength and other vGRF asymmetries suggest that, contrary to our hypothesis, individuals do not necessarily load their stronger limb more than the weaker limb in the take-off phase of a jump. This may be a result of the way in which the joints coordinate during movement. As vGRF represents the combined actions of primarily the lower extremity triple extensors (hip and knee extensors and ankle plantarflexors) during the force production portion of a jump, previous authors have suggested that asymmetric vGRF should not be interpreted as a direct result of lower limb strength asymmetries. Rather, it is argued that bilateral asymmetries in vGRF stem from the way the lower limbs, pelvis, and trunk are coordinated to perform the jump (Benjanuvatra et al. 2013, Chang et al. 2015). The comparatively large asymmetries in knee loading parameters (BAI 10.2 – 39.1%), when compared to those in vGRF (BAI 3.07 – 6.69%) suggest that compensations occur elsewhere in the kinetic chain (i.e. at the hip, ankle or trunk) to reduce the overall asymmetry in load. It may indicate that when considered bilaterally, the knee has a greater role in coordinating the action of the hip and ankle joints, rather than directly impacting jump performance itself. Perhaps instead it is therefore strength asymmetry at the ankle and/ or hip that are responsible for the loading asymmetries evident between limbs in jumping. This in turn may explain the lack of correlations apparent between quadriceps strength asymmetries and knee loading, and the large directional variability when variables are grouped by knee extensor strength.

5.4.1.3 Loading in Landing

During bilateral landing, rapid impact forces are typically dissipated by near synchronous joint flexion of both limbs and eccentric work of the quadriceps muscles. There was limited evidence of associations between maximum strength asymmetries and those in limb load, (i.e. there were no correlations, and differences in loading weren’t noticeable when grouped according to maximum strength), but the explosively strong limb presented with greater peak loads and loading rates (vGRF). This may be explained by previous research that has demonstrated an association between decreased muscular strength and reduced lower-limb eccentric torque production on landing (Sandler & Robinovitch 2001). It is this eccentric muscle action that decelerates the CoM on touchdown. As weaker muscles may not be able to attenuate the same loads as the stronger limb, this may result in a greater proportion of load being absorbed (demonstrated by greater vGRF) at the stronger limb. The greater loading rates experienced on the more explosive limb are logical when we consider that peak vGRF is greater on this limb.

The decrease in knee power at the more explosive limb may be largely explained by the decreased peak knee extensor moment on this side. However, while the more explosive quadriceps demonstrated decreased peak knee extension moment, and consequently, knee extensor moment rate, there was no difference in total impulse over the landing phase. This suggests that although the peak was smaller at the more explosive limb, the amount of force produced by the knee extensors during the entirety of the landing phase was similar to that of the less explosive quadriceps. Furthermore, as in the take-off phase of a CMJ, absolute loading BAIs were larger at the knee (BAI 15.7 – 26.4%), when compared to those in vGRF (BAI ~ 6%), suggesting that overall limb load asymmetry is reduced elsewhere in the loading chain. This is supported by the combination of decreased peak loading at the knee extensors of the explosively strong limb with greater peak vGRF (i.e. total limb load). This may be a result of compensations occur elsewhere in the kinetic chain: the knee may act primarily to coordinate the contributions of the ankle and hip, both of which are thought to be important in dissipating the load experienced early in landing (Rowley & Richards 2015). This in turn may explain the lack of correlations apparent between quadriceps strength asymmetries and knee loading asymmetries, and the large directional variability when variables are grouped by knee extensor strength. Alternatively, there is the possibility that the data analysed in this study was inappropriate to represent loading. For example, the use of EMG alongside loading analyses may allow the differentiation between reduced agonist, or increased antagonistic muscular activity, when interpreting reduced net joint moments.

Limb loading, and particularly loading rates, in landing are associated with injury (Gailey et al. 2008, Mundermann et al. 2005). It appears from the results of this study that the explosively strong and weak limbs may potentially perform different landing mechanics at both the knee joint and whole-limb level. Clinically, therefore, greater symmetry in explosive strength could potentially reduce injury risk in the weaker limb by more equal distribution of load relative to limb strength, resulting in landing which effectively mediates force bilaterally. Future research should look to explore the relationships between strength and loading asymmetries at other joints in the lower limb to explore this further.

5.5 Conclusion

This study investigated the association between loading asymmetries in a CMJ take-off and drop landing to maximal and explosive strength asymmetries in a healthy population with no training bias. vGRF asymmetries in both CMJ and drop landing were small (<10%), while knee loading BAIs were substantially larger (10 - 39%), suggesting that the role of the knee in both movements may be to coordinate the action of other joints in the kinetic chain (either proximally or distally to the knee) to produce a relatively symmetrical movement. The lack of correlations between strength and loading asymmetries in both CMJ take-off and landing from a drop suggests that, despite some of the inter-limb differences, these asymmetries are not proportional. The exception is the relationship between asymmetry in explosive strength and impulse in the lowering phase of take-off, although the correlation was weak. This may be a result of each individual performing movements in a different way within the constraints of their system – which may explain the large variability in each loading variable BAI_DIR. However, in landing, the significant differences between whole-limb (vGRF) and knee joint loading variables when organised by the explosively strong and weak limb suggest that symmetry in explosive strength may be important for loading symmetry, although the mechanisms of this are as yet unclear and require further investigation. The lack of associations between the two forms of asymmetry in jumping may be a result of the population tested not being habitually asymmetrical. It may be therefore that associations are more apparent in a pathological group with habitual asymmetry for both strength and movement in the same direction, such as ITTAs.

Chapter 6

The Association between Asymmetries in Strength and Gait in Unilateral Transtibial Amputees

6.1 Introduction

The loss of an ankle joint in individuals with unilateral transtibial amputations (ITTA) impacts their ability to walk. The ankle and surrounding musculature produce the majority of the propulsive power in gait (Winter & Sienko 1988), so ITTAs must adapt their motor control strategies to compensate for this loss of forward propulsion on one limb. As a result, walking speed – a key indicator of gait function – is often substantially slower in ITTAs compared to an able-bodied population (Bohannan 1997). Walking speed has been shown to be predictive of a range of outcomes in multiple populations including functional dependence (Purser et al. 2005, Shimada et al. 2013, Shinkai et al. 2000), institutionalisation (Woo et al. 1999) and falls (Montero-Odasso et al. 2005). In able-bodied populations, faster walking speeds are associated with longer strides (Murray et al. 1984). In ITTAs, the decreased strength of the amputated limb knee extensors when compared to the intact – i.e., the level of strength asymmetry – was most predictive of stride length, and as a result, walking speed (Powers 1996). Previous research in ITTAs has shown reduced amputated limb isometric and concentric knee extensor maximal muscle strength, resulting in asymmetries of 41 – 57% between limbs (Isakov et al. 1996, Renstrom et al. 1983, Pedrinelli et al. 2002, Lloyd et al. 2010). As muscle action at the knee is vital to coordinate mechanics of the ankle and hip throughout gait, and to help regulate joint movement through the eccentric control of knee flexion (Neptune et al. 2008) it is conceivable that strength asymmetry underpins walking speed. Currently, this has only been investigated in one study, which assessed habitual walking speeds only (Powers et al. 1996). As ITTAs should be encouraged to exercise, it is important that they can withstand increases in exercise intensity such as increased walking speed. Therefore, it would be beneficial to have a better understanding of the influence of muscular strength asymmetry on walking speed and overall gait function in ITTAs.

During fast limb movements, the short contraction time may not allow MVT to be reached (Krosshaug et al. 2007), and the ability to quickly increase torque is likely important. RTD has been shown to be important for postural corrections and balance (Behan, Pain & Folland 2018, Izquierdo 1999); fall avoidance (Pijnappels et al. 2008); and functional capacity in the elderly (Bassey et al. 1992); and positive outcomes in patients following total knee arthroplasty (Maffiuletti et al. 2010). In a control population, explosive strength asymmetry is of a similar magnitude to that of maximum strength, although more variable (Chapter 4). Currently, however, asymmetry in explosive strength has not been investigated in ITTAs, and relationships between asymmetries in RTD and gait in this population are unclear.

Chapter 5 provided evidence that in an able-bodied population, some associations were evident between asymmetries in the kinetics of landing and RTD, suggesting that explosive strength asymmetry may play a more important role in movement than maximum strength asymmetry. While strength asymmetry was not related to movement performance (i.e. jump height) in controls, the same may not be true of

ITTAs, who demonstrate larger, systematic asymmetries in both strength and movement. Indeed, evidence shows that ITTAs adapt to their partial limb loss by walking more asymmetrically (Sanderson & Martin 1997, Winter & Sienko 1988, Hak et al. 2013). In gait, ITTAs typically display reduced amputated limb stance time (Mattes et al. 2000, Nolan et al. 2003), stride length (Powers et al. 1996, Sanderson & Martin 1997), and contralateral (i.e. intact limb step length (Hak et al. 2014). Additionally, the amputated limb exhibits dec)reased peak vertical ground reaction force (vGRF; Mattes et al. 2000), and a reduction in knee extensor joint moments (Powers, Rao & Perry 1998, Silverman et al. 2008, Winter & Sienko 1988) when compared to the intact limb, suggesting reduced demand on the knee extensors to control knee flexion in braking on the amputated side. Thus, the asymmetrical knee kinetics and limb loading during gait (Beyaert et al. 2008, Schaarschmidt et al. 2012) may be a result of reduced amputated limb strength, requiring the intact limb to compensate to allow forward progression at a consistent pace. As increased strength asymmetry has previously been linked with altered gait mechanics in ITTAs (LaRoche et al. 2008, Lloyd et al. 2010, Powers et al. 1996) understanding how asymmetries in both MVT and RTD influence gait asymmetry may therefore be important in improving walking performance in ITTAs. This may be particularly important as muscular demand, and loading asymmetry, increases with increased walking speed (Nolan et al. 2003).

This study aimed to quantify the magnitude of asymmetries in knee extensor maximal and explosive strength in ITTAs, and to determine their relationship with walking speed and gait asymmetry for both habitual and fast walking speeds. This study also aimed to explore whether strength (maximal or explosive) was associated with changes in walking speed and gait asymmetry between habitual and fast walking speeds. It was hypothesised that (1) substantial asymmetries in maximum and explosive strength of the knee extensors would be present between the amputated and intact limbs, with the latter being greater; (2) gait asymmetries would be present at both walking speeds, but larger when walking fast; (3) greater strength asymmetries would be related to slower walking speeds and more asymmetrical gait patterns in unilateral ITTAs; and (4) gait asymmetries at faster walking speeds would be affected more than habitual speeds by larger strength asymmetries.

6.2 Methods

A comprehensive description participant recruitment, inclusion criteria and the methods followed for data collection and processing are given in Chapter 3, Sections 3.3 – 3.5.

6.2.1 Participant Information

Eight male ITTAs, who were moderately to highly physically active, took part in this study (Table 6.1). Participant inclusion and exclusion criteria, and detail about assessment of physical activity, can be found in Chapter 3, Section 3.2.

6.2.2 Data Collection

Briefly, participants visited the laboratory for 2 – 3 hours on three separate occasions, 3 – 7 days apart, to complete a familiarisation session, and two measurement sessions. The familiarisation session and first measurement session were identical and involved assessment of voluntary maximal and explosive muscle strength of the quadriceps (section *3.4.2.3 and 3.4.2.5*). Neuromuscular measures were taken on both legs and the first leg tested was randomised for each person but remained consistent between sessions. The second measurement session involved the collection of kinetic and kinematic data during walking at habitual and fast speeds (section *3.5.4.3*).

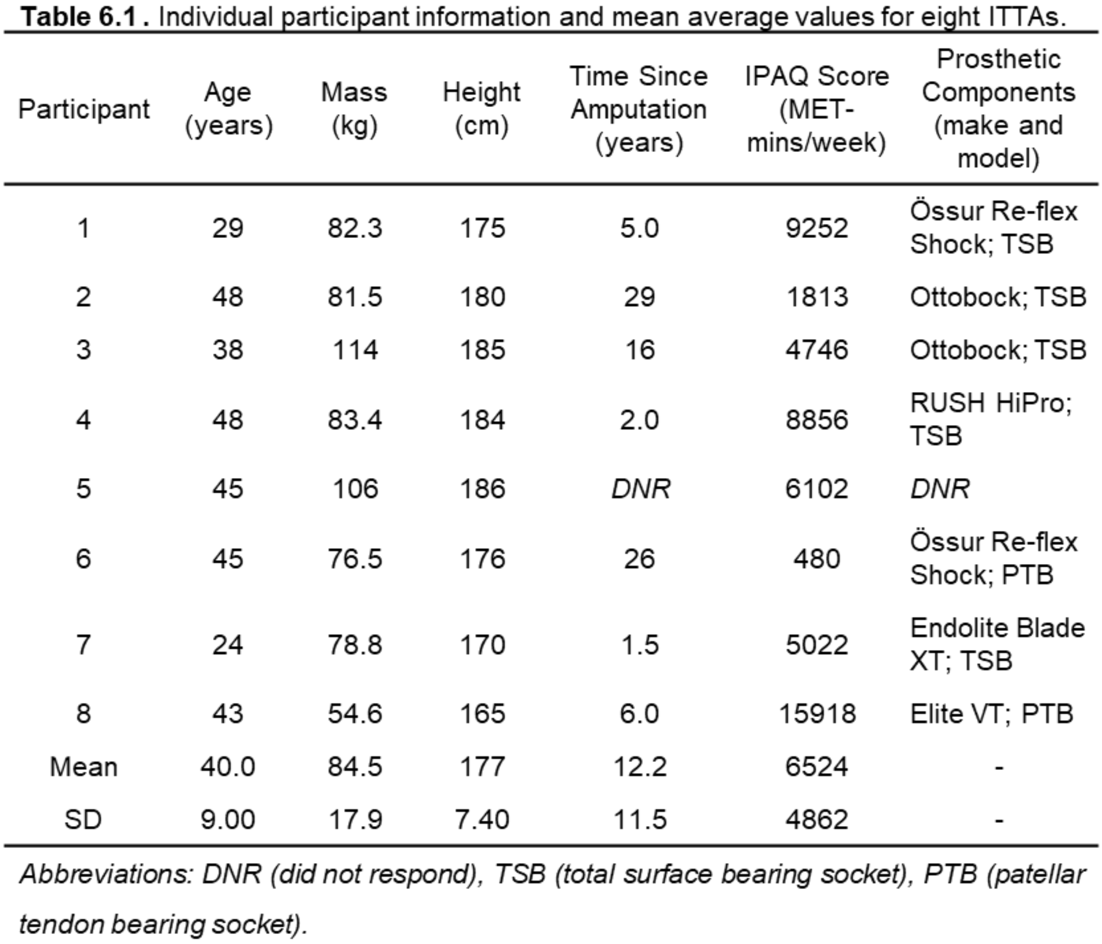


Table 6.1 . Individual participant information and mean average values for eight ITTAs.

Participant	Age (years)	Mass (kg)	Height (cm)	Time Since Amputation (years)	IPAQ Score (MET-mins/week)	Prosthetic Components (make and model)
1	29	82.3	175	5.0	9252	Össur Re-flex Shock; TSB
2	48	81.5	180	29	1813	Ottobock; TSB
3	38	114	185	16	4746	Ottobock; TSB
4	48	83.4	184	2.0	8856	RUSH HiPro; TSB
5	45	106	186	*DNR*	6102	*DNR*
6	45	76.5	176	26	480	Össur Re-flex Shock; PTB
7	24	78.8	170	1.5	5022	Endolite Blade XT; TSB
8	43	54.6	165	6.0	15918	Elite VT; PTB
Mean	40.0	84.5	177	12.2	6524	-
SD	9.00	17.9	7.40	11.5	4862	-

Abbreviations: DNR (did not respond), TSB (total surface bearing socket), PTB (patellar tendon bearing socket).

6.2.3 Data Extraction and Analysis

Further detail on specific data processing is given in sections *3.4.2.2* (maximal strength); *3.4.2.4* (explosive strength); and 3.5.5 (gait).

Strength data analysed were MVT and peak voluntary RTD, expressed as both an absolute value and relative to body mass (BM). Temporospatial (TS) gait parameters analysed were: cadence (steps per minute), single support time and step length (defined by the limb being stepped onto; Figure 6.1). Walking speed (calculated in Vicon Nexus) was extracted for each trial analysed, and the change in speed from habitual to fast (ΔSpeed) was calculated as

$$\Delta\text{Speed} = \text{Walking Speed}_{Fast} - \text{Walking Speed}_{Habitual} \quad (6.1)$$

Peak knee extension moments and net knee extension moment impulse were extracted from braking, while peak vGRF was calculated for the braking and propulsive phases individually. Values were recorded for each limb and averaged across the three trials selected for analysis. The braking (~0 – 50%) and propulsion (~50 – 100%) phases of stance were determined using the antero-posterior ground reaction forces (hGRF) – the first instance of positive hGRF indicating the end of the braking phase and beginning of propulsion (Figure 6.2).

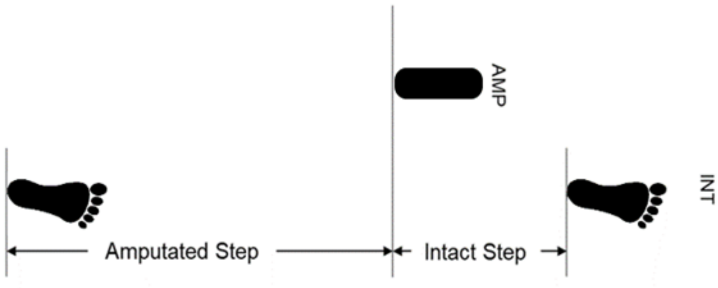


Figure 6.1. Illustration of amputated and intact step length. Note that step length is defined by the limb being stepped onto.

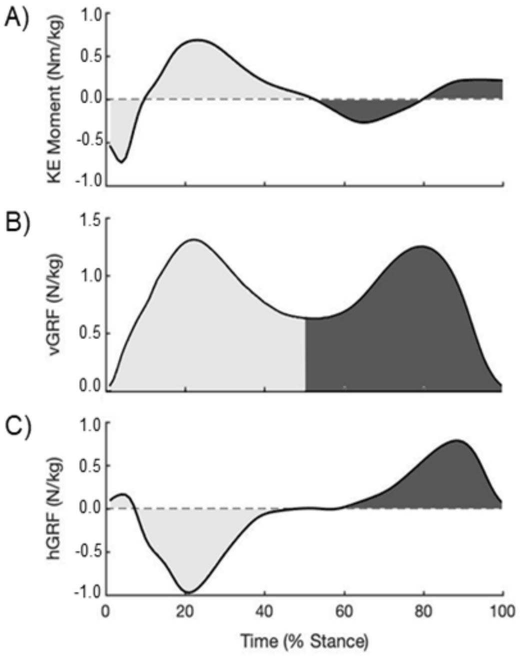


Figure 6.2. (A) Sagittal plane knee moments, (B) vertical ground reaction forces (vGRF) and (C) antero-posterior ground reaction forces (hGRF) during the stance phase of level walking gait for an example intact limb trial, expressed relative to body weight (BW). Positive values indicate knee extension moments. Shaded areas correspond to the braking (light grey) and propulsive (light grey) phases. Peak vGRF was recorded for both phases.

6.2.3.1 Asymmetry Calculation

Bilateral Asymmetry Index (BAI; Bishop et al. 2018) was generated for each variable of interest (x) using the formula

$$BAIx(\%) = \frac{(Intact_x - Amputated_x)}{(Intact_x + Ampuated_x)} \times 100 \tag{6.2}$$

resulting in a negative value if a greater value for a given variable was recorded on the amputated limb than the intact limb. A BAI of zero indicates perfect symmetry. The difference in BAI from habitual to fast walking speeds (ΔBAI) for a given variable was calculated as

$$\Delta\text{BAIx} = \text{BAI\_Fast}_x - \text{BAI\_Habit}_x \tag{6.3}$$

where BAI_Fast and BAI_Habit are the BAI for a given variable (x) at fast and habitual speeds, respectively.

6.2.3.2 Statistical Analysis

Data for the individual limbs (amputated and intact) and BAIs were reported as mean ± SD. Statistical analysis was completed using SPSS version 24, and the significance level was set at $p < 0.05$.

Levene’s test was used to check for equality of variances, and Shapiro-Wilkes assessed normality of the data prior to running all analyses. To determine if limbs were asymmetrical for strength variables, paired *t*-tests compared MVT and peak RTD between the intact and amputated limb. To assess if there was a difference in the magnitude of maximum and explosive strength asymmetries, BAI MVT vs. BAI RTD was also compared. To determine if gait asymmetries were present at either walking speed, a two-way ANCOVA was used to assess the effects of limb (amputated vs intact) and walking speed (habitual vs fast) on each gait parameter, while controlling for the variation in self-selected walking speed within each speed group. In the instance of a main or interaction effect, post-hoc Bonferroni corrected paired *t*-tests were performed to compare limbs at each speed. To determine whether there was a difference in the size of asymmetry between walking speeds, paired *t*-tests compared the magnitude of BAI for each dependent variable at habitual vs. fast walking speeds. Effect size (Hedges g, incorporating correction for small sample bias) was calculated for all comparisons, and interpreted as small (g = 0.2 – 0.5), medium (g = 0.5 – 0.8) and large effects (g > 0.8; Lakens 2013). Where variables were greater in the amputated than intact limb, g was correspondingly a negative value.

To determine the relationships between strength asymmetry and walking speed, Pearson's product moment correlations between BAI MVT and RTD were performed with (1) habitual and fast walking speeds; and (2) ΔSpeed. To determine if strength asymmetry was associated with gait asymmetry, correlations between BAI MVT and RTD were performed with both the BAI and ΔBAI for each gait variable. To ensure these latter relationships were independent of self-selected walking speed, the criteria outlined in Table 6.2 were used to select the type of correlation (i.e. bivariate, semi-partial, or partial). All relationships were interpreted as strong (r > 0.7), moderate (r = 0.5 – 0.7), weak (r = 0.3 – 0.5) or non-existent (r < 0.3; Mukaka 2012).

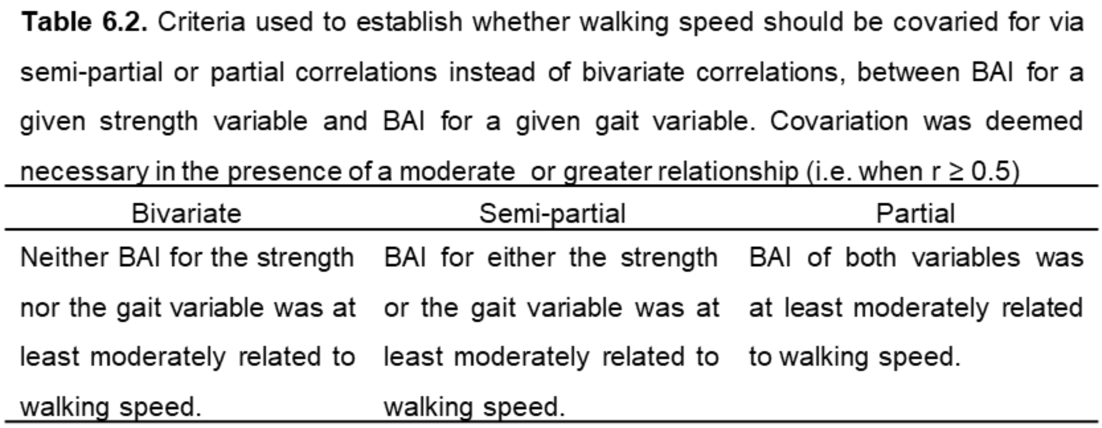


Table 6.2. Criteria used to establish whether walking speed should be covaried for via semi-partial or partial correlations instead of bivariate correlations, between BAI for a given strength variable and BAI for a given gait variable. Covariation was deemed necessary in the presence of a moderate or greater relationship (i.e. when $r \geq 0.5$)

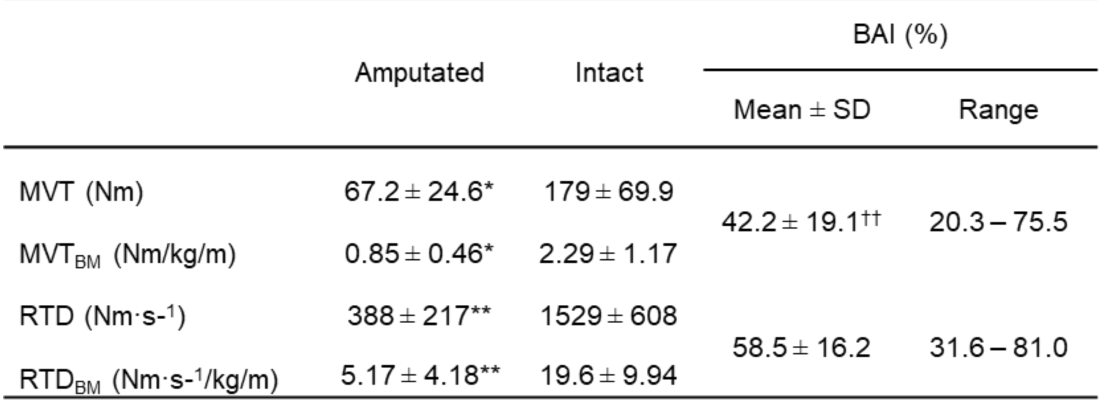
Bivariate	Semi-partial	Partial
Neither BAI for the strength nor the gait variable was at least moderately related to walking speed.	BAI for either the strength or the gait variable was at least moderately related to walking speed.	BAI of both variables was at least moderately related to walking speed.

6.3 Results

6.3.1 Maximal and Explosive Strength Asymmetries

There were considerable strength asymmetries, with both MVT ($p = 0.003$, g = 2.01) and RTD ($p < 0.001$, g = 2.36) being significantly greater in the intact compared to the amputated limb. Additionally, the asymmetry in RTD was significantly greater than the asymmetry in MVT ($p < 0.001$, g = 2.67; Table 6.3).

Table 6.3. Absolute, relative (to bodymass, BM) and BAI values for isometric MVT and peak RTD of the knee extensors in the amputated and intact limbs of unilateral transtibial amputees. Data are presented as mean ± SD (n = 8). Paired differences between the amputated and intact limbs are denoted by * ($p < 0.05$) or ** ($p < 0.001$), and between BAI MVT and BAI RTD by †† ($p \leq 0.001$).



	Amputated	Intact	BAI (%)	
			Mean ± SD	Range
MVT (Nm)	67.2 ± 24.6*	179 ± 69.9	42.2 ± 19.1††	20.3 – 75.5
MVT_{BM} (Nm/kg/m)	0.85 ± 0.46*	2.29 ± 1.17		
RTD ($Nm·s^{-1}$)	388 ± 217**	1529 ± 608	58.5 ± 16.2	31.6 – 81.0
RTD_{BM} ($Nm·s^{-1}$/kg/m)	5.17 ± 4.18**	19.6 ± 9.94		

BAI, Bilateral Asymmetry Index; MVT, maximum voluntary torque; RTD, rate of torque development.

6.3.2 Walking Speed and Gait Asymmetry

Self-selected fast walking speeds (1.70 ± 0.20 m/s) were significantly greater than habitual walking speeds (1.33 ± 0.15 m/s; $p < 0.001$, g = 1.88). Additionally, cadence was significantly greater at fast (124 ± 11.4 steps/min) when compared to habitual walking (109 ± 9.11 steps/min; $p < 0.001$, g = 1.41). Amputated and intact limb values and BAI for all gait parameters are presented in Table 6.4.

At habitual walking speeds, single support time was significantly longer in the intact limb ($p = 0.001$, g = 0.93), while step length was significantly greater in the amputated vs. intact limb ($p = 0.006$, g = 1.41). During fast walking, step length remained significantly greater in the amputated compared to the intact limb ($p < 0.001$, g = 1.26), but single support time was similar between limbs ($p = 0.218$, g = 0.32; Table 6.4).

Peak vGRF was significantly lower in the amputated compared to the intact limb in both phases of stance at habitual (braking: $p = 0.010$, g = 1.59; propulsion: $p = 0.002$, g = 2.18) and fast walking speeds (braking: $p = 0.002$, g = 1.59; propulsion: $p < 0.001$, g = 2.68; Table 6.4).

Knee moment waveforms throughout stance are presented in Figure 6.3. Significant differences were apparent in the braking phase at both walking speeds, between the amputated and intact limbs in peak knee extensor moment (habitual: $p = 0.002$, g = 1.72; fast: $p < 0.001$, g = 3.45; Table 6.4) and impulse (habitual: $p = 0.008$, g = 1.56; fast: $p < 0.001$, g = 2.80).

There were no significant differences in the size of BAI between habitual and fast walking speeds for any gait variable ($p = 0.085 – 0.365$; g = 0.16 – 0.46).

Table 6.4. BAI in temporo-spatial and kinetic parameters of gait between the amputated (AMP) and intact (INT) limb of unilateral transtibial amputees (n = 8), walking at a self-selected habitual and fast speed. Data are presented as mean ± SD. Paired differences between the amputated and intact limbs within a given walking speed are denoted by * ($p < 0.05$) or ** ($p < 0.001$).

	Habitual			Fast		
	AMP	INT	BAI (%)	AMP	INT	BAI (%)
Temporo-spatial parameters						
Single support time (s)	0.41 ± 0.03**	0.44 ± 0.03	3.65 ± 2.40	0.38 ± 0.05	0.39 ± 0.05	2.35 ± 4.62
Step length (m)	0.78 ± 0.05*	0.69 ± 0.07	-6.13 ± 2.34	0.86 ± 0.07**	0.78 ± 0.06	-5.03 ± 1.62
Braking Phase Kinetic parameters						
Peak KE Moment (Nm/kg)	0.27 ± 0.15*	0.68 ± 0.28	46.4 ± 24.7	0.32 ± 0.12**	1.10 ± 0.28	54.5 ± 14.3
KE Impulse (Nm/kg·s)	0.0084 ± 0.0059*	0.0224 ± 0.0104	47.4 ± 35.4	0.0076 ± 0.0038**	0.0305 ± 0.0102	60.6 ± 15.3
Peak vGRF (N/kg)	10.5 ± 0.70*	12.3 ± 1.36	7.76 ± 6.27	10.9 ± 1.50*	13.6 ± 1.64	10.8 ± 6.44
Propulsive Phase Kinetic parameters						
Peak vGRF (N/kg)	9.51 ± 0.89*	11.5 ± 0.87	9.69 ± 5.68	9.60 ± 0.99**	12.1 ± 0.80	11.8 ± 4.44

BAI, Bilateral Asymmetry Index; KF, knee flexion; vGRF, vertical ground reaction force

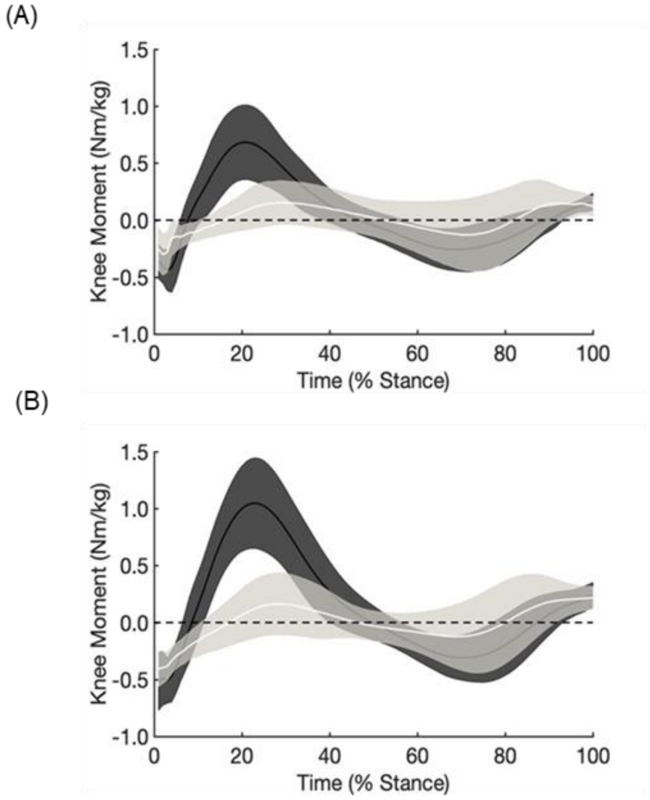


Figure 6.3. Sagittal plane knee moments during the stance phase of (A) habitual and (B) fast walking for the amputated (AMP, light grey line) and intact (INT, dark grey line) limbs of unilateral transtibial amputees. Knee joint moment is expressed as internal moment relative to body mass (BM). Positive and negative values indicate knee extension and flexion moments, respectively. Data are presented as mean ± SD for n = 8 (AMP and INT).

6.3.3 Relationships between Strength Asymmetry and Walking Speed

When considering the relationship between strength asymmetry and walking speed, weak, non-significant relationships were evident between BAI for MVT_{BM} and both habitual and fast walking speeds (Figure 6.4A and C). In contrast, there were strong significant negative relationships between BAI RTD_{BM} and both habitual and fast walking speeds (Figure 6.4B and D); i.e. at both speeds, amputees with greater asymmetry in RTD walked more slowly. Moderate, but non-statistically significant, negative relationships were observed between the difference in walking speed between habitual to fast (∆speed), and both BAI MVT_{BM} and BAI RTD_{BM} (Figure 6.4E and F), thus those with greater strength asymmetries had less ability to increase their walking speed from habitual to fast.

6.3.4 Relationships between Strength and Gait Asymmetries

6.3.4.1 TS Parameters

At habitual walking speed, the relationships between asymmetries in TS parameters of gait and knee extensor strength were weak to non-existent (Table 6.5). However, at a fast walking speed, BAI single support time was moderately related to BAI MVT_{BM} but not to BAI RTD_{BM} (i.e. those with greater maximum strength asymmetries displayed greater asymmetries in single support time), whilst BAI step length was moderately related to BAI RTD_{BM}, but not to BAI MVT_{BM} (Table 6.5). The latter relationship suggests that step length (which was negative; Figure 6.4B) became more symmetrical with increased explosive strength asymmetry.

When the difference in walking speed between habitual and fast walking was considered, there was a strong positive relationship between ΔBAI single support time and BAI MVT_{BM}, but only a weak relationship between ΔBAI step length and BAI MVT_{BM}. This implies that with greater maximum strength asymmetries, the asymmetry in single support time increased by a larger amount when walking speed changed from habitual to fast. The same held true when considering explosive strength asymmetry: both ΔBAI single support time and ΔBAI step length were moderately correlated with BAI RTD_{BM} (Table 6.5).

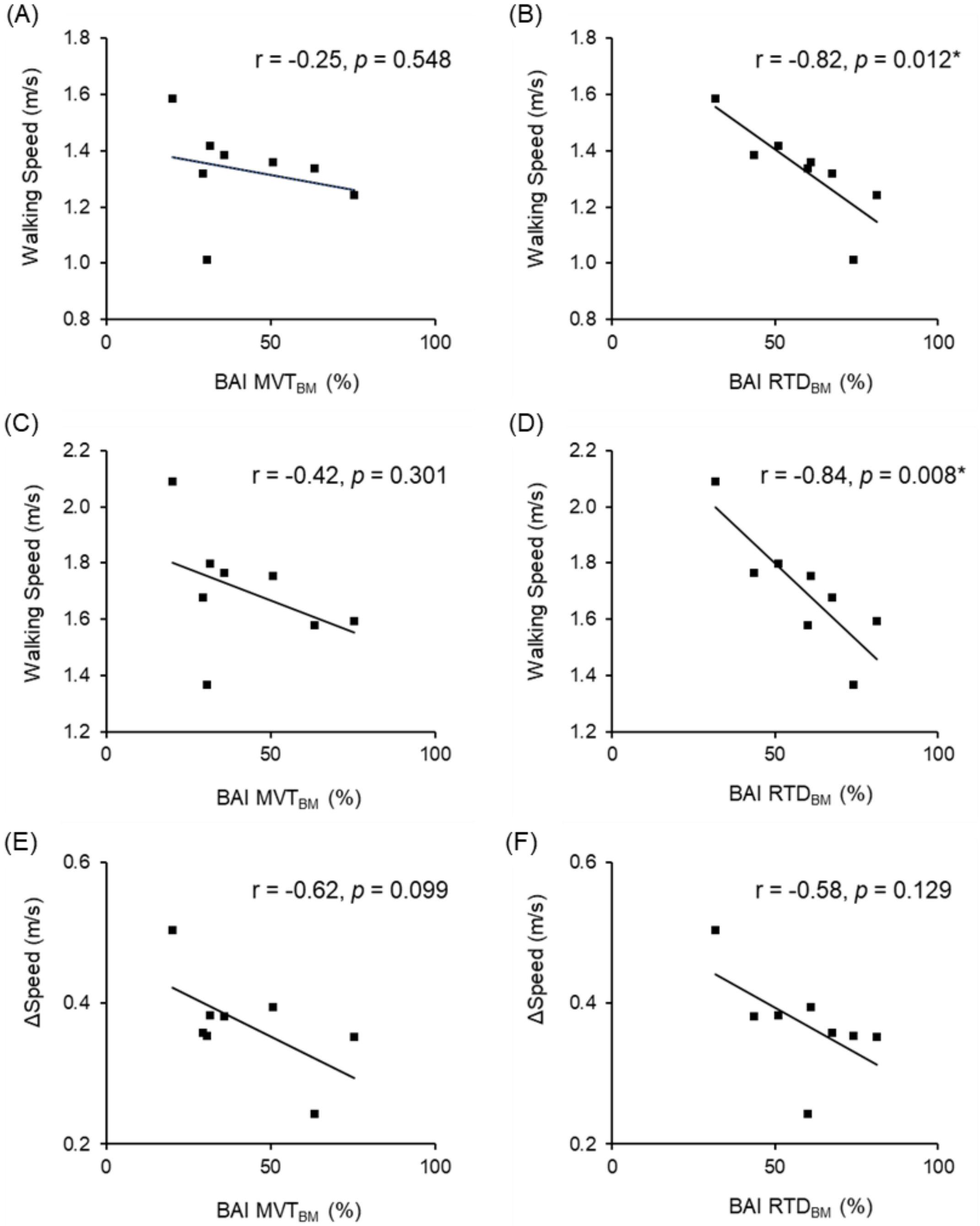


Figure 6.4. The relationships between walking speed and asymmetry in both maximal voluntary torque (BAI MVT_{BM}) and rate of torque development (BAI RTD_{BM}) of the knee extensors, in unilateral transtibial amputees. Walking speed was assessed during both self-selected habitual (A and C) and fast (B and D) speeds. Change in walking speed from habitual to fast walking (ΔSpeed; E and F) was also evaluated.

6.3.4.2 Kinetic Parameters

When ITTAs walked at their everyday habitual pace, BAI MVT_{BM} was either not correlated, or correlated weakly with BAIs for all kinetic parameters in both braking and propulsive phases of gait (Table 6.5). However, moderate negative relationships were apparent for BAI RTD_{BM} with BAI peak knee extension moment and impulse in the braking phase (Table 6.5), suggesting that as asymmetry in explosive strength increased, knee extension impulse became more symmetrical. Relationships between BAI RTD_{BM} and other kinetic variable BAIs at habitual walking speeds were weak or non-existent (Table 6.5).

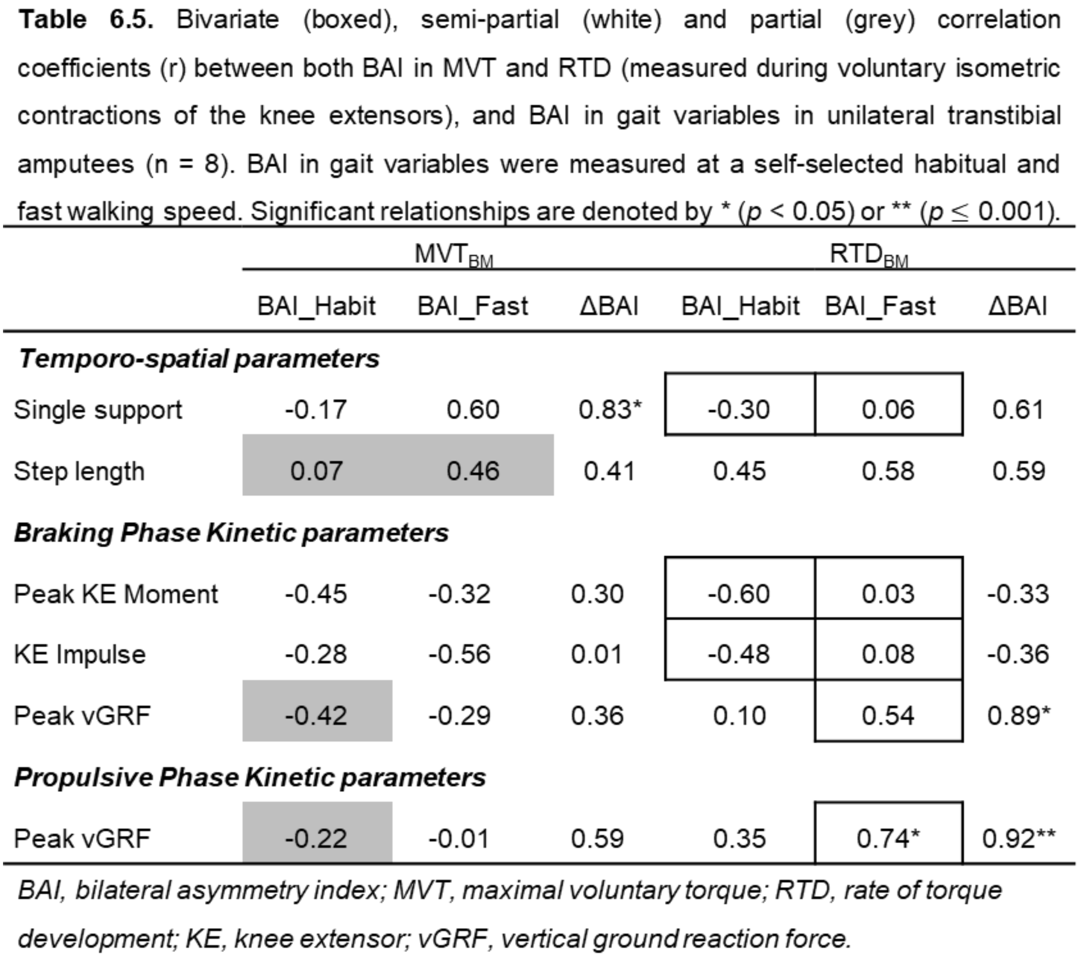


Table 6.5. Bivariate (boxed), semi-partial (white) and partial (grey) correlation coefficients (r) between both BAI in MVT and RTD (measured during voluntary isometric contractions of the knee extensors), and BAI in gait variables in unilateral transtibial amputees (n = 8). BAI in gait variables were measured at a self-selected habitual and fast walking speed. Significant relationships are denoted by * ($p < 0.05$) or ** ($p \leq 0.001$).

	MVT_{BM}			RTD_{BM}		
	BAI_Habit	BAI_Fast	ΔBAI	BAI_Habit	BAI_Fast	ΔBAI
Temporo-spatial parameters						
Single support	-0.17	0.60	0.83*	-0.30	0.06	0.61
Step length	0.07	0.46	0.41	0.45	0.58	0.59
Braking Phase Kinetic parameters						
Peak KE Moment	-0.45	-0.32	0.30	-0.60	0.03	-0.33
KE Impulse	-0.28	-0.56	0.01	-0.48	0.08	-0.36
Peak vGRF	-0.42	-0.29	0.36	0.10	0.54	0.89*
Propulsive Phase Kinetic parameters						
Peak vGRF	-0.22	-0.01	0.59	0.35	0.74*	0.92**

BAI, bilateral asymmetry index; MVT, maximal voluntary torque; RTD, rate of torque development; KE, knee extensor; vGRF, vertical ground reaction force.

During fast walking, BAI MVT_{BM} was moderately related to BAI knee extensor moment impulse during the braking phase (Table 6.5), but weakly, or not, related to BAIs for all other measured kinetic variables during gait; thus, at fast speeds, those with greater maximum strength asymmetries displayed decreased knee extension impulse asymmetry in braking. In contrast, BAI RTD_{BM} was moderate to strongly correlated with the BAI for peak vGRF in both the braking and propulsive phase of gait. Weak or non-existent relationships were evident between BAI RTD_{BM} and other BAIs for kinetic variables during fast walking (Table 6.5).

When considering the difference in walking speed between habitual and fast, there was a moderate positive correlation between ΔBAI peak propulsive vGRF and BAI MVT_{BM}, whilst there were strong positive relationships between BAI RTD_{BM} and ΔBAI peak vGRF during both braking and propulsive (Table 6.5) phases. Thus, those with greater strength asymmetries had greater increases in vGRF asymmetries when they walked at a faster speed compared to their habitual speed. BAI MVT_{BM} and BAI RTD_{BM} were both weakly related to ΔBAI for other kinetic variables**.**

6.4 Discussion

This study provides novel evidence that, in addition to significant asymmetry in knee extensor maximal strength (42%), substantially larger asymmetry was present in explosive strength (59%) in ITTAs. Explosive strength asymmetry was directly related to walking speed, and both forms of strength asymmetry were related to the ability to increase self-selected walking speed from habitual to fast. As walking speed is a key indicator of gait function, this finding is fundamental for the promotion of health, exercise and quality of life in ITTAs. Furthermore, relationships between asymmetries in strength and single support time suggest that some aspects of ITTA gait become more asymmetrical with larger between-limb strength differences. Strength asymmetry was also related to some kinetic asymmetry, in particular, change in vGRF

BAI with faster walking speeds. This suggests that those ITTAs with larger strength BAIs are more asymmetrical in loading with faster walking speeds, which may have implications for the development of degenerative neuromuscular conditions – such as osteoarthritis – which are potentially related to loading asymmetries and are highly prevalent in this population.

6.4.1 Strength Asymmetry and Walking Speed

The amputated limb knee extensors of the ITTAs that participated in this study consistently performed with reduced maximal and explosive strength than the intact limb, resulting in substantial strength asymmetries. Asymmetry between limbs reported in this study of 42% for MVT (Table 6.3) is much greater than that found in able-bodied controls (10%; Chapter 4) but of a smaller magnitude to that found in previous amputee research, where decreases in maximum strength of the amputated limb resulted in asymmetry (BAI) of up to 57% (Isakov et al. 1996a, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Renstrom, Grimsby & Larsson 1983). This may be in part because some of these earlier studies measured concentric, not isometric, MVT, but potentially the increased activity levels of our ITTA also contributed. No previous research has examined the asymmetries in explosive strength in ITTAs. In this study we found significantly larger asymmetry in explosive (BAI 59%, Table 6.3) compared to maximal strength in the knee extensors of ITTAs, and explosive strength was significantly related to a greater number of gait asymmetry features. In other populations, past research has reported similar findings, for example, substantial deficits in RTD but not MVT six months in the involved limb post-ACL reconstruction (Angelozzi et al. 2012); positive associations between RTD, but not MVT, and patient-reported outcomes and walking speed post-joint arthroplasty (Cobian et al. 2017, Suetta et al. 2007); and relationships between asymmetry in RTD, but not MVT, and subjective knee function in total knee arthroplasty (Maffiuletti et al. 2010). Furthermore, Chapter 4 found that RTD asymmetry in able-bodied populations was more variable than that in MVT, despite similar magnitudes. Currently, knee extensor MVT strength assessment is used as the gold standard to identify muscle weakness in multiple populations, often in the form of an asymmetry index (e.g. LaRoche et al. 2008, Palmieri-Smith & Lepley 2015, Rannama et al. 2015). However, our results of greater BAI for RTD suggest it is a more sensitive measure of functional capacity in ITTAs and should perhaps be assessed as well as, if not instead of, MVT BAI. This is further highlighted by the relationship seen between explosive, but not maximal, strength asymmetry and walking speed – a key measure as an indicator of walking ability in multiple populations (van Velzen et al. 2006). However, to the author's knowledge no previous studies have investigated the association between knee extensor explosive strength asymmetry and walking speed in ITTAs. Whilst cause and effect cannot be assumed from correlation, the results of this study suggest that explosive strength asymmetry is a significant limiting factor for functional capacity during walking in this population (Figure 6.4B, D & F). The importance of MVT symmetry for walking speed is less clear; while at habitual and fast walking speeds correlations with BAI MVT were only small-to-moderate (Figure 6.4A & C), there was a moderate association with change in walking speed from habitual to fast (Figure 6.4E). Smaller asymmetry in both maximal and explosive strength may therefore enable ITTAs to better increase their walking speed, which is fundamental for exercise and daily living.

6.4.2 Influence of Strength Asymmetry on Gait

Significant differences in single support time were evident between the amputated and intact limbs at habitual, but not fast, walking speeds (Table 6.4). This supports the work of Lloyd et al. (2010), who reported that while ITTAs tend to spend less time in single support on the amputated limb, and experience prolonged load transfer from the intact onto the amputated limb, asymmetries in single support time decrease with increasing speed. Despite this, no relationships were apparent between asymmetries in single support time and RTD. Instead, asymmetries in single support time during fast walking were moderately correlated with asymmetry in maximum strength, and strongly related to the difference in speed between habitual and fast walking (Table 6.5). This suggests that maximum, rather than explosive, strength asymmetry of the knee extensors is more important in an ITTA's ability to sustain their body weight on a single limb when walking at speeds greater than their habitual pace. Previous research has demonstrated significant increases in support contributions from the knee extensor muscles with increases in walking speed from habitual to fast (Fey, Silverman & Neptune 2010, Liu et al. 2008). In the presence of knee extensor strength asymmetry in ITTAs, it follows that as the muscular demand of gait increases via increased walking speeds, the weaker amputated limb is less able to perform its role in supporting the body during single support. As a result, the ITTA would spend less time in this phase on the amputated limb, which in turn would lead to such asymmetries in single support time between the limbs as reported in this study.

ITTAs were observed to have a significantly reduced step length on the intact compared to amputated limb (and therefore negative BAIs; Table 6.4) which supports the findings of past research (Barnett et al. 2009, Isakov et al. 1996, Mattes et al. 2000). While this pattern was evident at both speeds, the effect was greater in fast (g = 1.26) when compared to habitual walking (g = 0.93). Previous research has found increases in step times (which combine a period of single support and its preceding double support), with increasing speed (Lloyd et al. 2010). This was thought to reflect the increasing muscular and loading demands of ITTA walking at higher speed, and may partially explain the results of the current study. At habitual walking speeds, relationships between strength and gait asymmetries were minimal; however, stronger associations became apparent at fast speeds, and/or when considering the difference between fast and habitual speeds (ΔBAI). These asymmetries in step length were moderately related to BAI RTD in fast walking (although not habitual) and in the difference in walking speed between habitual and fast (Table 6.5). The increase in step length symmetry with increased explosive strength asymmetry may be a result of adaptations ITTAs perform when walking to maintain a stable gait. Previous research has found evidence that both able-bodied individuals and ITTAs increase walking speed by increasing step frequency and step length (Roerdink et al. 2012). However, as shorter steps are thought to be more stable because the CoM is closer to the moving base of support (Epsy et al. 2010); it may therefore be that in order to maintain gait stability, ITTAs preferentially increase their step frequency, but not step length, when walking faster. Furthermore, links between decreased rapid force production capabilities and poor control of postural sway have been found (Izquierdo et al. 1999); when considered in relation to explosive strength asymmetry, therefore it could be that those ITTAs with larger strength asymmetries increase their step frequency (but not length) to maintain stability while reducing the load borne by the amputated limb when stepping onto the intact. In contrast, those with more symmetrical explosive strength may be able to take larger steps as their increased amputated limb strength means that they are able to sustain additional loads on this limb. In combination with a constant step frequency, this also helps to explain why those ITTAs who are more symmetrical in explosive strength walk faster.

Asymmetries in limb loading of up to 11% (peak vGRF braking phase) and 12% (peak vGRF propulsive phase; Table 6.4) present in this study were of a comparable magnitude to those reported previously in ITTAs (4-10%; e.g. Menard et al. 1992, Powers 1994, Sanderson & Martin 1997) but substantially larger than those reported in an able-bodied population (LSI_3 ~0.05% [see Appendix A for calculation]; Polk et al. 2017), demonstrating that ITTAs load their amputated limb less than the intact in these phases during gait. There was no difference in the magnitude of the asymmetry in any loading variables in either braking or propulsion phases between the two walking speeds. This supports the work of previous authors who have reported that while faster walking significantly affected gait parameters at the individual limbs in ITTAs, the level of asymmetry between them remained unchanged (Isakov et al. 1996, Nolan et al. 2003, Silverman 2008). Despite this, the correlations between BAIs in strength and vGRF highlight the variability in individual responses. In the braking phase, moderate to strong relationships were evident between asymmetries in RTD and peak vGRF in fast walking and as speed changed, suggesting that more symmetrical explosive strength may allow the amputated limb to become more involved as walking demand increases. Furthermore, in propulsion, strong significant relationships were present between asymmetries in explosive strength and peak vGRF during fast walking. As speed changed from habitual to fast, peak propulsive phase vGRF was also related strongly to BAI RTD, and moderately with BAI MVT (Table 6.5). This suggests that, when increasing demand by changing pace and/ or walking faster, ITTAs with greater strength symmetry are more likely to experience more symmetrical loads, implying that the amputated limb may become more involved in the generation of propulsive force. This is an important consideration, particularly in those ITTAs who aim to be more active and need to withstand increases in exercise intensity such as increased walking speed, due to the link between loading asymmetry and the high prevalence of degenerative neuromuscular conditions in this population (Macfarlene et al. 1991, Lloyd et al 2010, Schmalz et al. 2001).

This study also examined asymmetry in knee joint kinetics in gait. Significant differences were apparent between limbs in both peak knee extension joint moment and total knee extension impulse in the braking phase, resulting in substantial BAIs at both speeds in early stance. This agrees with the findings of Sanderson & Martin (1997) who reported that in braking, amputated limb knee moment was reduced to the point that it remained flexor during the entirety of early stance. There were no significant increases in the size of asymmetry in knee kinetics in braking when habitual and fast walking were compared, which is consistent with the findings of Silverman et al. (2008). Unlike previous research, this study additionally assessed the relationship of knee kinetics to knee extensor strength; several negative associations were found between BAIs in strength and knee kinetic variables. While logically we might expect the reverse to be true (i.e., increased strength asymmetry associated with increased kinetic asymmetry), these relationships could be a result of increased co-contraction of the amputated limb hamstrings in ITTAs with smaller strength BAIs. Such muscular activity has been observed in previous studies (Isakov et al. 2001, Mattes et al. 2001, Powers et al. 1998, Silverman et al. 2008) to aid braking and provide increased propulsion (Liu et al. 2006, Neptune, Zajac & Kautz 2004) but with the result of reducing the net knee joint moments.

6.5 Conclusion

Significant strength asymmetries were found in ITTAs – importantly, explosive strength asymmetry, which has not previously been measured in this population, was substantially larger than the asymmetry in maximal strength. Additionally, knee extensor explosive strength asymmetry was an excellent predictor of walking speed, and asymmetries in both MVT and RTD were associated with the ability to increase walking speed in this population, which is vital for health, exercise and quality of life. When related to movement, those ITTAs with greater symmetry in maximal, and more particularly, explosive strength of the knee extensors showed greater symmetry in single support time and whole-limb loading, although this was not true for all conditions. This knowledge may be useful in the design of rehabilitation programmes for ITTAs, where neuromuscular training to target increases in maximal and explosive strength and muscle size may be incorporated, with the goal of improving functional gait capacity, and decreasing the risk of degenerative neuromuscular conditions in this population. Furthermore, the substantial asymmetries in both maximal and explosive strength, together with those of gait, in this population of ITTAs allows us a novel opportunity. Namely, by investigating the neuromuscular characteristics of their amputated and intact limbs, we may be able to answer some broader physiological questions, such as the effect of long-term disuse on strength and neuromuscular function.

Chapter 7

The Effects of Long-Term Disuse on Neuromuscular Function in Unilateral Transtibial Amputees

7.1 Introduction

Chapter 6 showed large strength asymmetries in ITTAs that were closely associated with gait asymmetry. Previous studies have also shown substantial asymmetries in ITTA movement: during movements such as walking, jumping, and stair ascent/ descent, they adopt an asymmetrical loading pattern characterised by considerably shorter stance time, lower vGRFs, and knee extensor moments on the amputated compared to the intact limb (Fey & Neptune 2012, Schmalz, Blumentritt & Marx 2007, Schoeman, Diss & Strike 2012). Collectively, these results suggest that the quadriceps of the amputated limb are chronically disused. While cause and effect is hard to pinpoint, it is likely a vicious cycle, with muscular weakness leading to reduced use and movement asymmetry. This is then likely to result in further disuse and muscular maladaptations. It is therefore not surprising that studies on ITTAs have observed considerably lower (~50%) quadriceps maximum strength (Isakov et al. 1996, Lloyd et al. 2010, Pedrinelli et al. 2002) and size (Moirenfeld et al. 2000) in the amputated, compared to the intact and control limbs. Comparison of quadriceps neuromuscular function in the amputated vs. intact limb of ITTAs may therefore offer new insight into the long-term effects of habitual disuse.

Prolonged disuse of skeletal muscle poses a considerable threat to muscle tissue and neuromuscular function (Narici & de Boer 2011) and as a result, functional capacity and health (Kortebein et al. 2008). Just nine days of disuse causes observable declines in maximum strength (Rozier et al. 1979), and within two weeks, deficits in explosive strength are apparent (Bamman et al. 1998). As reduced or limited physical activity is implicated in multiple clinical populations, continuous muscle disuse is a default position for many (Brown et al. 2004), thus it is important to understand the effects of long-term disuse on both maximum and explosive strength, and their neuromuscular determinants.

The knee extensor muscles are particularly susceptible to degenerative changes resulting from disuse (Campbell et al. 2019) owing to their large contributions to locomotion on land, and so are frequently investigated in typical study models of disuse including spaceflight (e.g. Tesch et al. 2005), unilateral lower-limb suspension (ULLS; e.g. Campbell et al. 2013), limb immobilisation (e.g. Deschenes et al. 2002) and bed rest (e.g. Berg et al. 1997). Studies show reductions in quadriceps maximum strength of approximately 2% per day for the first ten days (Berg & Tesch 1996, Gamrin et al. 1998, Rozier et al. 1979, Thom et al. 2001), slowing to ~1% per week up to 30 days, with an eventual plateau resulting in average strength losses of around 23% after 120 days of disuse (Dirks et al. 2013, 2016, Horstman et al. 2012, Narici & de Boer 2011, Suetta et al. 2012, Wall et al. 2013). Unfortunately, it is unclear how maximum strength may change with more long-term, habitual disuse, as typical disuse study models last <90-120 days for logistical and ethical reasons. Furthermore, explosive strength has not been widely studied in disuse studies. In order to further explore the mechanisms of reduced maximum and explosive strength with chronic unloading, there is therefore a need for a study model with an internal control independent from the effects of ageing and disease states.

ITTAs may provide a useful model for studying the effects of chronic disuse. However, currently it is unclear whether the intact limb provides an internal control that is unaffected by the amputation and comparable to the limb of an able-bodied control, which would support the efficacy of ITTAs as a study model of long-term disuse. Previous studies in ITTAs have shown lower maximum strength in the intact limb compared to able-bodied participant limbs (Isakov et al. 1996, Lloyd et al. 2010, Pedrinelli et al. 2002, Powers et al. 1996); however, these studies did not control for other factors known to independently affect muscle strength between the groups such as ageing, health, and sedentary lifestyle. It is possible that, by comparing knee extensor muscle load during habitual walking (by assessing knee extensor moments and impulse), we may be able to quantify the level of disuse of the amputated and intact limbs quadriceps when compared to those of a control group.

Six studies (Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002, Powers et al. 1996, Renstrom et al. 1983) have previously measured quadriceps maximum strength, albeit isokinetically, in ITTAs. None have assessed the changes in explosive strength in this population. Furthermore, the neuromuscular mechanisms of the considerable strength loss in the amputated limb of ITTAs have not been investigated. Short-term (< 89 days) disuse studies have reported decreases (e.g. Alkner & Tesch 2004, Deschenes et al. 2002, Kawakami et al. 2001) or no change (de Boer et al. 2007, Campbell et al. 2013) in neural activation, and a shift to faster contractile properties in the disused limb (measured as increased involuntary RTD relative to peak torque; Lambertz et al. 2001). Furthermore, 21-30 days of disuse have elicited declines in muscle size (≤10%), pennation angle (≤13%), and fascicle length (≤9%) (e.g. Campbell et al. 2013, de Boer et al. 2007, Wall et al. 2013). Determining the degree of change in these neuromuscular determinants of muscle strength with long-term habitual disuse may allow better targeting of preventative and rehabilitative interventions for populations subject to muscular disuse.

The aims of this study were firstly to assess the efficacy of unilateral ITTAs as a model to study long-term disuse. This was achieved by comparing neuromuscular function and loading during walking gait of the intact limb with a control limb in an able-bodied population, where both groups are healthy, young, and active. Secondly, we aimed to assess maximum and explosive strength, and the mechanisms underpinning these (neural activation, contractile properties and muscle architecture) in the disused quadriceps muscles of ITTAs, in comparison to both the intact and an able-bodied control limb.

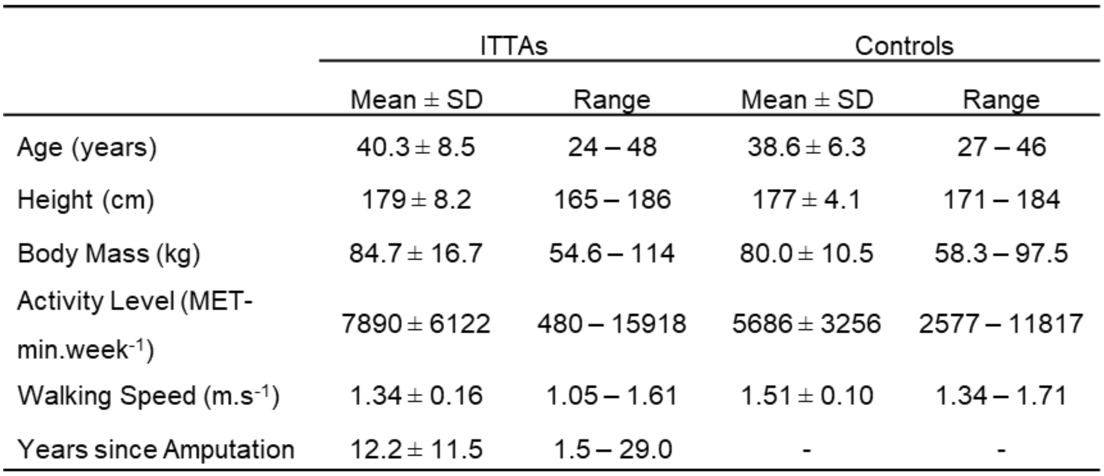
7.2 Methods

A comprehensive description participant recruitment, inclusion criteria and the methods followed for data collection and processing are given in Chapter 3, Sections 3.3 – 3.5.

7.2.1 Participant Information

Nine male ITTAs and nine male controls took part in this study (Table 6.1). The control participants were selected prior to analysis of gait, strength or neuromuscular data to ensure group means were matched for age, height, body mass, and physical activity, all of which are factors known to independently affect muscle strength and neuromuscular function (Hannah et al. 2012, Narici & de Boer 2011, Pincivero et al. 2004, Sacchetti et al. 2013). As a result, the groups had similar age, height, body mass and physical activity scores ($p \geq 0.354$; g = 0.10 – 0.64; Table 7.1). Participant inclusion and exclusion criteria, and detail about assessment of physical activity, can be found in Chapter 3, Section 3.2.

Table 7.1. Participant information. Data are presented as mean ± SD. Data is presented for n = 9 for both groups, except walking speed (n = 8 ITTAs and n = 9 controls).



	ITTAs		Controls	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	40.3 ± 8.5	24 – 48	38.6 ± 6.3	27 – 46
Height (cm)	179 ± 8.2	165 – 186	177 ± 4.1	171 – 184
Body Mass (kg)	84.7 ± 16.7	54.6 – 114	80.0 ± 10.5	58.3 – 97.5
Activity Level (MET-min.week^{-1})	7890 ± 6122	480 – 15918	5686 ± 3256	2577 – 11817
Walking Speed (m.s^{-1})	1.34 ± 0.16	1.05 – 1.61	1.51 ± 0.10	1.34 – 1.71
Years since Amputation	12.2 ± 11.5	1.5 – 29.0	-	-

7.2.2 Data Collection

Briefly, participants visited the laboratory for 2-3 hours on three separate occasions, 3-7 days apart, to complete a familiarisation session, and two measurement sessions. The familiarisation session and first measurement session were identical and involved assessment of voluntary maximal and explosive muscle strength and intrinsic contractile properties of the quadriceps, and muscle architecture of the VL muscle (section 3.4). Neuromuscular measures were taken on both legs and the first leg tested was randomised for each person but remained consistent between sessions. The second measurement session involved the collection of kinetic data during walking at a habitual speed. The loading on the knee (peak knee extensor moments and impulse in stance) was used to quantify disuse of the amputated limb (section *3.5.4.3*).

7.2.3 Data Processing and Extraction

Full detail on specific data processing is given in sections *3.4.2.3* and *3.4.2.5* (strength), *3.4.2.4* and *3.4.2.5* (neural drive), *3.4.2.2* (intrinsic contractile properties), *3.4.2.1* (muscle architecture) and 3.5.5 (gait analysis). Values were recorded for each limb and averaged across the three trials selected for analysis

Strength data extracted were MVT and peak voluntary RTD. Both were analysed as an absolute value and relative (MVT relative to BM, MVT_{BM}; RTD relative to MVT, RTD_{MVT}). Neuromuscular data extracted included knee extensor evoked twitch and octet peak torques and RTD; voluntary activation (VA), EMG at MVT, and explosive EMG from 0-100 ms. Muscle architectural variables (muscle thickness, pennation angle and fascicle length of VL) were extracted using Tracker software (available from http://physlets.org/tracker/).

In walking, internal peak knee extension moment (Figure 7.1) and knee extension moment impulse were extracted (i.e. the area under the torque-time curve for the entire stance phase) to quantify the comparative disuse of the amputated and intact limbs when compared to the control. Gait kinetic data were reported both as an absolute value and relative to body mass (BM).

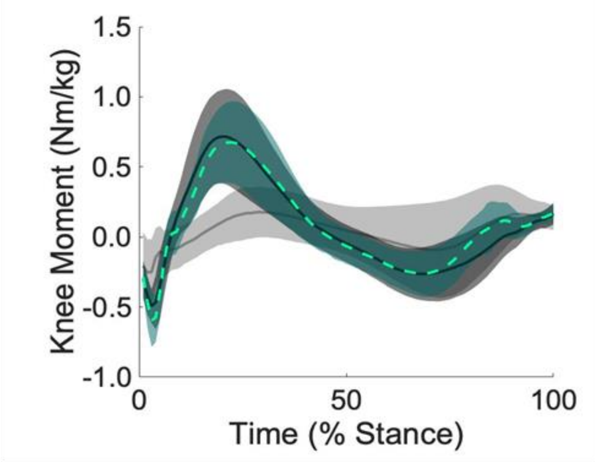


Figure 7.1. Sagittal plane knee moments during the stance phase of walking for the amputated (AMP, light grey line) and intact (INT, dark grey line) limbs of unilateral transtibial amputees, and of an able-bodied control limb (CON, green dashed line). Joint moment is expressed as internal moment. Positive and negative values indicate knee extension and flexion moments, respectively. Data are presented as mean ± SD for n = 8 (AMP and INT) and n = 9 (CON).

7.2.4 Statistical Analysis

Paired t-tests revealed no differences in either MVT or peak RTD between dominant vs. non-dominant (MVT: p = 0.775, g = 0.07; RTD: p = 0.237, g = 0.43) limbs in the control group, where the dominant limb was defined as the one in which the participant would favour to kick a ball. Given the lack of differences in strength variables between limbs, and the substantial variability in the relative direction of strength and neuromuscular asymmetries in a control group in Chapter 4, each dependent variable was averaged between the dominant and non-dominant limbs in the control group. Thus, comparisons are made between the mean of the control limbs (CON) vs the amputated limb of ITTAs (AMP) vs the intact limb (INT).

Levene’s test was used to check for equality of variances, and Shapiro-Wilkes assessed normality of the data prior to running all analyses. A one-way ANOVA was used to analyse the influence of limb for independent comparisons (AMP vs. CON and INT vs. CON) on each dependent variable. In the instance of a main effect, post-hoc *t*-tests were performed. Paired *t*-tests compared dependent variables between the intact and amputated limb. Effect size (specifically Hedges *g*, incorporating correction for small sample bias; Lakens 2013) was calculated for paired comparisons, and interpreted as small (0.2 – 0.5), medium (0.5 – 0.8) and large effects (> 0.8). Statistical analysis was completed using SPSS version 24, and the significance level was set at $p < 0.05$. Data are reported as mean ± standard deviation (SD), with absolute percentage difference (BAI) in values between each condition.

7.3 Results

Due to an injury that occurred between laboratory visits two and three, one ITTA participant did not complete biomechanical data collection. For this reason, while neuromuscular data are presented for 9 ITTAs (with the exception of VA, where n = 8 due to participant withdrawal), gait data is presented for 8 ITTAs. One control withdrew from evoked train stimuli, so control data for is given for 9 participants excepting octet and doublet stimulation (where n = 8). There was a large effect size for the controls to have a faster walking speed (g = 1.21), although this difference was not statistically significant ($p = 0.616$; Table 7.1).

7.3.1 Knee Kinetics in Gait

Knee moment waveforms throughout stance for each limb are presented in Figure 3.10. Both absolute and relative peak knee extensor moment during the stance phase of gait was significantly lower in the AMP compared to INT (-59 to -60%, $p < 0.011$, g = 1.77 – 1.78) and CON (-54 to -59%, absolute $p = 0.005$, g = 1.61; BM $p = 0.006$, g = 1.72) limbs, but similar between INT and CON ($p = 1.000$; g = 0.05 – 0.14; Table 7.2). While there was no main effect of limb on absolute or relative knee extensor moment impulse during stance ($p > 0.069$), there were medium to large effects for it to be 36% and 27% lower in AMP than INT (absolute g = 0.99, BM g = 1.15) and CON (absolute g = 0.56, BM g = 0.90), respectively (Table 7.2).

7.3.2 Maximal and Explosive Strength

Although ITTA strength (n = 8) was reported in Chapter 6, it is reported again here as (1) data in this chapter are presented for n = 9; and (2) for the comparison against the control limb.

MVT (both absolute and relative to body mass) was significantly lower in AMP than INT (~-60%, $p < 0.002$, g = 1.74 – 1.97) and CON (~-64%, $p < 0.001$, g = 2.05 – 2.33). There were no differences between INT and CON in absolute ($p = 1.000$, g = 0.35) or relative ($p = 1.000$, g = 0.28; Table 7.2) MVT.

Absolute peak voluntary RTD (Table 7.2) was ~75% lower in AMP than INT ($p = 0.001$, g = 2.22), ~76% lower in AMP than CON ($p < 0.001$, g = 2.36), but similar between INT and CON ($p = 1.000$, g = 0.14). When expressed relative to MVT, peak RTD was significantly smaller in AMP than INT (-43%, $p = 0.027$, g = 1.37) and CON (-39%, $p = 0.031$, g = 1.09), while INT and CON were similar ($p = 1.000$, g = 0.23; Table 7.2).

Table 7.2. Knee extensor kinetics throughout the stance phase of gait, muscle strength and neuromuscular function in the amputated (AMP) and intact (INT) limbs of unilateral transtibial amputees, and in an able-bodied control limb (CON). Data are presented as mean ± SD for n = 9 (AMP and INT) and n = 9 (CON). Data in italics correspond to those variables where n = 8. No significant differences were apparent between INT and CON for any variable. Differences compared to AMP are denoted by * ($p < 0.05$) or ** ($p < 0.001$).

	AMP	INT	CON
Gait Knee Extension Kinetics			
Moment (Nm)	_26.1 ± 13.3_	_65.4 ± 38.1_*	57.0 ± 13.7*
Moment$_{BM}$ (Nm.kg^{-1})	_0.30 ± 0.14_	_0.75 ± 0.31_*	0.71 ± 0.24*
Impulse (Nm·s)	_1.14 ± 0.84_	_2.23 ± 1.21_	1.75 ± 0.43
Impulse$_{BM}$ (Nm·s.kg^{-1})	_0.013 ± 0.009_	_0.025 ± 0.011_	0.022 ± 0.008
Muscle Strength			
MVT (Nm)	74.6 ± 32.0	182 ± 62.6 *	202 ± 37.1 **
MVT$_{BM}$ (Nm.kg^{-1})	0.93 ± 0.49	2.31 ± 1.10*	2.57 ± 0.58**
RTD (Nm.s^{-1})	427 ± 235	1678 ± 722 **	1757 ± 307 **
RTD$_{MVT}$ (MVT.s^{-1})	5.39 ± 2.27	9.50 ± 3.58 *	8.83 ± 1.62 *
Neural Drive			
Voluntary Activation (%)	_50.6 ± 12.7_	89.2 ± 5.75 **	_90.4 ± 4.07_ **
RMS EMG$_{MVT}$ (% M$_{max}$)	5.19 ± 1.20	9.10 ± 2.39 **	7.64 ± 1.47 *
Explosive RMS EMG$_{0-100}$ (% M$_{max}$)	5.38 ± 3.12	7.92 ± 3.66	7.00 ± 1.75
Evoked Twitch			
PT (Nm)	11.6 ± 6.00	30.8 ± 11.6 **	39.0 ± 11.9 **
Absolute RTD (Nm.s^{-1})	223 ± 171	650 ± 247 **	808 ± 243 **
Relative RTD (PT.s^{-1})	16.7 ± 3.23	20.4 ± 1.79 *	21.1 ± 4.60 *
Evoked Octet			
PT (Nm)	47.1 ± 31.2	94.5 ± 32.3 **	_116 ± 28.0_ **
Absolute RTD (Nm.s^{-1})	609 ± 387	1647 ± 541 **	_1840 ± 365_ **
Relative RTD (PT.s^{-1})	13.3 ± 1.62	17.7 ± 1.66 **	_16.0 ± 1.43_ *
Muscle Architecture			
Muscle Thickness (mm)	15.4 ± 5.19	26.3 ± 6.38 *	25.0 ± 3.34 *
Pennation Angle (°)	12.0 ± 1.66	13.9 ± 3.79	13.7 ± 1.46
Fascicle Length (mm)	73.8 ± 23.2	117 ± 50.8 **	96.5 ± 14.8

MVT, Maximal Voluntary Torque; RTD, peak rate of torque development; subscript BM, relative to body mass; subscript MVT, relative to MVT; RMS EMG$_{MVT}$, root mean squared electromyography at MVT; M$_{max}$, maximal M-wave; PT, peak torque.

7.3.3 Neural Drive

Both VA and RMS EMG_{MVT} (Table 7.2) were lower in AMP than INT (-44% for VA, *p* < 0.001, g = 3.63; and -43% for EMG_{MVT}, *p*< 0.001, g = 1.97) and CON (-43% for VA, *p* < 0.001, g = 3.54; -32% for EMG_{MVT}, *p* = 0.021, g = 1.23), but similar between INT and CON for either VA (*p* = 1.000, g = 0.14) or EMG_{MVT} (*p* = 0.271, g = 0.70).

There was no significant difference main effect of limb in the amplitude in explosive RMS EMG_{100} (*p* = 0.304; Table 7.2). However, there was a moderate effect for $EMG_{0\text{-}100}$ to be greater in INT than AMP (g = 0.75), but only small to moderate effects for differences for other comparisons (AMP vs. CON, g = 0.30; INT vs. CON, g = 0.45).

7.3.4 Intrinsic Contractile Properties

PT in both evoked twitch and octet contractions (Table 7.2) was lower in AMP compared to INT (twitch PT: -62%, *p* < 0.001, g = 1.97; octet PT: -50%, *p* = 0.001, g = 1.43); AMP compared to CON (twitch PT: -70%, *p* < 0.001, g = 2.84; octet PT: -68%, *p* = 0.004, g = 2.07), but similar between INT and CON (*p* ≥ 0.284 , g = 0.68; Table 7.2).

Absolute peak RTD for both twitch and octet was lower in AMP compared to INT (twitch RTD: -62%, *p* < 0.001, g = 1.94; octet RTD: -63%, *p* < 0.001, g = 1.43); AMP compared to CON (twitch RTD: -72%, *p* < 0.001, g = 2.62; octet RTD: -70%, *p* < 0.001, g = 2.49); but statistically similar in INT and CON (twitch RTD: *p* = 0.433, g = 0.62; octet RTD: *p* = 0.497, g = 0.40; Table 7.2). When expressed relative to PT, twitch RTD was 18% lower (*p* = 0.006, g = 1.35), and octet RTD 25% lower (*p* < 0.001, g = 2.60) in AMP when compared to INT (Table 7.2). Relative twitch and octet RTD were also both 14% lower in AMP compared to CON (twitch RTD: *p* = 0.036, g = 1.59; octet RTD: *p* = 0.037, g = 1.63). Despite being statistically similar, there was a large effect for relative octet RTD to be greater in INT than CON (*p* = 0.120, g = 1.03; Table

7.2), whilst relative twitch RTD was similar between INT and CON (*p* = 1.000, g = 0.18).

7.3.5 Muscle Architecture

There was no main effect (*p* = 0.226) of limb on pennation angle (Table 7.2). However, muscle thickness in AMP was lower than both INT (-41%, p = 0.030, g = 1.78) and CON (-38%, p = 0.002, g = 1.58; Figure 7.2), but similar between INT and CON (p = 1.000, g = 0.23; Table 7.2). Fascicle length was shorter in AMP than INT (-36%, *p* < 0.001, g = 0.95), but similar between AMP and CON (*p* = 0.187; g = 0.50), and INT and CON (*p* = 1.000; g = 0.49).

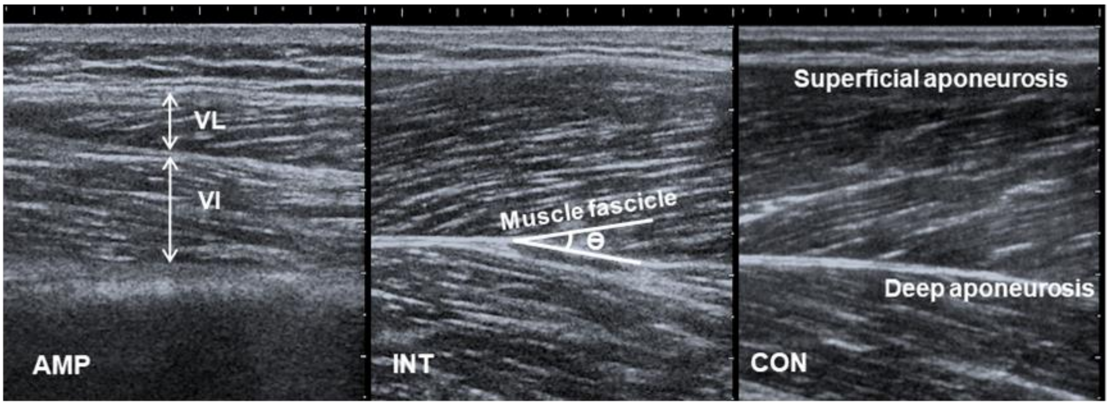


Figure 7.2. Static B-mode ultrasound image of the Vastus Lateralis muscle for the amputated (AMP) and intact (INT) limb of one TTA, and one control limb (CON). Architectural measures taken included pennation angle relative to the deep aponeurosis (a), extrapolated fascicle length (b) and muscle thickness, measured between the superficial and deep aponeuroses (c). Significant reduction in amputated limb muscle thickness is evident, while similarities in pennation angle in all three limbs, and muscle thickness between INT and CON, can clearly be seen.

7.4 Discussion

In this study, we compared quadriceps strength and neuromuscular function in the amputated limb of ITTAs with their intact limb and a control group limb. The intact and control group limbs were comparable for all gait and neuromuscular measures, suggesting that the intact limb is a good internal control. ITTAs therefore provide a novel model for studying the long term (>1.5 years) effects of disuse. Long-term disuse of the amputated limb in ITTAs was evidenced from the ~60% lower peak knee extensor moments during walking compared to the intact and control limbs. This disuse was accompanied by ~60% lower maximal strength and ~75% lower explosive strength in the amputated limb, which are much greater differences than may be predicted from short-term disuse studies. Declines in MVT appeared to be largely due to reduced muscle size (evidenced by lower muscle thickness in AMP) and neural drive (evidenced by lower VA and EMG_{MVT} in AMP). Declines in RTD appeared to be due primarily to declines in MVT and a shift towards slower intrinsic contractile properties, with neural drive in explosive contractions being unaffected in AMP.

7.4.1 ITTAs as a model for long-term disuse

In the current study, there were large effects for knee extensor kinetics during gait to be lower in amputated than intact or control limb, suggesting that the knee extensors of the amputated limb undergo substantially less habitual loading during walking. This is evidenced by significant differences and large effects in knee extensor kinetics between the amputated and intact, and amputated and control limbs confirming their efficacy as a chronic disuse model. These data are supported by the findings of previous studies, which reported decreased knee moments (Powers, Rao & Perry, 1998, Winter & Sienko, 1988); powers (Powers, Rao & Perry, 1998, Winter & Sienko, 1988); and work (Silverman & Neptune, 2012) on the amputated limb in walking. The knee extensors of the intact limb in the ITTAs did not differ from those of an able-bodied population for kinetics during walking, maximal or explosive strength, or any of the neuromuscular determinants of strength. This suggests the intact limb of the ITTAs provides an ideal internal control for comparison to the amputated limb, from which to draw conclusions about the effects of long-term disuse. Furthermore, the

ITTAs in this study were young, healthy, and moderate-highly active indicating that the effects of disuse on strength and neuromuscular function are isolated from factors such as ageing, disease, and sedentary behaviour.

7.4.2 Changes in Strength

The declines in maximum strength found in the amputated limb of ITTAs when compared to the intact limb (-59%; measured isometrically) are comparable, albeit at the high end, of differences observed in previous amputee studies (-33 to -57%, measured isokinetically; Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002), but considerably greater than the reduction in strength typically observed after a period of short-term disuse of up to 120 days (~23%; Narici & de Boer, 2011). Short-term intervention studies suggest that maximum strength decreases exponentially over time following unloading, plateauing out after ~90 days; however, the results of this study suggest that the strength declines with longer-term disuse are considerably more than could be predicted from short-term intervention studies.

To the authors' knowledge, only two previous studies have investigated the effect of disuse on voluntary explosive strength of the knee extensors, reporting 54% (Bamman et al., 1998) and 42% (de Boer et al., 2007) decreases in RTD after 16 days of bed rest, and 23 days of ULLS, respectively. The considerable reductions in peak RTD (-75%) in the amputated vs. intact limb are important, as explosive strength is considered more functionally relevant than maximum strength, in many sports-specific and daily tasks, such as sprinting, jumping, and balance recovery (Behan, Pain & Folland 2018, Pijnappels et al. 2008, Tillin, Pain & Folland 2013a). Peak RTD expressed relative to MVT was significantly reduced in the amputated compared to the non-amputated limbs. Thus, the reduction in MVT appears only partially contributed to the reduction in peak RTD, which was likely also influenced by the slowing of the contractile properties (discussed in more detail below).

7.4.3 Mechanisms of Strength Differences

7.4.3.1 Neural Drive

A broad suppression in neuromuscular activity at maximal force production – indicated by reduced amputated limb VA (~44%) and EMG_{MVT} (~38%) compared to non-amputated limbs – likely contributes to the reduction in amputated limb maximum strength. Whilst many previous studies have reported reduced quadriceps EMG amplitude (-16 to -35%; Alkner & Tesch 2004, Deschenes et al. 2002) and VA (-7%; Kawakami et al. 2001), others have not observed changes in these measurements (de Boer et al. 2007, Campbell et al. 2013, Horstman et al. 2012), following periods of disuse of up to 89 days. Thus, the large limb effects on VA and EMG responses observed in the present study suggest that reductions in neural drive with disuse become more pronounced and observable over time. Of note is the specificity of the neural deficits in the ITTAs to the amputated limb. Evidence from unilateral injury and training studies suggest a cross-over effect of neural function, in that neural drive adaptations occur at the contralateral, as well as the injured/ trained limb (Hart et al. 2010, Bogdanis et al. 2019, Tillin, Pain & Folland 2011). In this study, however, there was no evidence that the reduced neural drive on the amputated side had affected neural drive on the intact side, which was similar to the control limb. Perhaps this is because ITTAs rely more heavily on the intact limb for most activities of daily living and exercise (e.g. Mattes et al. 2000, Fey et al. 2010, Winter & Sienko 1988), which may negate any cross-over effects of reduced neural drive from the amputated to the intact limb.

Despite the substantial differences between the amputated and non-amputated limbs evident in neural drive during maximum force production, no such differences were observed in this study in explosive $EMG_{0\text{-}100}$, although moderate to large effects were noticeable (g = 0.45 – 0.71; Table 7.2). This suggests that altered neural drive does not explain the lower RTD in the amputated limb, which is interesting given that neural drive is a key determinant of explosive strength, (del Vecchio et al. 2019a, Folland, Buckthorpe & Hannah 2014). The large variability in EMG, even after normalisation to M_{max} (Buckthorpe et al. 2012), greater variability in explosive torque compared to MVT (Folland, Buckthorpe & Hannah 2014, Tillin, Pain & Folland 2013a), and small sample sizes (n = 9 per limb) may have reduced the chances of observing a significant effect. Alternatively, the amputated limb’s role in ambulation may explain the lack of differences in neural drive during the explosive contractions. Specifically, whilst the knee extensors of the amputated limb experience reduced load compared to the intact during ambulation, the amputated side does contribute to stability and postural correction, for which RTD appears to be important (Behan et al. 2018). Thus, typical physical activity in the amputees may provide sufficient stimulus to maintain the neural drive during short, rapid contractions, which typically underpins RTD.

7.4.3.2 Muscle Architecture

The VL muscle was 41% thinner in the amputated limb compared to the intact, which is a larger difference than the declines in magnetic resonance imaging (MRI) and computed tomography scanner measurements of muscle size (-3 to -18%) observed in short-term disuse studies (Alkner & Tesch, 2004, Campbell et al. 2013, de Boer et al., 2007, Dirks et al. 2016). Thus, similar to the changes observed for strength and neural drive, reductions in muscle size with long-term disuse are much greater than could be predicted from short-term disuse studies. Muscle size is considered an important determinant of MVT (Blazevich et al. 2009), and thus the reduction in muscle thickness is likely to contribute to the declines in both maximum and explosive strength in the amputated limb.

Fascicle length was reduced by 36% in the amputated limb compared to the intact. Again, this difference is considerably greater than the decline in knee extensor fascicle length (6 – 9%) typically observed with short-term unloading (Campbell et al. 2013, de Boer et al. 2007). ITTAs walk with a comparatively stiff knee joint on the amputated limb (Powers, Rao & Perry 1998, Winter & Sienko 1988), which would theoretically isolate loading to shorter fascicle lengths, and limit the stimulus likely required to maintain longer fascicle lengths. Decreases in fascicle length may reduce maximum shortening velocities and power (Blazevitch & Sharp 2006) and shift the torque-angle relationship towards more extended knee positions (Blazevich et al. 2009). Given our strength measurements were made at a typical plateau region of the torque-angle relationship (Chow et al. 1999), a shift away from this region in the amputated limb may have partly contributed to the observed differences in maximum and explosive strength.

In contrast to the results of previous research which demonstrated decreases in pennation angle during short periods of ULLS (de Boer et al. 2007, 2008, Campbell et al. 2013), our results appear to suggest that pennation angle does not change with long-term disuse. In healthy populations, angles of pennation of the VL muscle have been reported to be 6 – 27° (Blazevich et al. 2006, Rutherford & Jones 1992); the pennation angle of all three limbs in this study (~12 – 14°) falls within this range. This suggests that the structural re-modelling that seems to take place in the early phases of disuse are not representative of long-term adaptations. It is possible that muscle thickness declines at a faster rate than fascicle length with short term disuse, causing a decline in pennation angle; whilst over longer periods of disuse, fascicle length reductions “catch-up” with muscle thickness loss, causing a return to baseline pennation angle. However, this hypothesis cannot be tested with our data.

7.4.3.3 Intrinsic Contractile Properties

The significant reductions in evoked (twitch and octet) contractile peak torque in the amputated compared to the intact and control limbs (Table 7.2) are reflective of the reduced capacity of the amputated limb knee extensors for torque production. These changes were accompanied by reductions in RTD, both absolute and relative to peak torque, reflecting a shift towards slower contractile properties in the amputated limb. This is in contrast to the results of short-term disuse studies, which have reported a shift towards faster contractile properties owing to a greater expression of fast-contracting MHCs (Bamman et al. 1998, Trappe et al. 2004). The results of the current study therefore provide novel evidence that changes in intrinsic contractile properties with long-term disuse are more characteristic of ageing muscle, which also displays a slowing of the contractile properties (Roos et al. 1999). This slowing may be due to preferential atrophy of type II muscle fibres, and potentially also to an increased dominance of type I MHC in fibres co-expressing MHCs commonly seen with old age (Lexell et al. 1988). The slower contractile properties in the amputated limb likely contributed to the lower explosive strength also observed in this limb, as twitch and octet RTD are importance determinants of explosive strength.

7.5 Conclusion

This study was the first to utilise ITTAs as a novel study model to investigate the effects of chronic muscle disuse on strength and neuromuscular function, in young, healthy, and active adults. The ITTAs, who displayed chronic disuse of the quadriceps muscle on the amputated side characterised by considerably less (internal) loading of the quadriceps during walking, recorded considerable reductions in maximal strength (-60%), and to a greater extent explosive strength (-75%), in the amputated compared to the intact limb. These differences were substantially greater than previously reported in short-term unloading studies suggesting strength loss with long-term disuse is considerably greater than can be predicted from short-term disuse studies. The reductions in MVT were likely due to the considerable declines in muscle size and neural drive, whilst the reductions in explosive strength appeared due to the decline in MVT coupled with a slowing of the contractile properties. The slower contractile properties and the observed similarities in pennation angle between limbs contrast with previous findings of short-term disuse studies, which have observed faster contractile properties and a decline in pennation angle.

Chapter 8

General Discussion

The purpose of this thesis was to investigate strength asymmetry, its underpinning neural and mechanical determinants, and to determine its influence on movement. The results of this thesis have provided further insight into strength and movement asymmetries by (1) comparing the magnitude, relative direction and variability of knee extensor maximum and explosive strength asymmetries, and their potential underlying determinants in a healthy, active, control population, discussed in Section 8.1; (2) investigating the influence of knee extensor strength asymmetries on movement asymmetries in two populations with different levels of inherent strength asymmetries, discussed in Section 8.2; and (3) utilising a known asymmetric ITTA population to assess the effects of long-term disuse on strength and neuromuscular function of the quadriceps muscle, discussed in Section 8.3.

8.1 Strength Asymmetry and its Determinants

Strength asymmetries at the quadriceps have previously been linked to ageing (Skelton, Kennedy & Rutherford 2002), pathology (e.g. Lloyd et al. 2010, Sandroff, Sosnof & Motl 2013, Suetta et al. 2007) and increased risk of musculoskeletal injury (Knapik et al. 1991, Impellizzeri et al. 2007). Up until now, however, research in healthy populations has only considered asymmetries in maximal strength. When comparing the strong and weak limbs, Chapter 4 found greater magnitudes of maximal strength asymmetry compared to those previously reported in a healthy able-bodied population (10% [Table 4.1] vs. 1 – 5%; Kobayashi et al. 2013, Lanshammar & Ribom 2010, Schiltz et al. 2009), although it should be noted that the results are not directly comparable: the previous studies performed dynamic maximum strength testing at various speeds, and calculated asymmetry using a dominant/ non-dominant limb split. Based on past research indicating the functional importance of RTD, and its large inter-individual variability (Behan, Pain & Folland 2018, Folland, Buckthorpe & Hannah 2014, Pijnappels et al. 2008, Tillin, Pain & Folland 2013a), we hypothesised that magnitudes of RTD asymmetry would be larger than those in MVT; however, this did not appear to be the case. Instead, Chapter 4 found that there was no difference in the size of maximum and explosive strength asymmetry in a control population (10% vs. 13%, respectively), although the latter was more variable (range BSA MVT ~17%, BSA RTD ~35%). Furthermore, although asymmetries in the two aspects of strength were related, they were not always in the same direction (Figure 4.2). In comparison, ITTAs demonstrated significantly greater asymmetry in RTD than MVT (Chapter 6) that was consistently in the same direction – i.e. the intact limb was always the strong limb.

The difference in the magnitudes of strength asymmetry in these two populations is reflected in asymmetries in neuromuscular function in controls and ITTAs. In an able-bodied population demonstrating relatively small strength asymmetries in maximal and explosive strength, neither was explained by a systematic combination of asymmetries in the factors which are thought to determine strength. In other words, the coordination of neuromuscular function between limbs for force production is different between individuals. This appears to be supported by the large inter-individual variability inherent in the data (Tables 4.1 and 4.3). As previous research has suggested that a combination of factors such as load distribution mechanisms (e.g. in ITTAs, Chapters 6 & 7), training background (Maloney 2018), and environmental interactions (including injuries, e.g. Knezevic et al. 2014) determines the presence and magnitude of bilateral asymmetries in strength, the substantial variability demonstrated in the bilateral asymmetries of both aspects of strength (discussed above) and neuromuscular function in this population is perhaps unsurprising.

In contrast, in ITTAs, the differences in determinants between limbs clearly explain asymmetries in both aspects of strength (Chapter 7). The difference in the results is likely a result of the substantial and systematic nature of the strength asymmetries evident in ITTAs. In this population, asymmetries in maximum strength are strongly associated with reductions in muscle size (-41%) and neural drive (VA: -44%, EMG_{MVT}: -43%; Table 7.2) at the amputated limb when compared to the intact. Asymmetries in explosive strength were associated with deficits in MVT coupled with a slowing of the contractile properties on the amputated limb, but not, surprisingly, explosive neural drive, which is the primary determinant of later-stage explosive torque (Folland, Buckthorpe & Hannah 2014). These asymmetries – both in strength and neuromuscular function – are presumably reflective of the effects of long-term disuse (discussed in detail in Section 8.3).

8.2 Strength Asymmetry and Movement Performance

While the effect of movement asymmetry on sporting performance is a popular topic in research literature, we currently have little understanding of the underpinning mechanisms of these asymmetries (Bishop, Turner & Read 2018). This thesis aimed to better understand the association between asymmetries in strength and movement. To that end, Chapters 5 and 6 explored the relationships of strength and movement asymmetries in two populations with substantially different levels of asymmetry: a control group and a group of ITTAs. To aid comparisons between these chapters, both groups were young, healthy and active.

The knee plays a key role in both a jump take-off and landing from a drop. Furthermore, both are planar movements that seem to be partially determined by maximal and, to a greater extent, explosive quadriceps strength (Chang et al. 2015, de Ruiter et al. 2007, Sandler and Robinovitch 2001), which makes these movements a useful means of assessing the relationships between movement and strength asymmetries in a healthy population. However, Chapter 5 presented limited evidence for relationships between strength asymmetries and jump height (Figure 5.3) or kinetic asymmetries (whole-limb or at the knee specifically; Table 5.1) in the take-off phase of a CMJ. We hypothesised that this was reflective of joint coordination, i.e. that rather than being the prime mover in a jump take-off, the primary role of the knee extensors was instead to coordinate the actions of the ankle and hip. This in turn may explain why there is such large directional variability when variables are grouped by knee extensor strength for this movement. In contrast, while asymmetries in MVT were not associated with landing from a drop, explosive strength symmetry did appear to be important for kinetic symmetry in this task. The latter associations were assessed by between-limb differences, indicating that the asymmetries were in the same direction; however, they were not correlated. It seems likely that this is because each individual performs movements in a different way within the constraints of their system. The results of Chapter 5, which further echo the substantial variability in asymmetry in a healthy, able-bodied population (as seen in Chapter 4), highlight the importance of personalised data analysis when attempting to quantify an individual's strength and/ or movement asymmetry.

Given the postulated difficulty in identifying consistent bilateral asymmetries within a group of individuals unless they are highly specialised to an asymmetric task (Lawson et al. 2006) – which was indeed apparent in the results of Chapters 4 and 5 – Chapter 6 allowed a further opportunity to establish the relationships between asymmetries in strength and movement in greater detail. ITTAs have previously been established to be asymmetrical for both strength and movement (Lloyd et al. 2010, Winter & Sienko 1988), which was confirmed by the results of Chapter 6. Furthermore, as the asymmetries in strength in ITTAs are in the same direction as those in movement (i.e. the amputated limb is consistently weaker and accepts less load in a variety of movements; Sanderson & Martin 1997, Schoeman, Diss & Strike 2012, 2013), we hypothesised that associations between asymmetries in strength and movement would be more observable in this population.

Adequate gait function is fundamental for daily living and exercise, and as a result, health and quality of life. ITTAs are not necessarily able to perform more intense exercises (e.g. running) due to the greater musculoskeletal demand on the weaker amputated limb; therefore, this thesis utilised walking as a model to investigate associations between strength and movement asymmetries. Explosive strength asymmetry was directly related to walking speed, and both forms of strength asymmetry were related to the ability to change self-selected walking speed from habitual to fast (Figure 6.2). As walking speed is a key indicator of gait function (van Velzen et al. 2006), this finding is important for the promotion quality of life in ITTAs. The magnitude of asymmetry in RTD should therefore be an additional key outcome measure in rehabilitation protocols alongside the commonly measured asymmetry in MVT. Furthermore, ITTAs who were more symmetrical for strength (and particularly explosive strength) demonstrated greater symmetry in single support time and gait kinetics. As mechanical overloading of a joint is thought to place an individual at an increased risk of developing joint degenerative diseases (Farrokhi et al. 2016), and the intact limb of ITTAs is at a 25 – 28% greater risk of joint degeneration compared to the general population (Struyf et al. 2009, Norvell et al. 2005), this may have important implications for rehabilitation. Specifically, as more symmetrical limb loading may be important to reduce likelihood of degenerative conditions, it may be that we could mitigate this risk through improving explosive strength asymmetry. The results of Chapters 5 and 6, when taken together (i.e. the presence of relationships between strength and movement asymmetries in ITTAs but not controls, who have smaller levels of asymmetry), also suggest that perhaps strength asymmetry becomes more important for movement performance at higher levels of asymmetry.

8.3 ITTAs as a Model for Long-Term Disuse

By investigating the neuromuscular characteristics of populations with known asymmetries, we can answer some broader physiological questions. Chapter 7 was the first study to utilise ITTAs as an experimental model to explore the effect of long-term disuse on strength and neuromuscular function. The knee extensors of the intact limb in the ITTAs did not differ from those of an able-bodied population for kinetics during walking, maximal or explosive strength, or any of the neuromuscular determinants of strength. This suggests the intact limb of the ITTAs provides an ideal internal control for comparison to the amputated limb, from which to draw conclusions about the effects of chronic disuse. Furthermore, as the ITTAs in this study were young, healthy, and moderate-highly active, so the effects of disuse could be isolated from factors such as ageing, disease, and sedentary behaviour, which are known to independently affect muscle strength and function (Narici & de Boer 2011, Pincivero et al. 2004, Sacchetti et al. 2013). The results of this study may have applications to multiple clinical populations that experience chronic muscular disuse as a result of, for example, prolonged bedrest, injury or pathological conditions.

The ITTAs, who displayed chronic disuse of the quadriceps muscle on the amputated side characterised by considerably less (internal) loading of the quadriceps during walking, recorded considerable reductions in maximal strength (-60%), and to a greater extent explosive strength (-75%), in the amputated compared to the intact limb. These differences were substantially greater than previously reported in short-term unloading studies (maximum strength ~23%; explosive strength ~48%; Bamman et al. 1998, de Boer et al. 2007; Narici & de Boer, 2011). Short-term intervention studies suggest that maximum strength decreases exponentially over time following unloading, plateauing out after ~90 days; however, the results of this study suggest that, with longer periods of disuse, both maximum and explosive strength declines considerably more than would be predicted from short-term intervention studies. As explosive strength is considered more relevant for function in many sports-specific and functional daily situations (e.g. sprinting, jumping, recovery from balance perturbation) than maximum strength (Maffiuletti et al. 2010, Pijnappels et al. 2008, Tillin, Pain & Folland 2013a), these findings have significant implications for the rehabilitation of both ITTAs and those who have been subject to long-term disuse. It is important for clinicians to note that the training stimuli for increases in explosive strength are distinct from the stimuli for changes in MVT (Tillin & Folland 2014), so both should be factored into any programming to combat muscular changes resulting from disuse.

This recommendation is underpinned by the findings regarding alterations in the neuromuscular determinants of strength (as discussed in Section 8.1) which were different and distinct for MVT and RTD. Interestingly, this study provides novel evidence that intrinsic muscular changes with chronic disuse are characteristic of ageing muscle, which also displays a slowing of the contractile properties (Doherty & Brown, 1997, Roos et al. 1997, Roos et al. 1999), in contrast to the increase in contractile speed (measured as relative evoked RTD) evident in short-term disuse studies (Narici & de Boer 2011). While perhaps surprising given that neural drive is a key determinant of explosive strength (del Vecchio et al. 2019a, Folland, Buckthorpe & Hannah 2014), there were no differences between limbs in explosive $EMG_{0\text{-}100}$. This suggests that altered neural drive does not explain the lower explosive torques and RTD in the amputated limb. Two potential explanations for this have been highlighted. Firstly, the large variability in EMG, even after normalisation to M_{max} (Buckthorpe et al. 2012), greater variability in explosive torque compared to MVT (Folland, Buckthorpe & Hannah 2014, Tillin, Pain & Folland 2013a), and small sample sizes (n = 9 per limb) may have reduced the chances of observing a significant effect. Alternatively, whilst the amputated limb is loaded less than the intact during ambulation, it may be that the amputated side contributes to postural control and stability – for which RTD appears to be important (Behan, Pain & Folland 2018). Thus, typical physical activity in the amputees may provide sufficient stimulus to maintain the neural underpinnings of RTD.

8.4 Limitations

Limitations of this thesis include the small sample size, which was restricted by the stringent inclusion criterial applied to ITTA participants, and the assumptions of inverse dynamics. The results of ITTA studies (Chapters 6 and 7) should be generalised with care, primarily due to the low sample size which reflects the lack of availability of healthy, young, active traumatic unilateral amputees. Furthermore, it is important to note that the population of ITTAs that participated in this study were ideal for studying strength and neuromuscular changes without the confounding influences of age, inactivity or disease, was nevertheless not necessarily reflective of ITTAs in general. The majority of ITTAs in the UK tend to be older, less active, and have amputations arising from vascular conditions (British Society of Rehabilitation Medicine 2018); however, they are likely to be more asymmetrical in strength (Isakov et al. 1996, Lloyd et al. 2010, Moirenfeld et al. 2000, Pedrinelli et al. 2002) and thus the associations with movement asymmetries potentially stronger.

Additionally, the assumptions of inverse dynamics – specifically, that there is no co-contraction of agonist and antagonist muscles during movement – may be considered a limitation of this study, which assessed net joint moments in walking, CMJs and drop landings. The use of EMG alongside kinetic analyses may allow the differentiation between reduced quadriceps muscular activity, or increased antagonistic (hamstring) activity, when interpreting reduced net knee joint moments. Despite this, inverse dynamics is an easy, non-invasive approach to estimating muscle function. Furthermore, further work is required to understand the effect of modelling on joint moments on amputees. Initial research is being conducted by a research group lead by Silverman (Strike 2019, personal communication) and indicates little evidence of a significant effect.

The isometric measurement of strength could be considered a limitation of this study, due to (1) its reduced ecological validity, and (2) as Chapters 5 and 6 involved correlating isometric measures of strength with dynamic movements. However, isometric measures of RTD are necessary to remove the confounding influence of joint angle changes that are inherent in isokinetic contractions (Maffiuletti et al. 2016). As the isokinetic dynamometer measures external torque (rather than force which is later converted to torque by consideration of external moment arm) it is unlikely that the placement of the adaptor on the amputated compared to the intact and control limbs affected the results. It may, however, be possible that there are other, unidentified factors that may have affected the groups differently because of where the adaptor was placed. Nevertheless, measurements not affected by the isokinetic dynamometer setup (i.e. muscle thickness and fascicle length, VA, EMG_{MVT} and gait kinetic parameters) were, similarly to torque measures, significantly reduced on the amputated limb compared to the two healthy limbs. This suggests that the overall picture of the results (i.e. that strength and neuromuscular function is significantly impaired in the amputated limb of ITTAs, and by extension, in long-term disuse) is reliable.

Finally, changes in fascicle length such as those evident in the amputated limb of ITTAs may result in a shift in the torque-angle relationship which may have partly contributed to the observed differences in maximum and explosive strength in ITTAs. However, we tested strength close to a plateau region of the torque-time curve (Chow et al. 1999), and differences apparent between the amputated and control limbs were much greater than could be explained by the small differences on part of the torque-angle curve.

8.5 Potential Implications

As both maximum and explosive strength asymmetry are variable, not necessarily in the same direction, and underpin different functional capabilities of muscle, both aspects of strength should be considered for a complete profile for diagnosis and monitoring of muscle strength asymmetry in able-bodied populations. In ITTAs, as the magnitude of asymmetry in RTD appears to be a more sensitive marker of functional gait capacity than the commonly measured asymmetry in MVT, the former should be an additional key outcome measure in rehabilitation protocols. These should emphasise strengthening of the amputated limb knee extensors in order to improve both maximal and explosive strength symmetry.

In addition to improving walking speed and functional capacity, targeting RTD asymmetry in ITTAs could reduce the risk of asymmetrical loading that may predispose to degenerative disorders (Macfarlene et al. 1991, Lloyd et al 2010, Schmalz et al., 2001). As the reductions in explosive strength with long-term unloading are significantly greater than deficits in maximum strength, populations who experience long-term disuse could also benefit from explosive strength training. In particular, this may be important for individuals who frequently perform movements such as landing from a jump, which involves the rapid deceleration of the CoM. This is because explosive strength asymmetry appears to be associated with loading asymmetry in able-bodied populations. Greater symmetry in explosive strength could therefore potentially reduce injury risk in the weaker limb by more equal distribution of load relative to limb strength, resulting in landing which effectively mediates force bilaterally. However, it is important to note that the training stimulus for increases in explosive strength is specific and different to that for MVT (Tillin & Folland, 2014), so both will need to be factored into the programming. Given the associations between explosive strength asymmetry and landing from a drop in a control population (as evidenced by the significant differences apparent between limbs when split strong vs. weak), including drop landings in rehabilitation could be a good way to target this deficit.

8.6 Future work

Possible suggestions for further work to build on that of this thesis are as follows:

1) Investigate the determinants of strength asymmetry in more specialised populations with systematic asymmetries (e.g. unilateral injury, the elderly), given that clearly, they exist for ITTAs, if not for a control population. This may be useful for targeting rehabilitation protocols in such groups of individuals.

2) Further explore the mechanisms of the association between explosive strength asymmetry and landing. This could be done by investigating the mechanics of the motion at the other joints in the kinetic chain (i.e. ankle, hip, pelvis and trunk) and/ or utilising EMG to establish how the joints coordinate to rapidly attenuate the load associated with a landing task.

3) Building on the above, a prospective study design could be utilised to explore the associations between explosive strength, load distribution asymmetry in movement, and injury. This may help provide a better understanding of the role that strength asymmetries play in injury risk.

4) Given the significance of explosive strength asymmetry that was identified in Chapters 5, and 7, and the very large asymmetries in explosive strength

present in ITTAs whose amputated limb was chronically disused (Chapters 6 and 7), investigate the response of ITTA muscle to a period of a) maximal and b) explosive strength training, and the subsequent effect on movement patterns. This may allow us to establish more effective rehabilitation protocols for ITTAs/ populations that undergo chronic muscular disuse.

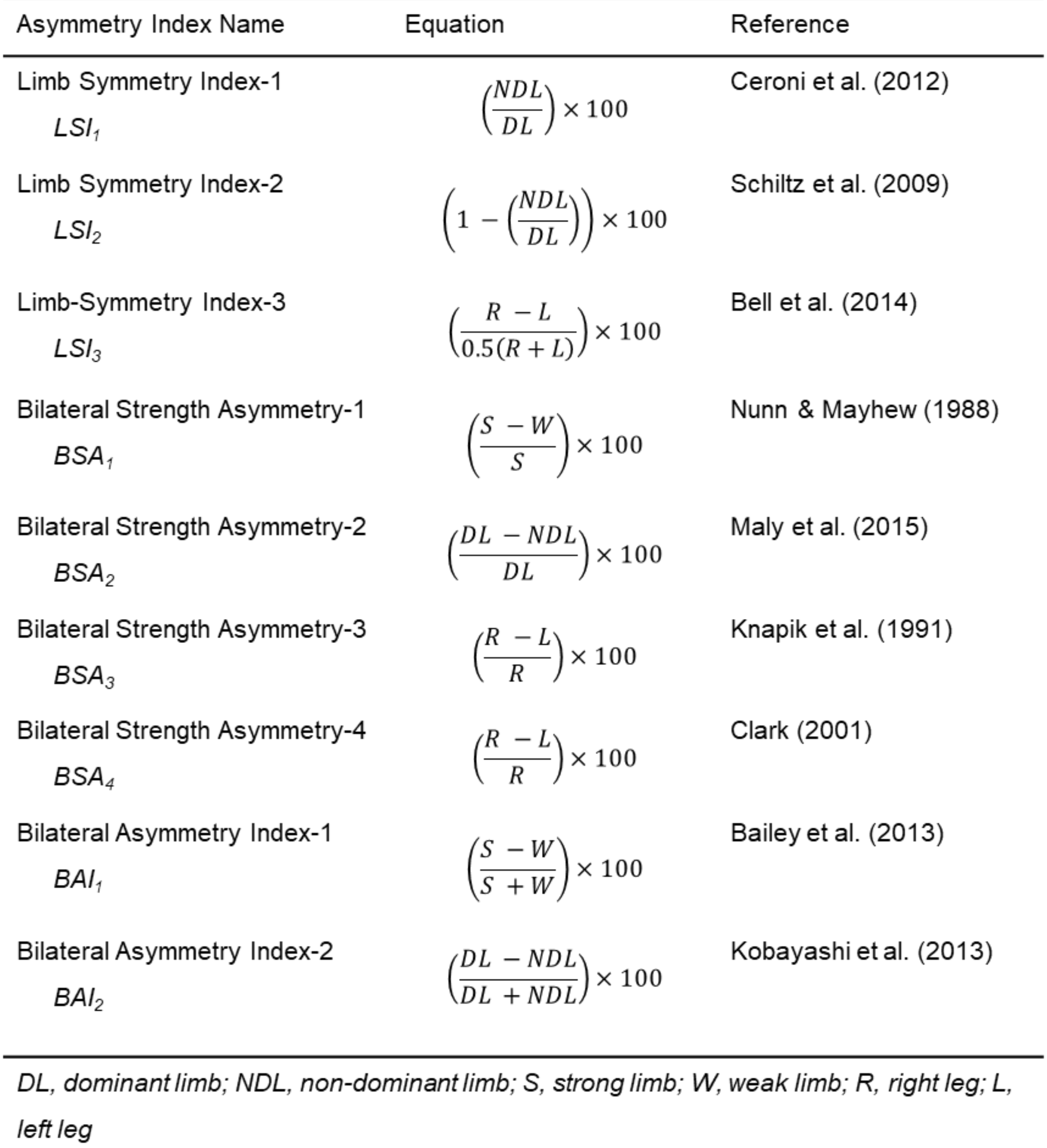
5) Utilise ITTAs as a disuse model to investigate the associations between long-term disuse and factors such as protein synthesis, muscle morphology, and metabolic health. Furthermore, imaging methodologies such as MRI could be used to quantify the effect of long-term disuse on internal muscle-tendon forces.

Appendix A

Calculation of Asymmetry

Strength asymmetries are typically reported as a percentage, with limbs split as dominant/ non-dominant, left/ right, strong/ weak in order to provide a reference limb for the calculation. Table A.1 gives the seven equations that have been used to calculate strength asymmetries. Note that each equation has been given an acronym as suggested by Bishop et al. (2016, 2018) to differentiate between calculations, as the available literature often refers to different equations by the same name.

Table A1. Equations that have been used in literature to calculated strength asymmetries. Adapted from Bishop et al. (2016, 2018).



Asymmetry Index Name	Equation	Reference
Limb Symmetry Index-1 *LSI₁*	$\left(\frac{NDL}{DL}\right) \times 100$	Ceroni et al. (2012)
Limb Symmetry Index-2 *LSI₂*	$\left(1 - \left(\frac{NDL}{DL}\right)\right) \times 100$	Schiltz et al. (2009)
Limb-Symmetry Index-3 *LSI₃*	$\left(\frac{R - L}{0.5(R + L)}\right) \times 100$	Bell et al. (2014)
Bilateral Strength Asymmetry-1 *BSA₁*	$\left(\frac{S - W}{S}\right) \times 100$	Nunn & Mayhew (1988)
Bilateral Strength Asymmetry-2 *BSA₂*	$\left(\frac{DL - NDL}{DL}\right) \times 100$	Maly et al. (2015)
Bilateral Strength Asymmetry-3 *BSA₃*	$\left(\frac{R - L}{R}\right) \times 100$	Knapik et al. (1991)
Bilateral Strength Asymmetry-4 *BSA₄*	$\left(\frac{R - L}{R}\right) \times 100$	Clark (2001)
Bilateral Asymmetry Index-1 *BAI₁*	$\left(\frac{S - W}{S + W}\right) \times 100$	Bailey et al. (2013)
Bilateral Asymmetry Index-2 *BAI₂*	$\left(\frac{DL - NDL}{DL + NDL}\right) \times 100$	Kobayashi et al. (2013)

DL, dominant limb; NDL, non-dominant limb; S, strong limb; W, weak limb; R, right leg; L, left leg

A.1 Importance of Reference Limb

For example, Bilateral Strength Asymmetry-1 (Table A.1) distinguishes between limbs as strong and weak. However, the same equation has also been used in the literature but with the limbs split as dominant and non-dominant (Bilateral Strength Asymmetry-2). In a situation where the participant's strong limb is not the dominant, the choice of calculation will give two different asymmetry values.

Consider a participant who has a maximum strength score of 200 Nm for their right leg, and 150 Nm for their left. However, they have identified the left as their dominant limb.

$$BSA_1\ (\%) = \frac{200 - 150}{200} \times 100\ = 25.0\%$$

$$BSA_2\ (\%) = \frac{150 - 200}{150} \times 100\ = -33.3\%$$

The different results given by what is essentially the same equation highlights the importance of the careful selection of reference limb. Further discussion of reference limb selection will be given in context of the individual equations in Section A.2 below.

A.2 The Difference between Asymmetry Calculations

Conversely, some equations give the same asymmetry value, regardless of the differences in the equation.

$$LSI_2 = \left(1\ - \left(\frac{150}{200}\right)\right) \times 100 =\ -33.3\%$$

Other equations will give still different values.

$$LSI_3 = \left(\frac{200\ - 150}{0.5(200 + 150)}\right) \times 100 = 28.5\%$$

$$BAI_1 = \left(\frac{200\ - 150}{200 + 150}\right) \times 100 = 14.2\%$$

Consideration therefore needs to be given when selecting the equation for use.

A.3 Interpretation of Asymmetry Calculations

Equations that produce the same result have been grouped together for further discussion. Sections A.3.1 and A.3.2 will discuss equations that could potentially be used to quantify asymmetries in a unilateral task, given that force is produced solely on the designated test leg potentially providing a more accurate representation of ‘true’ inter-limb asymmetry. Section A.3.3 will discuss equations that could be used to quantify asymmetries in a bilateral task, whereby the influence of the contralateral limb must be taken into account (Bishop et al. 2018).

A.3.1 LSI_1, LSI_2, BSA_1, BSA_2

LSI_1 used by Ceroni et al. (2012) gives a value of limb symmetry rather than asymmetry. When compared with LSI_2 (Schiltz et al. 2009) and BSA_2 (Maly et al. 2015), it gives a value at the opposite end of the ‘asymmetry spectrum’. Perfect symmetry is represented by 0% for LSI_2, BSA_1, BSA_2, but 100% for LSI_1. For the earlier example, while LSI_2 and BSA_2 give an asymmetry score of 25%, LSI_1 gives a score of 75%.

BSA_1 (Impellizzeri et al. 2007, Nunn & Mayhew 1988) differs from the other equations purely as a result of using a different reference limb. Bishop et al. (2016) suggests that always putting the strong limb first (i.e. BSA_1) will pose problems for longitudinal analysis due to this always resulting in a positive value. They argue that a later testing date the strong limb could become weaker (e.g. as a result of injury, or exposure to training), and the criteria in this equation do not take this into account. This could have implications for reliability of the asymmetry measure. Furthermore, having only positive values for asymmetry would be problematic when calculating percentiles, as the distribution would be skewed. Classifying limbs by left/ right or dominant/non-dominant, in contrast, would result in a negative value if the stronger limb changed. However, classifying limbs by dominance has its own problems. Limb dominance is used to standardise limb comparisons. The most common method to determine limb dominance is by using self-reported kicking leg preference, although the validity of this in situations other than where kicking is a key demand (e.g. football, kickboxing, some rugby positions) has been questioned. Kicking preference may not accurately categorise limb dominance in relation to strength, power, proprioception, or other variables that relate to biomechanical movement strategies (Mulrey et al. 2018). Perhaps because of this, Schlumberger et al. (2006) have suggested that limb dominance should not be used for classifying asymmetry, as it may not have sufficient external validity.

A.3.2 LSI_3

In order to circumvent some of the issues surrounding the definition of limb dominance, LSI_3 (Bell et al. 2014) defines inter-limb asymmetry as between right and left. However, as limb dominance is known to be task-specific (Maloney et al. 2019), and laterality (i.e. the preferential use of one side of the body when presented with a motor task to perform) manifests dependent on sport-specific demands (Maloney 2018), some sports (e.g. fencing) which are very asymmetrical in nature are likely to dictate which limb is dominant in key sport-specific actions. In these contexts, grouping limbs by right and left may not give a true account of the inter-limb asymmetry present in the population being studied.

A.3.3 BAI_1, BAI_2

These two equations produce substantially smaller asymmetry scores than any of the previously discussed methods. However, unlike the above equations, which account for the lack of contribution from the opposing limb in a unilateral task, these equations are useful when considering the asymmetry in a bilateral task such as a CMJ or drop landing (Chapter 5), as the differences in force between limbs are always relative to the sum force value.

Appendix B

Data Tables (Figure 2.10)

Table B1. Summary of studies investigating the effect of unloading on knee extensor muscle strength as depicted in Figure 2.10. Adapted from Narici & de Boer (2011).

Model of Unloading	Reference	Duration (Days)	% Strength Decrease
Bed rest	Alkner & Tesch (2004)	45	45
	Bamman et al. (1998)	15	15
	Berg et al. (1997)	24	24
	Berg et al. (2007)	20	20
	Dirks et al. (2016)	7	8
	Dudley et al. (1989)	19	19
	Funato et al. (1997)	26	26
	Gogia et al. (1988)	19	19
	Kawakami et al. (2001)	11	11
	Kubo et al. (2004)	20	20
	Kubo et al. (2000)	19	19
	LeBlanc et al. (1992)	31	31
	Mulder et al. (2006)	17	17
	Trappe et al. (2004)	43	43
Limb Immobilisation	Dirks et al. (2013)	5	9
	Hespel et al. (2001)	22	22
	Hortobagyi et al. (2000)	45	45
	Rozier et al. (1979)	13	13
	Suetta et al. (2012)	4	13
		14	20
	Thom et al. (2001)	42	42
	Velduizen et al. (1993)	53	53
	Wall et al. (2013)	14	23
ULLS	Adams et al. (1994)	12	12
	Berg & Tesch (1996)	13	13
	Berg et al. (1993)	17	17
	Berg et al. (1991)	20	20
	Campbell et al. (2013)	26	26
	Clark et al. (2006)	17	17
	Clark et al. (2007)	25	25
	de Boer et al. (2007)	15	15
		21	21
	Deschenes et al. (2002)	22	22
	Dudley et al. (1992)	42	42
	Gamrin et al. (1998)	17	17
	Horstman et al. (2012)	21	21
	Ploutz-Snyder et al. (1995)	35	35
	Schulz et al. (2002)	17	17

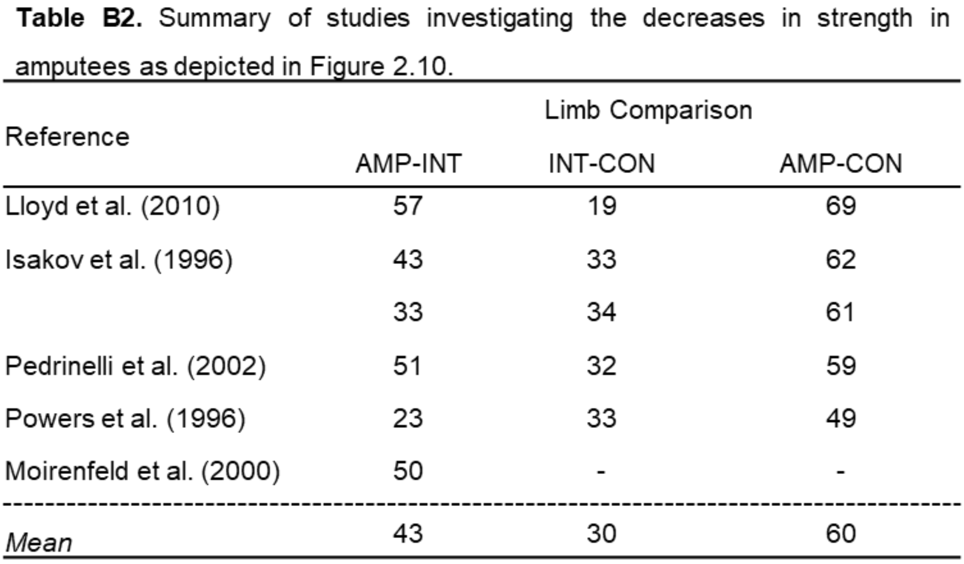


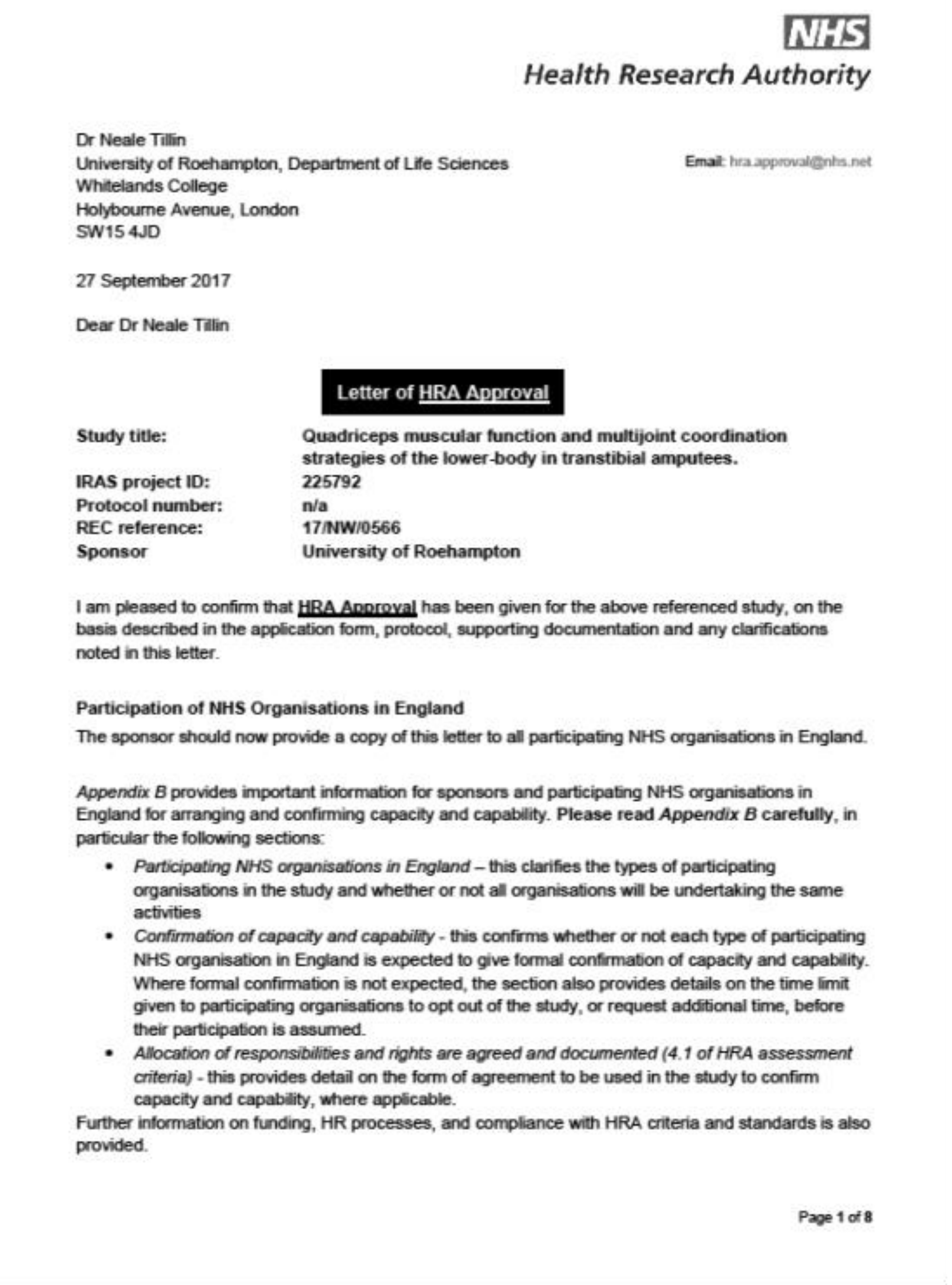
Table B2. Summary of studies investigating the decreases in strength in amputees as depicted in Figure 2.10.

Reference	Limb Comparison		
	AMP-INT	INT-CON	AMP-CON
Lloyd et al. (2010)	57	19	69
Isakov et al. (1996)	43	33	62
	33	34	61
Pedrinelli et al. (2002)	51	32	59
Powers et al. (1996)	23	33	49
Moirenfeld et al. (2000)	50	-	-
Mean	43	30	60

Appendix C

Ethical Approval

The research for this project was submitted for ethics consideration under the reference LSC 16/176 in the Department of Life Sciences and was approved under the procedures of the University of Roehampton's Ethic Committee on 11th July 2016.



NHS
Health Research Authority

Dr Neale Tillin
University of Roehampton, Department of Life Sciences
Whitelands College
Holybourne Avenue, London
SW15 4JD

Email: hra.approval@nhs.net

27 September 2017

Dear Dr Neale Tillin

Letter of HRA Approval

Study title:	**Quadriceps muscular function and multijoint coordination strategies of the lower-body in transtibial amputees.**
IRAS project ID:	**225792**
Protocol number:	**n/a**
REC reference:	**17/NW/0566**
Sponsor	**University of Roehampton**

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

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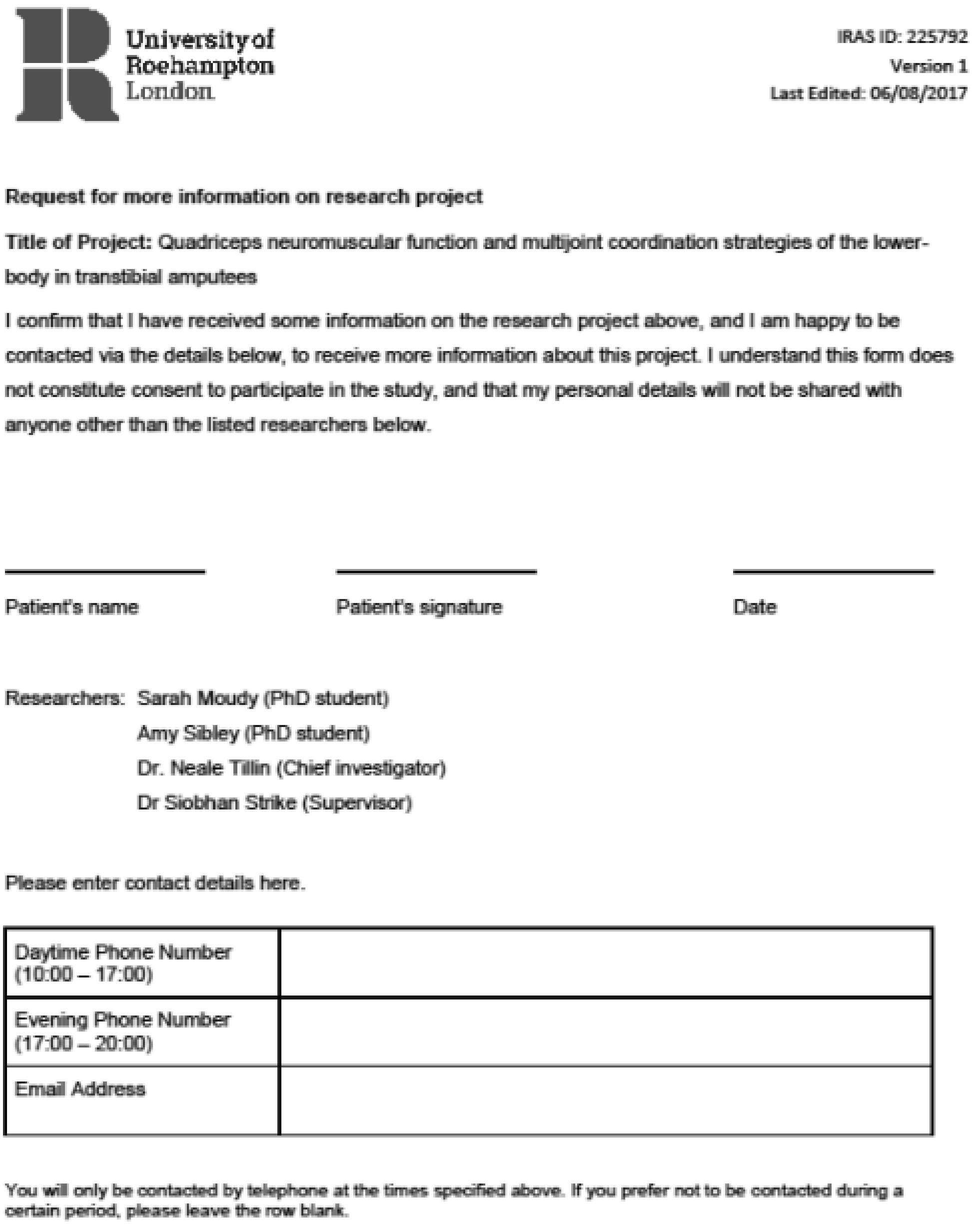
Appendix D

Participant Documentation



University of Roehampton London

IRAS ID: 225792
Version 1
Last Edited: 06/08/2017

Request for more information on research project

Title of Project: Quadriceps neuromuscular function and multijoint coordination strategies of the lower-body in transtibial amputees

I confirm that I have received some information on the research project above, and I am happy to be contacted via the details below, to receive more information about this project. I understand this form does not constitute consent to participate in the study, and that my personal details will not be shared with anyone other than the listed researchers below.

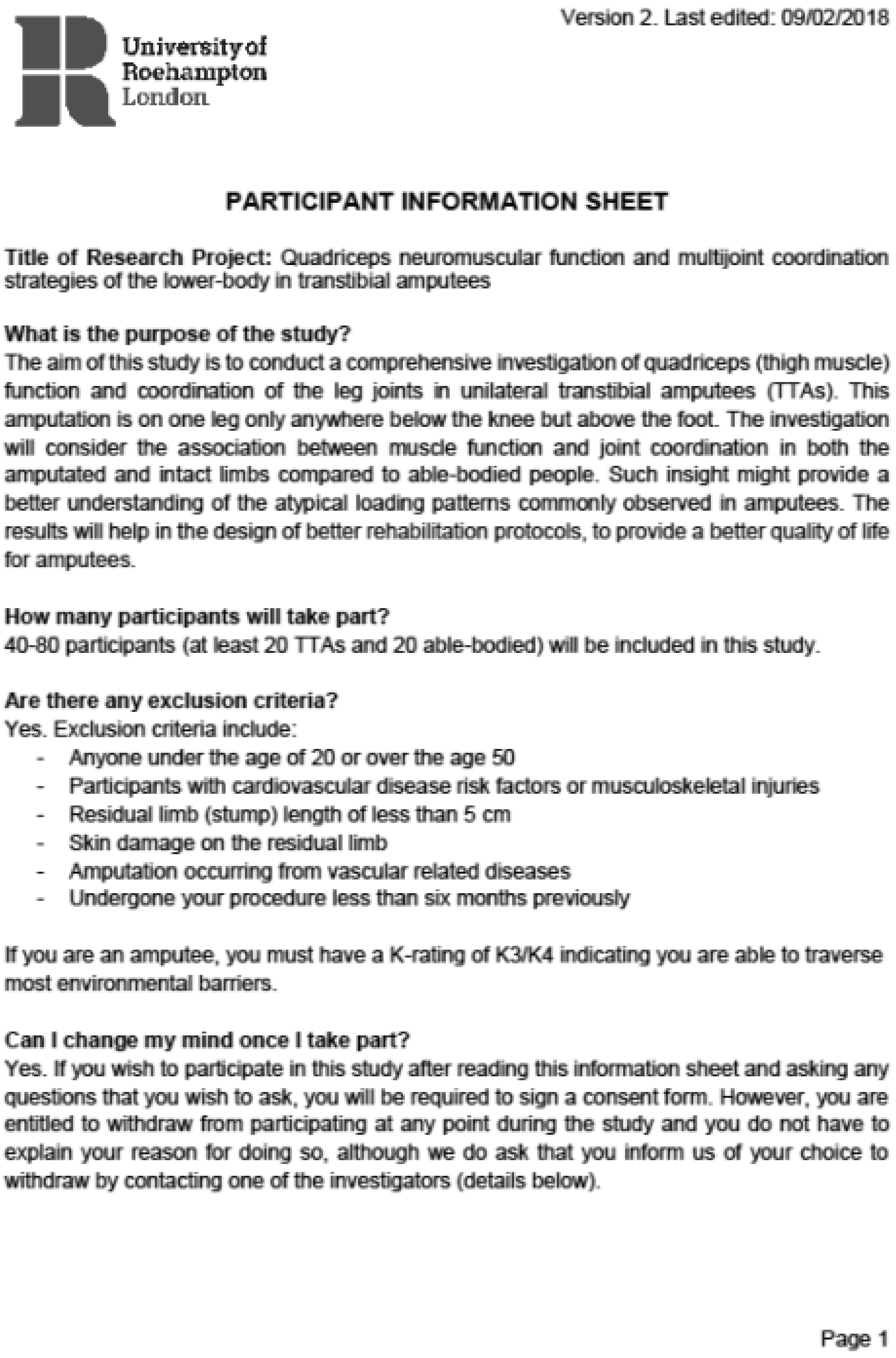
Patient's name	Patient's signature	Date

Researchers: Sarah Moudy (PhD student)
Amy Sibley (PhD student)
Dr. Neale Tillin (Chief investigator)
Dr Siobhan Strike (Supervisor)

Please enter contact details here.

Daytime Phone Number (10:00 – 17:00)	
Evening Phone Number (17:00 – 20:00)	
Email Address	

You will only be contacted by telephone at the times specified above. If you prefer not to be contacted during a certain period, please leave the row blank.



Version 2. Last edited: 09/02/2018

University of
Roehampton
London

PARTICIPANT INFORMATION SHEET

Title of Research Project: Quadriceps neuromuscular function and multijoint coordination strategies of the lower-body in transtibial amputees

What is the purpose of the study?

The aim of this study is to conduct a comprehensive investigation of quadriceps (thigh muscle) function and coordination of the leg joints in unilateral transtibial amputees (TTAs). This amputation is on one leg only anywhere below the knee but above the foot. The investigation will consider the association between muscle function and joint coordination in both the amputated and intact limbs compared to able-bodied people. Such insight might provide a better understanding of the atypical loading patterns commonly observed in amputees. The results will help in the design of better rehabilitation protocols, to provide a better quality of life for amputees.

How many participants will take part?

40-80 participants (at least 20 TTAs and 20 able-bodied) will be included in this study.

Are there any exclusion criteria?

Yes. Exclusion criteria include:

- Anyone under the age of 20 or over the age 50
- Participants with cardiovascular disease risk factors or musculoskeletal injuries
- Residual limb (stump) length of less than 5 cm
- Skin damage on the residual limb
- Amputation occurring from vascular related diseases
- Undergone your procedure less than six months previously

If you are an amputee, you must have a K-rating of K3/K4 indicating you are able to traverse most environmental barriers.

Can I change my mind once I take part?

Yes. If you wish to participate in this study after reading this information sheet and asking any questions that you wish to ask, you will be required to sign a consent form. However, you are entitled to withdraw from participating at any point during the study and you do not have to explain your reason for doing so, although we do ask that you inform us of your choice to withdraw by contacting one of the investigators (details below).

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How many times will I visit the lab?

You will visit the biomechanics laboratory (B047) at Roehampton University on three separate occasions. The lab is on the basement level of Parkstead House. There is lift access to the lab. Each visit will last between two to three hours.

Is there anything I need to do before I visit the lab?

Please refrain from drinking alcohol for 24 hours, or undertaking any strenuous exercise for 36 hours prior to each laboratory visit. Please avoid using body lotions/ moisturisers on the day of your visit as these products make it difficult to maintain adequate contact between the measurement equipment and your skin.

Please bring a pair of loose fitting shorts with you to wear during the first two lab sessions. Running or football shorts are ideal. For females, non-underwired bras are recommended to prevent discomfort in the strength testing chair. For the third session, tight fitting shorts, training top and training shoes should be worn. Females are requested to wear sports bra where possible, as this will allow for more comfortable execution of the movements required. There are private changing rooms and showers at the lab that are available for your use.

Please try to avoid clothing with reflective markings on them as these can interfere with the cameras we'll be using during your third visit. As it is necessary for the cameras to see all of the reflective markers throughout the movement, tops/t-shirts will either need to be taped up to waist height or removed during data collection in order that the view of markers on your torso is not obstructed. When data is not being collected, you may remain fully clothed.

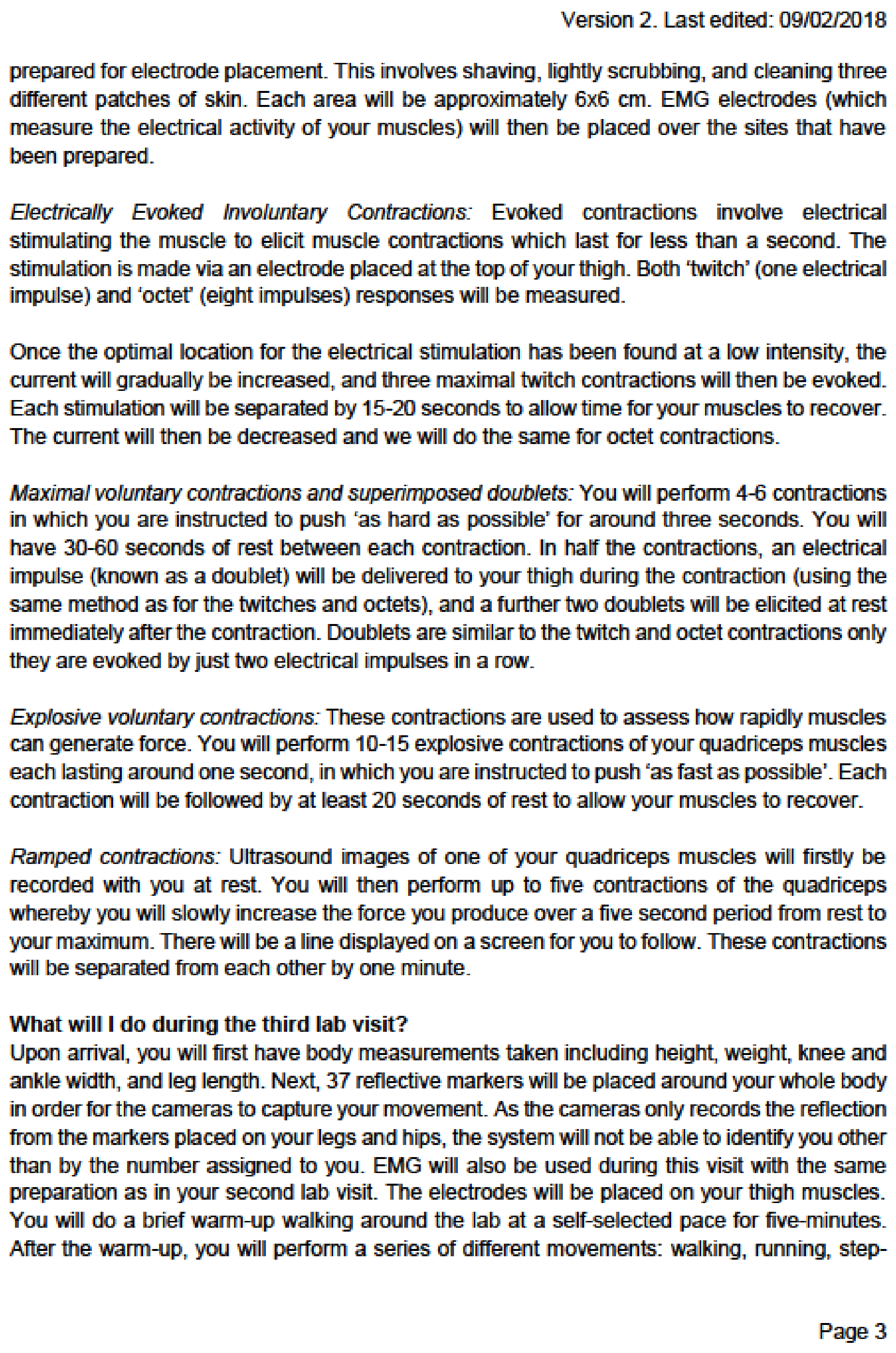
What will I do during the first lab visit?

The first visit will be used to familiarise yourself with the protocols and measurements that will be taken during subsequent visits. To begin, you will be verbally taken through the procedures for each of the visits. Health/activity questionnaires and consent forms will be completed during this time and are expected to take no longer than 15 minutes to complete. Amputees will also need to complete a questionnaire giving details of their amputation history. You will then perform a series of voluntary and electrically evoked involuntary isometric (static) contractions of the thigh (quadriceps) muscles in a strength testing chair, similar to a knee extension resistance exercise machine that you might find in a gym. Full information about the procedures in the strength testing chair is given below. In this initial visit, data will be collected following the protocol outlined below – this is a chance for you to get to know the measurements (including electrical stimulation) that will be taken during your second visit.

What will I do during the second lab visit?

You will be seated in the strength testing chair, and will perform a warm-up of contractions that gradually increase in force for approximately two minutes. Procedures for the lab visits are as follows:

EMG skin preparation and electrode placement: This will take place for your second session only. When you first arrive in the lab, the skin over the surface of three thigh muscles will be



prepared for electrode placement. This involves shaving, lightly scrubbing, and cleaning three different patches of skin. Each area will be approximately 6x6 cm. EMG electrodes (which measure the electrical activity of your muscles) will then be placed over the sites that have been prepared.

Electrically Evoked Involuntary Contractions: Evoked contractions involve electrical stimulating the muscle to elicit muscle contractions which last for less than a second. The stimulation is made via an electrode placed at the top of your thigh. Both 'twitch' (one electrical impulse) and 'octet' (eight impulses) responses will be measured.

Once the optimal location for the electrical stimulation has been found at a low intensity, the current will gradually be increased, and three maximal twitch contractions will then be evoked. Each stimulation will be separated by 15-20 seconds to allow time for your muscles to recover. The current will then be decreased and we will do the same for octet contractions.

Maximal voluntary contractions and superimposed doublets: You will perform 4-6 contractions in which you are instructed to push 'as hard as possible' for around three seconds. You will have 30-60 seconds of rest between each contraction. In half the contractions, an electrical impulse (known as a doublet) will be delivered to your thigh during the contraction (using the same method as for the twitches and octets), and a further two doublets will be elicited at rest immediately after the contraction. Doublets are similar to the twitch and octet contractions only they are evoked by just two electrical impulses in a row.

Explosive voluntary contractions: These contractions are used to assess how rapidly muscles can generate force. You will perform 10-15 explosive contractions of your quadriceps muscles each lasting around one second, in which you are instructed to push 'as fast as possible'. Each contraction will be followed by at least 20 seconds of rest to allow your muscles to recover.

Ramped contractions: Ultrasound images of one of your quadriceps muscles will firstly be recorded with you at rest. You will then perform up to five contractions of the quadriceps whereby you will slowly increase the force you produce over a five second period from rest to your maximum. There will be a line displayed on a screen for you to follow. These contractions will be separated from each other by one minute.

What will I do during the third lab visit?

Upon arrival, you will first have body measurements taken including height, weight, knee and ankle width, and leg length. Next, 37 reflective markers will be placed around your whole body in order for the cameras to capture your movement. As the cameras only records the reflection from the markers placed on your legs and hips, the system will not be able to identify you other than by the number assigned to you. EMG will also be used during this visit with the same preparation as in your second lab visit. The electrodes will be placed on your thigh muscles. You will do a brief warm-up walking around the lab at a self-selected pace for five-minutes. After the warm-up, you will perform a series of different movements: walking, running, step-descent, and jumping. You will be given sufficient time to practice each movement being examined.

Walking: For each trial, you will walk at a self-selected speed (determined by your practice walks) along a 10m walkway. In order to maintain the same pace, we will instruct you to walk either faster or slower after each pass. Around 20-30 trials will be collected.

Step-Descent: This movement will be performed on a custom-built platform that is the same height as a standard kerb. You will walk at a self-selected speed (same as your walking speed) across the platform and step down onto the ground and continue walking. The movement will be performed by both limbs stepping off the platform and you will complete around 10-15 trials for each leg.

Running: Similar to walking, you will run along the same 10 m walkway at a pace necessary to complete 5 km in 30-35 min. You will not be asked to run 5 km, only to run the length of the 10-m walkway. You will be instructed to either increase or decrease the pace after each pass. Around 20-30 trials will be collected.

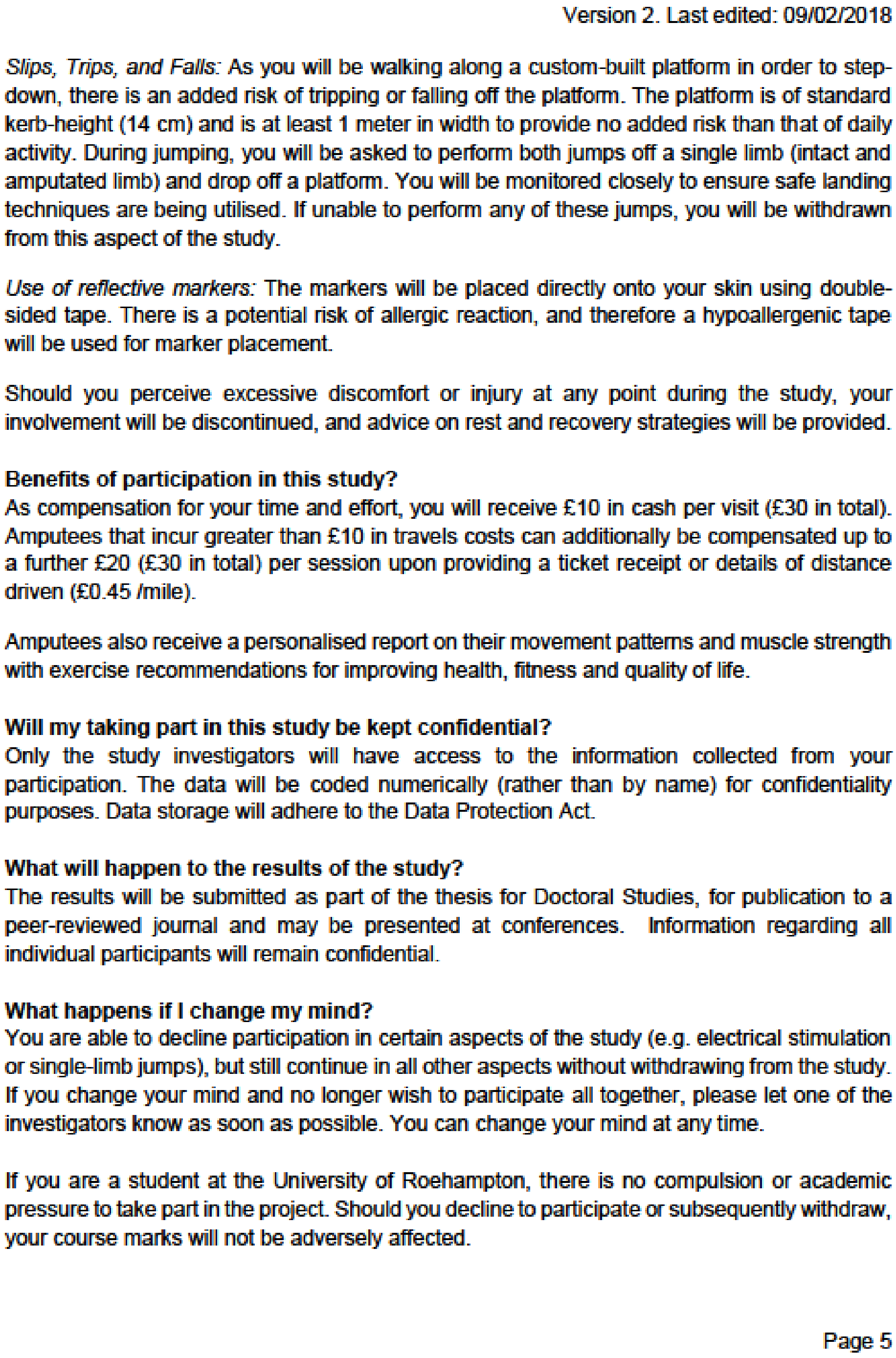
Jumping: You will be performing two different types of jumps: maximal effort vertical jumps and a drop landing. The vertical jumps will be performed firstly with both limbs pushing off the floor (without using your arms), and then also using each limb singly in turn to complete the movement. The drop landings will involve you hanging from a bar by your hands, and letting go to drop to the floor. The bar will be moved so that your feet are 25 cm from the ground. You will be allowed to practice these jumps before data collection takes place. Data from three successful (full recovery after landing) maximal effort jumps and drop landings will be collected.

Are there any risks or side effects in participating?

Muscle soreness and strain: This may occur after the measurement sessions and last for up to 72 hours. The risk and severity of muscle soreness will be no greater than that experienced with physical exercise performed for recreation and health (e.g., playing a team sport or resistance exercise in the gym), and should not prevent you from performing normal daily activities.

Electromyography skin preparation: Shaving and cleaning your skin may cause soreness and redness, similar to that experienced when shaving other areas of the body, but these effects should wear off within a couple of days.

Involuntary contractions: The electrical stimulation procedures involved in the involuntary contractions can be uncomfortable at high intensities, such as those that will be used during the octet contractions of this study, but this discomfort is very short term (i.e., lasts for <1 second), and there are no known long term side-effects apart from potential muscle soreness. There is a very small risk of you experiencing nausea or dizziness/ faintness as a result of this stimulation. If this occurs, you will be withdrawn from any further stimulation aspects of the study.



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Slips, Trips, and Falls: As you will be walking along a custom-built platform in order to step-down, there is an added risk of tripping or falling off the platform. The platform is of standard kerb-height (14 cm) and is at least 1 meter in width to provide no added risk than that of daily activity. During jumping, you will be asked to perform both jumps off a single limb (intact and amputated limb) and drop off a platform. You will be monitored closely to ensure safe landing techniques are being utilised. If unable to perform any of these jumps, you will be withdrawn from this aspect of the study.

Use of reflective markers: The markers will be placed directly onto your skin using double-sided tape. There is a potential risk of allergic reaction, and therefore a hypoallergenic tape will be used for marker placement.

Should you perceive excessive discomfort or injury at any point during the study, your involvement will be discontinued, and advice on rest and recovery strategies will be provided.

Benefits of participation in this study?

As compensation for your time and effort, you will receive £10 in cash per visit (£30 in total). Amputees that incur greater than £10 in travels costs can additionally be compensated up to a further £20 (£30 in total) per session upon providing a ticket receipt or details of distance driven (£0.45 /mile).

Amputees also receive a personalised report on their movement patterns and muscle strength with exercise recommendations for improving health, fitness and quality of life.

Will my taking part in this study be kept confidential?

Only the study investigators will have access to the information collected from your participation. The data will be coded numerically (rather than by name) for confidentiality purposes. Data storage will adhere to the Data Protection Act.

What will happen to the results of the study?

The results will be submitted as part of the thesis for Doctoral Studies, for publication to a peer-reviewed journal and may be presented at conferences. Information regarding all individual participants will remain confidential.

What happens if I change my mind?

You are able to decline participation in certain aspects of the study (e.g. electrical stimulation or single-limb jumps), but still continue in all other aspects without withdrawing from the study. If you change your mind and no longer wish to participate all together, please let one of the investigators know as soon as possible. You can change your mind at any time.

If you are a student at the University of Roehampton, there is no compulsion or academic pressure to take part in the project. Should you decline to participate or subsequently withdraw, your course marks will not be adversely affected.

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I have some more questions – who should I contact?

If you have any further questions please contact one of the lead investigators, Amy Sibley or Sarah Moudy. Alternatively, you may contact either the Directors of Studies for both researchers – Dr Siobhán Strike – or the Supervisor for both – Dr Neale Tillin (details below).

Amy Sibley
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What if I have any concerns and would prefer not to contact the investigator?

If you would prefer to contact someone independent to the study you should contact the head of the department of Life Sciences at Roehampton University, Dr Caroline Ross.

Dr. Caroline Ross
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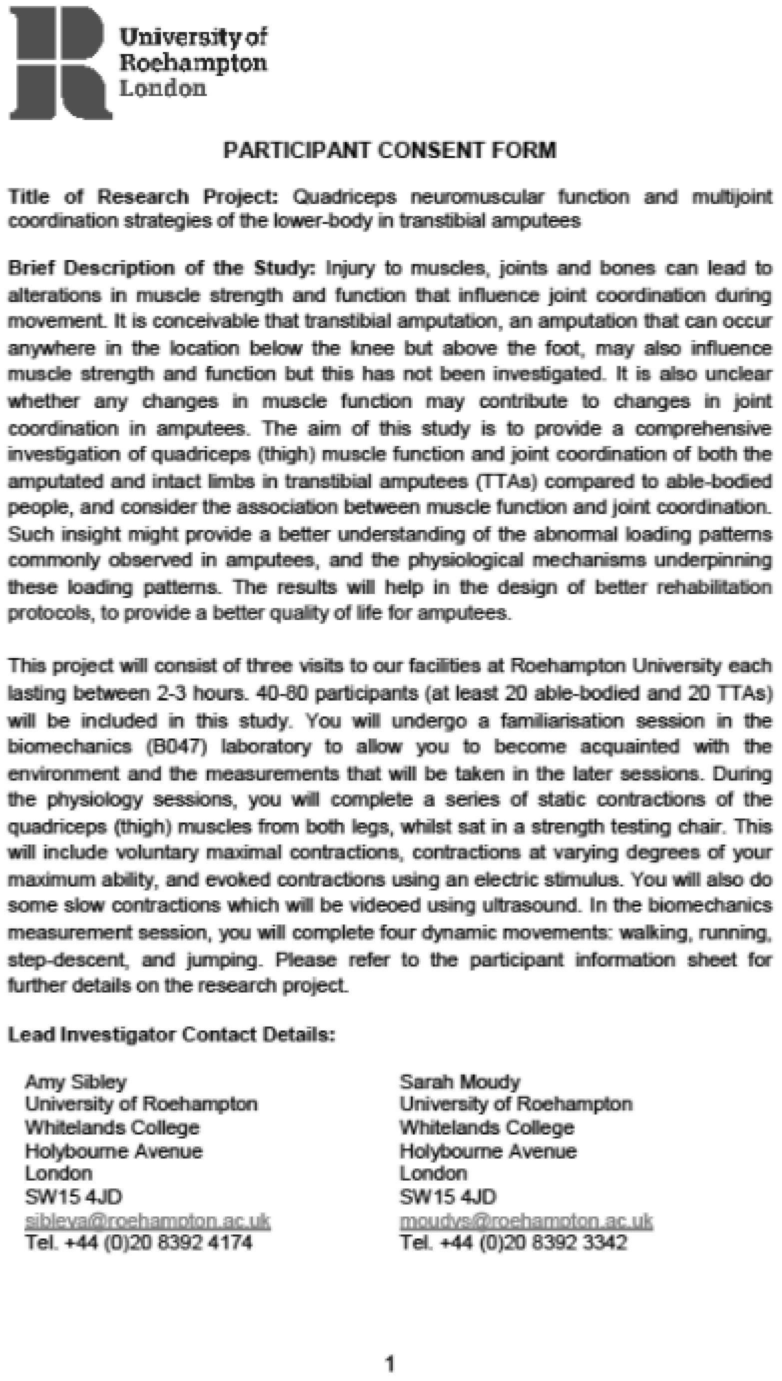

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London

PARTICIPANT CONSENT FORM

Title of Research Project: Quadriceps neuromuscular function and multijoint coordination strategies of the lower-body in transtibial amputees

Brief Description of the Study: Injury to muscles, joints and bones can lead to alterations in muscle strength and function that influence joint coordination during movement. It is conceivable that transtibial amputation, an amputation that can occur anywhere in the location below the knee but above the foot, may also influence muscle strength and function but this has not been investigated. It is also unclear whether any changes in muscle function may contribute to changes in joint coordination in amputees. The aim of this study is to provide a comprehensive investigation of quadriceps (thigh) muscle function and joint coordination of both the amputated and intact limbs in transtibial amputees (TTAs) compared to able-bodied people, and consider the association between muscle function and joint coordination. Such insight might provide a better understanding of the abnormal loading patterns commonly observed in amputees, and the physiological mechanisms underpinning these loading patterns. The results will help in the design of better rehabilitation protocols, to provide a better quality of life for amputees.

This project will consist of three visits to our facilities at Roehampton University each lasting between 2-3 hours. 40-80 participants (at least 20 able-bodied and 20 TTAs) will be included in this study. You will undergo a familiarisation session in the biomechanics (B047) laboratory to allow you to become acquainted with the environment and the measurements that will be taken in the later sessions. During the physiology sessions, you will complete a series of static contractions of the quadriceps (thigh) muscles from both legs, whilst sat in a strength testing chair. This will include voluntary maximal contractions, contractions at varying degrees of your maximum ability, and evoked contractions using an electric stimulus. You will also do some slow contractions which will be videoed using ultrasound. In the biomechanics measurement session, you will complete four dynamic movements: walking, running, step-descent, and jumping. Please refer to the participant information sheet for further details on the research project.

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1

Consent Statement: I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.

Name ....

Signature ....

Date ....

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with one of the lead investigators. You can also contact the Director of Studies for both researchers – Dr Siobhan Strike – or the Supervisor for both researchers – Dr Neale Tillin. However, if you would like to contact an independent party please contact the Head of Department.

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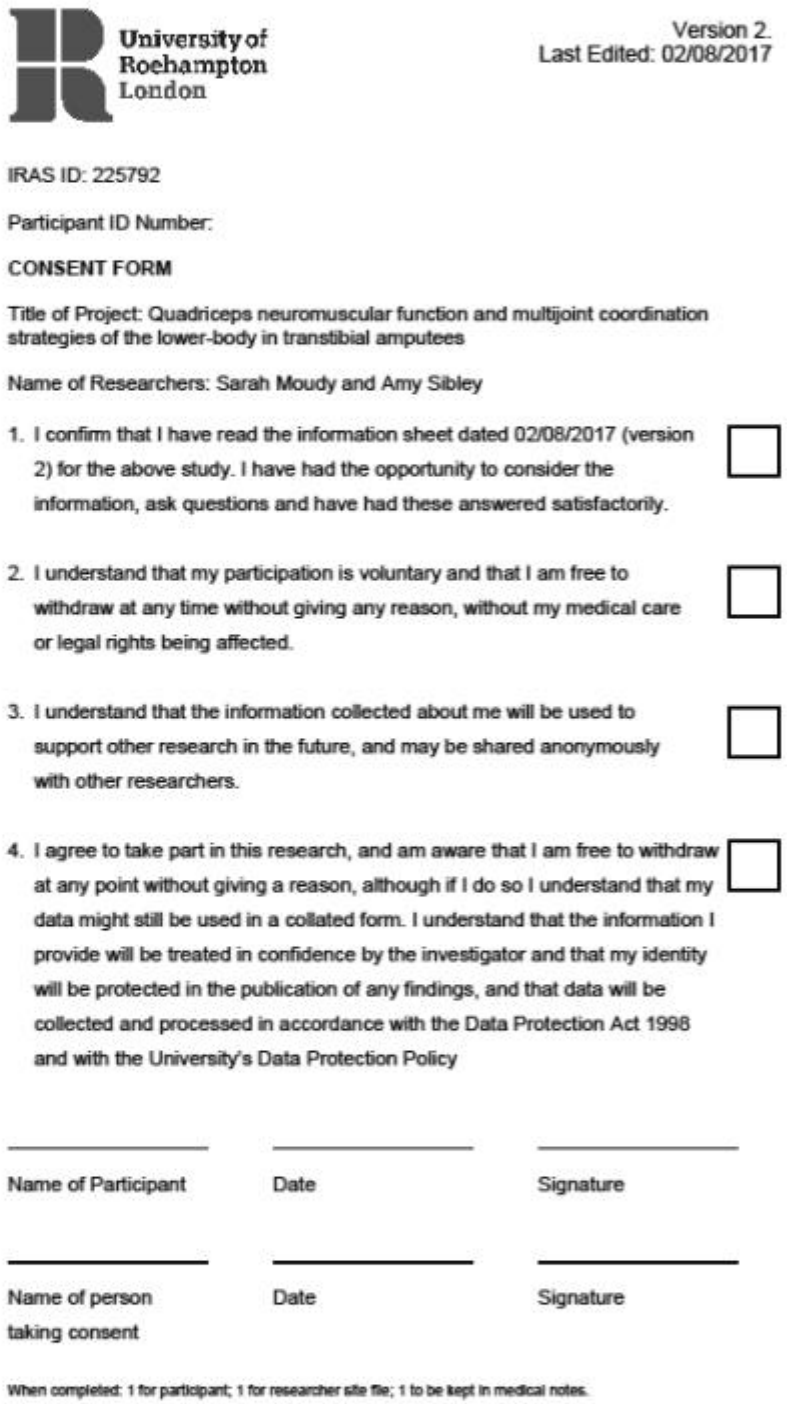

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Version 2.
Last Edited: 02/08/2017

IRAS ID: 225792

Participant ID Number:

CONSENT FORM

Title of Project: Quadriceps neuromuscular function and multijoint coordination strategies of the lower-body in transtibial amputees

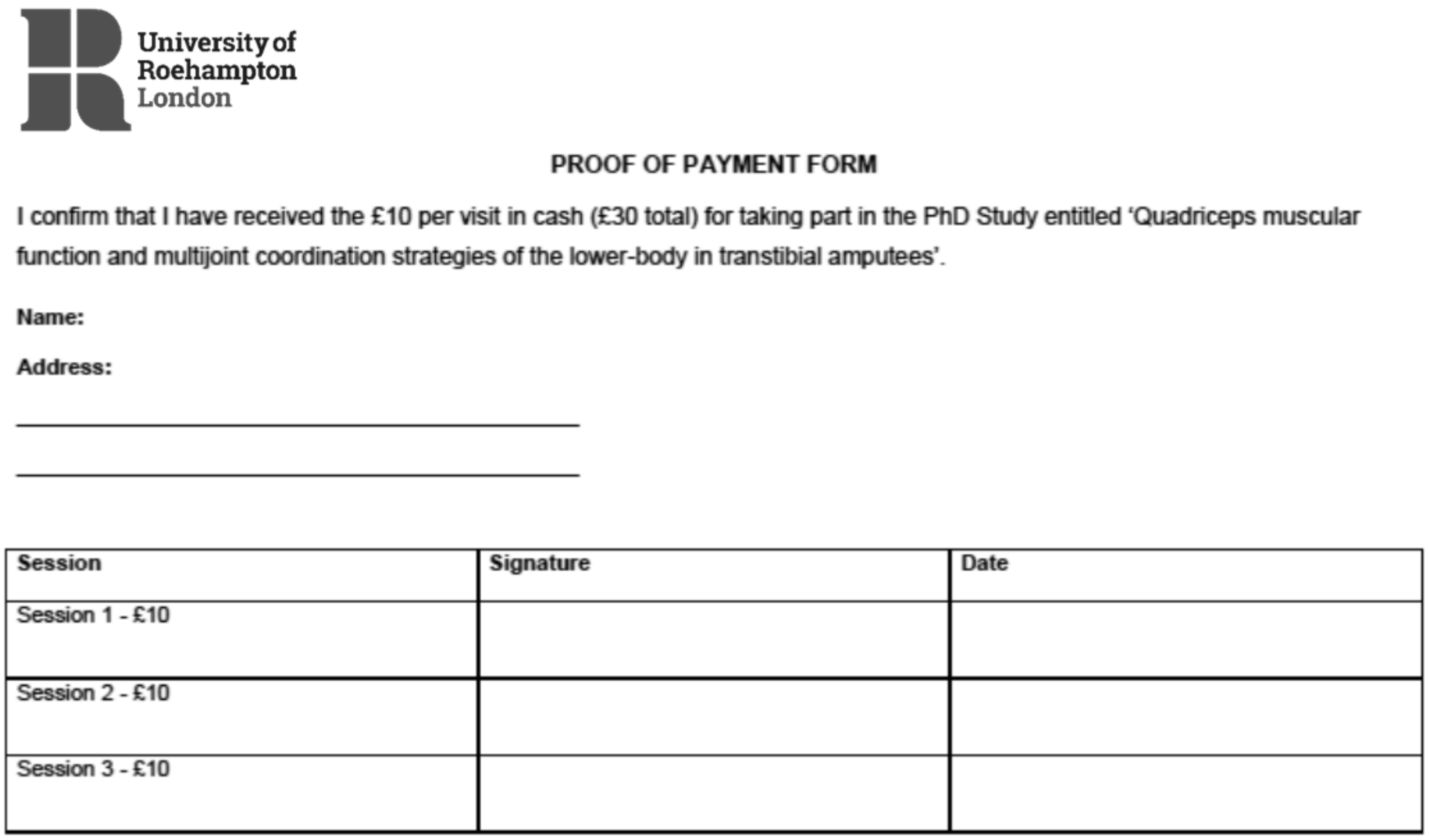
Name of Researchers: Sarah Moudy and Amy Sibley

1. I confirm that I have read the information sheet dated 02/08/2017 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
4. I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy ☐

Name of Participant	Date	Signature
Name of person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 to be kept in medical notes.

Appendix D4. Proof of Payment Form



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PROOF OF PAYMENT FORM

I confirm that I have received the £10 per visit in cash (£30 total) for taking part in the PhD Study entitled ‘Quadriceps muscular function and multijoint coordination strategies of the lower-body in transtibial amputees’.

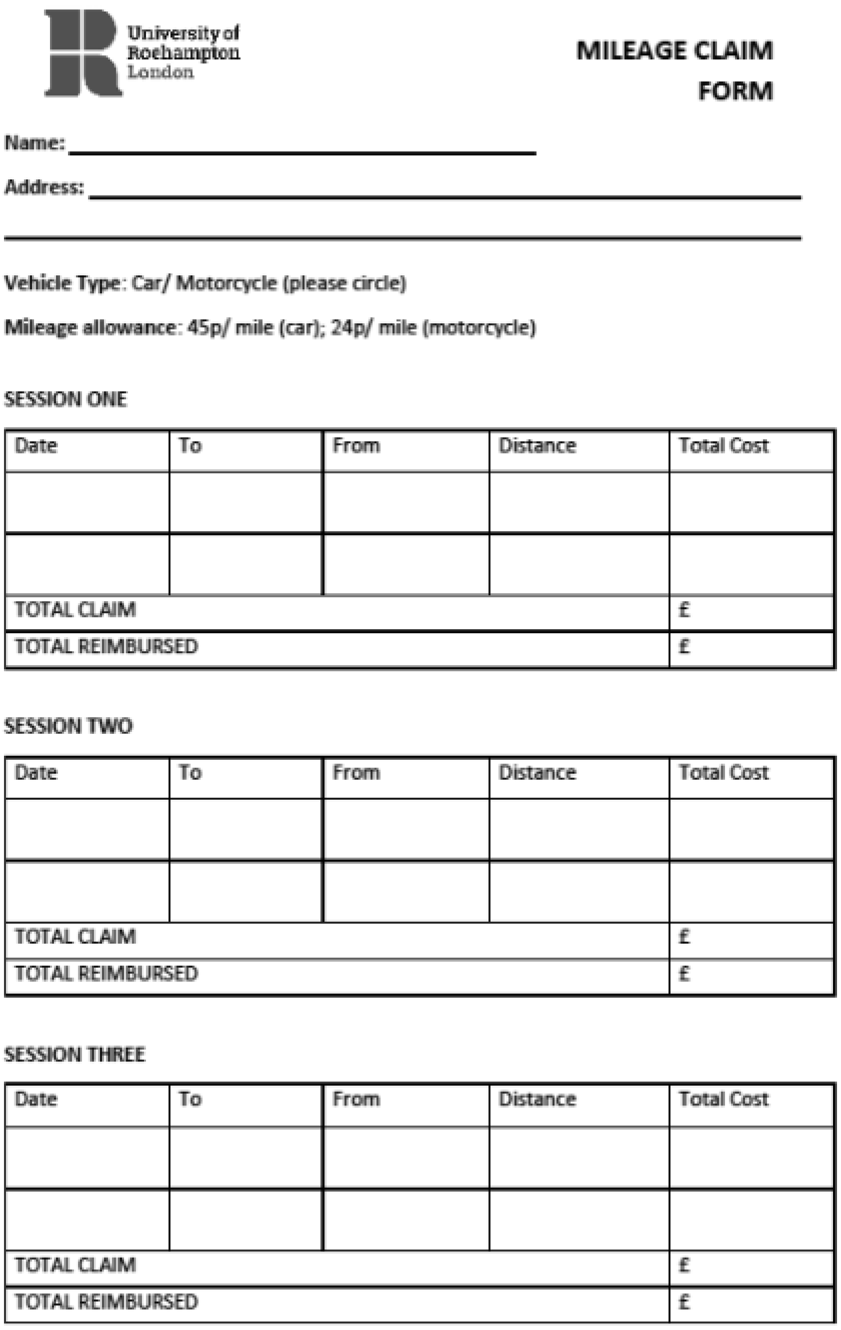
Name:

Address:

____

____

Session	**Signature**	**Date**
Session 1 - £10		
Session 2 - £10		
Session 3 - £10		



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MILEAGE CLAIM FORM

Name: ______________________________

Address: ______________________________

____

Vehicle Type: Car/ Motorcycle (please circle)

Mileage allowance: 45p/ mile (car); 24p/ mile (motorcycle)

SESSION ONE

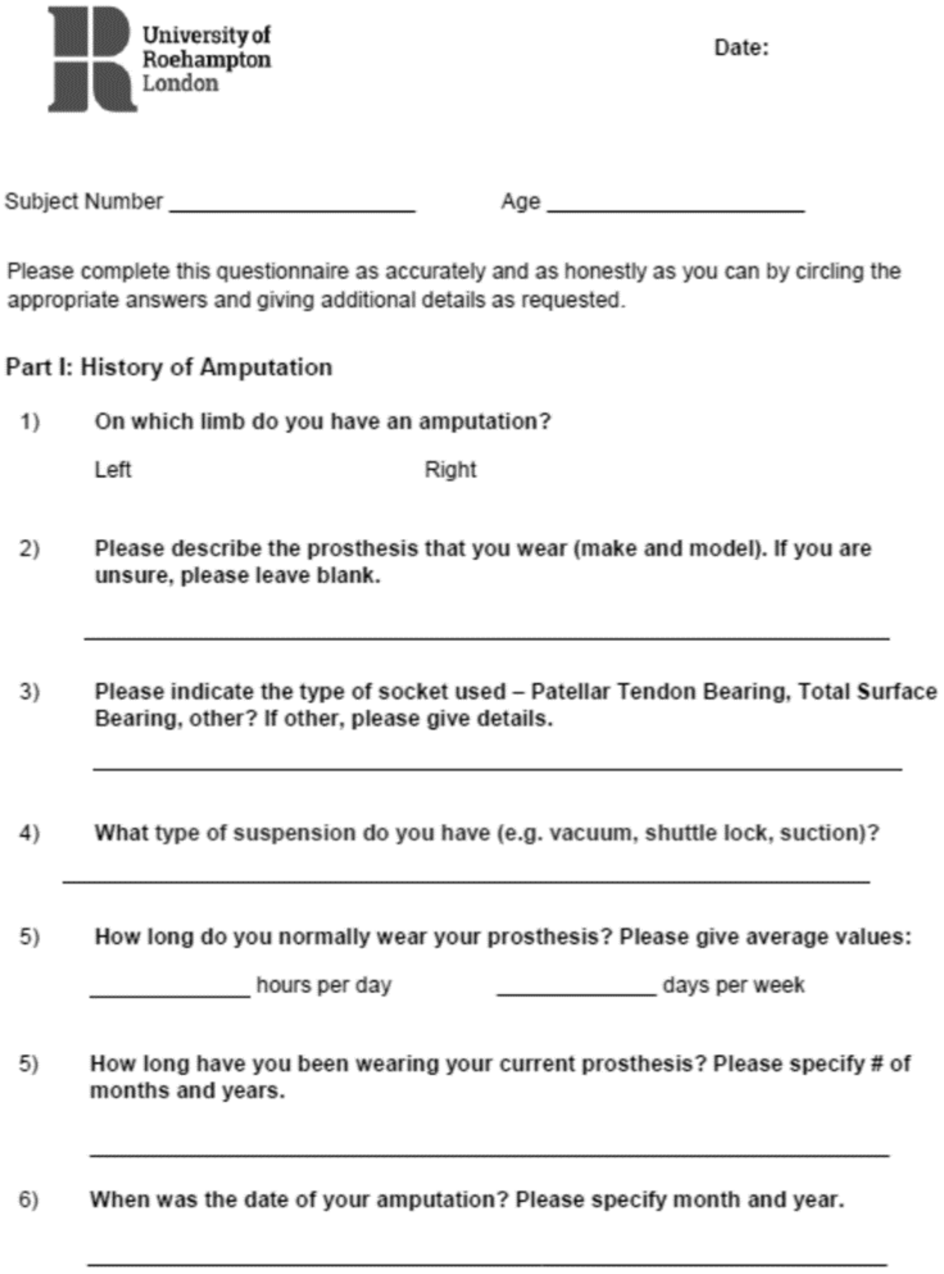
Date	To	From	Distance	Total Cost
TOTAL CLAIM				£
TOTAL REIMBURSED				£

SESSION TWO

Date	To	From	Distance	Total Cost
TOTAL CLAIM				£
TOTAL REIMBURSED				£

SESSION THREE

Date	To	From	Distance	Total Cost
TOTAL CLAIM				£
TOTAL REIMBURSED				£



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Date:

Subject Number ____________________ Age ____________________

Please complete this questionnaire as accurately and as honestly as you can by circling the appropriate answers and giving additional details as requested.

Part I: History of Amputation

1) **On which limb do you have an amputation?**

Left Right

2) **Please describe the prosthesis that you wear (make and model). If you are unsure, please leave blank.**

____

3) **Please indicate the type of socket used – Patellar Tendon Bearing, Total Surface Bearing, other? If other, please give details.**

____

4) **What type of suspension do you have (e.g. vacuum, shuttle lock, suction)?**

____

5) **How long do you normally wear your prosthesis? Please give average values:**

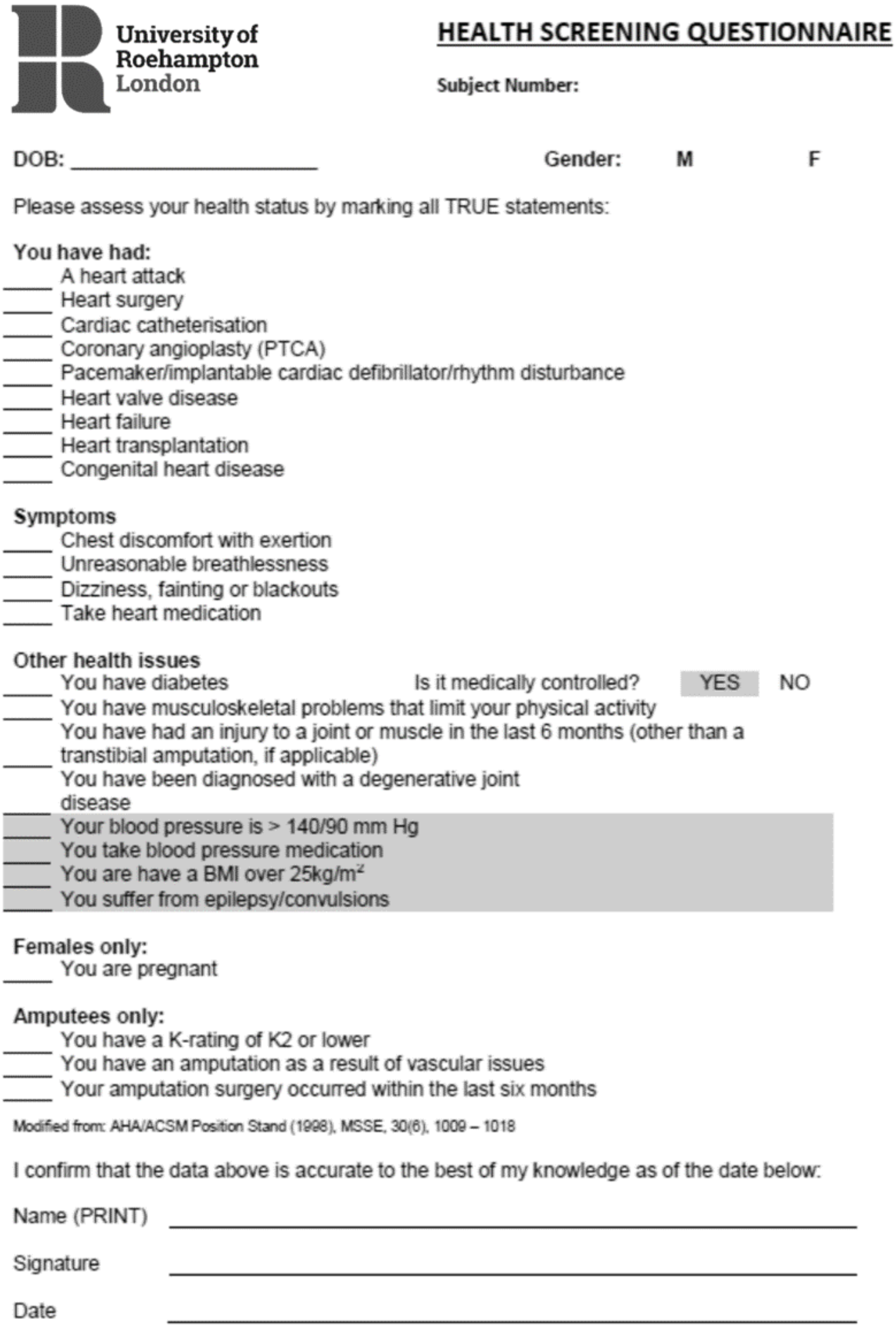
____________ hours per day ____________ days per week

5) **How long have you been wearing your current prosthesis? Please specify # of months and years.**

____

6) **When was the date of your amputation? Please specify month and year.**

____



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HEALTH SCREENING QUESTIONNAIRE

Subject Number:

DOB: ______________________ **Gender:** **M** **F**

Please assess your health status by marking all TRUE statements:

You have had:

____ A heart attack
____ Heart surgery
____ Cardiac catheterisation
____ Coronary angioplasty (PTCA)
____ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
____ Heart valve disease
____ Heart failure
____ Heart transplantation
____ Congenital heart disease

Symptoms

____ Chest discomfort with exertion
____ Unreasonable breathlessness
____ Dizziness, fainting or blackouts
____ Take heart medication

Other health issues

____ You have diabetes Is it medically controlled? YES NO
____ You have musculoskeletal problems that limit your physical activity
____ You have had an injury to a joint or muscle in the last 6 months (other than a transtibial amputation, if applicable)
____ You have been diagnosed with a degenerative joint disease
____ Your blood pressure is > 140/90 mm Hg
____ You take blood pressure medication
____ You are have a BMI over 25kg/m^2
____ You suffer from epilepsy/convulsions

Females only:

____ You are pregnant

Amputees only:

____ You have a K-rating of K2 or lower
____ You have an amputation as a result of vascular issues
____ Your amputation surgery occurred within the last six months

Modified from: AHA/ACSM Position Stand (1998), MSSE, 30(6), 1009 – 1018

I confirm that the data above is accurate to the best of my knowledge as of the date below:

Name (PRINT) ______________________________

Signature ______________________________

Date ______________________________

Appendix E

Isokinetic Dynamometer Adaptations

Figure E1.1. Posterior (A) and anterior (B) view of the custom-made, rigid knee pad for the isokinetic dynamometer. This could be tightly clamped down using the screws to remove unnecessary rotation (usually present in dynamometers to assist dynamic movement) around the shaft that connects knee pad to the crank arm.

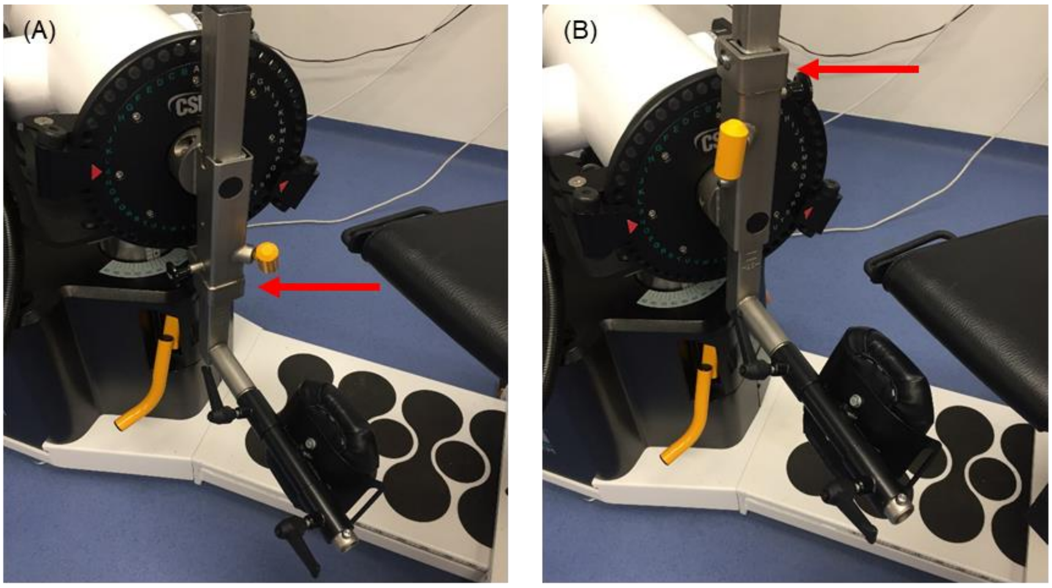

Figure E2.1. Normal (A) and flipped (B) position of the isokinetic crank arm. The red arrow indicates the bottom of the crank arm. Normal positioning was utilised for control limbs and the intact limb in ITTAs. The crank arm was flipped by 180° for the ITTA amputated limb, allowing the ankle adaptor to be placed higher to account for the shortened tibia.

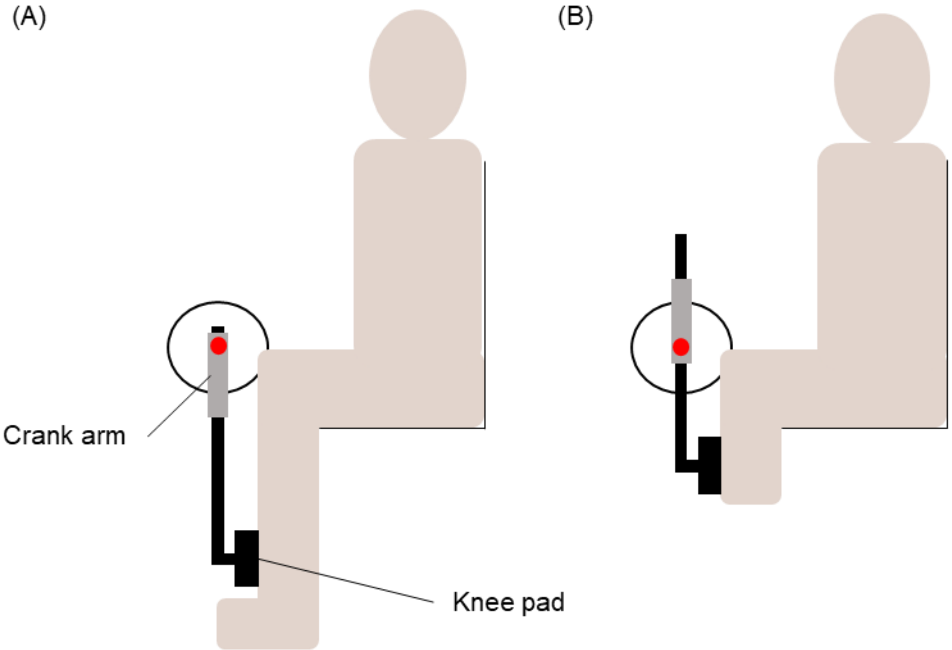

Figure E2.2. Schematic showing the placement of the knee pad and relative position of the isokinetic crank arm for (A) the control and intact limbs, and (B) the amputated limb. The red circle indicates the point of rotation of the crank arm.

Appendix F

Neuromuscular Methods Test-Retest Reliability

F.1 Rationale

The purpose of this pilot study was to determine the test-retest reliability of the methods used for assessing neuromuscular function.

F.2 Methods

Following their participation in the main study, eight able-bodied participants visited the laboratory for a fourth session to collect quadriceps strength and neuromuscular data on a single limb. This session was identical to the neuromuscular data collection session (Chapter 3, Section 3.4), other than that data were collected for the right leg only. One participant withdrew from performing octet contractions, so contractile data are presented for n = 7 (octet) and n = 8 (twitch). An issue with an EMG transducer during some testing sessions means that EMG data are presented for n = 4.

F.2.1 Statistical Analysis

Statistical analysis was completed in in SPSS version 24. Test and re-test values were correlated using Pearson's product moment correlation coefficients. Intraclass Correlation Coefficient (ICC) was also calculated. Coefficient of Variation (CV) for each variable was calculated for each participant's test-retest scores and the mean presented. Significance level was set at $p < 0.05$.

F.3 Results

Results are presented in Table F1.

Table F1. Test-retest reliability of measures of knee extensor muscle strength, assessed during voluntary isometric contractions; intrinsic contractile properties, assessed during evoked twitch and octet contractions; neural drive, assessed as VA and during voluntary contractions using EMG; and muscle architecture, assessed using B-mode ultrasonography of the Vastus Lateralis. Data are presented for n = 8 for all measures other than evoked octet (n = 7) and EMG measures (n = 4).

Measure		Attempt	Mean ± SD	Reliability coefficient		CV%	ICC (95% CI)
				r	*p*		
MVT (Nm)		Test	198 ± 75.0	0.966	<0.001	6.32	0.95 (0.78 – 0.99)
		Re-test	188 ± 62.7				
RTD (Nm.s^{-1})		Test	1645 ± 679	0.898	0.002	7.76	0.87 (0.48 – 0.97)
		Re-test	1692 ± 521				
RTD_{MVT} (MVT.s^{-1})		Test	8.48 ± 2.10	0.967	<0.001	7.64	0.89 (0.56 – 0.98)
		Re-test	9.12 ± 1.40				
Twitch	PT (Nm)	Test	34.9 ± 14.3	0.979	<0.001	4.66	0.98 (0.90 – 1.00)
		Re-test	35.3 ± 14.3				
	RTD (Nm.s^{-1})	Test	730 ± 296	0.940	0.001	6.99	0.94 (0.73 – 0.99)
		Re-test	741 ± 287				
Octet	PT (Nm)	Test	116 ± 40.4	0.963	<0.001	4.29	0.95 (0.73 – 0.99)
		Re-test	110 ± 32.9				
	RTD (Nm.s^{-1})	Test	1948 ± 728	0.933	0.002	5.80	0.93 (0.65 – 0.99)
		Re-test	1918 ± 662				
VA (%)		Test	90.5 ± 7.57	0.959	<0.001	1.44	0.96 (0.80 – 0.99)
		Re-test	89.7 ± 7.16				
RMS EMG_{MVT} (% M-wave)		Test	8.31 ± 2.42	0.703	0.297	13.0	0.70 (0.46 – 0.98)
		Re-test	7.45 ± 2.62				
Explosive RMS $\text{EMG}_{0\text{-}100}$ (% M-wave)		Test	7.36 ± 1.62	0.947	0.208	11.7	0.84 (0.55 – 1.00)
		Re-test	6.80 ± 2.69				
Muscle Thickness		Test	29.2 ± 5.28	0.959	<0.001	4.35	0.92 (0.64 – 0.98)
		Re-test	29.0 ± 7.17				
Pennation Angle		Test	14.0 ± 3.40	0.717	0.045	9.28	0.84 (0.18 – 0.97)
		Re-test	12.8 ± 3.44				
Fascicle Length		Test	127 ± 23.8	0.284	0.495	10.9	0.28 (-)
		Retest	135 ± 18.7				

95% CI, 95% Confidence Interval; MVT, Maximal Voluntary Torque; RTD, Rate of Torque Development; RTD_{MVT}, RTD relative to MVT; PT, peak torque; VA, Voluntary Activation; RMS EMG_{MVT}, Root Mean Square Electromyography at MVT; RMS $\text{EMG}_{0\text{-}100}$, Root Mean Square Electromyography from 0-100 ms of an explosive voluntary contraction

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