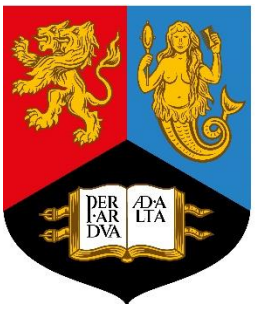

UNIVERSITY OF BIRMINGHAM

INVESTIGATING MUSCULOSKELETAL TISSUE STIFFNESS AND ITS RESPONSE TO EXERCISE

By

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A thesis submitted to the University of Birmingham

for the degree of

DOCTOR OF PHILOSOPHY

School of Sport, Exercise and Rehabilitation Sciences

College of Life and Environmental Sciences

University of Birmingham

October 2024

Abstract

The aim of this thesis is to investigate the non-invasive estimation of musculoskeletal (MSK) tissue stiffness to develop understanding of its mechanical properties and to determine the efficacy of doing so in an elite sport setting. The following objectives were set to achieve this aim and based on the gaps identified in the literature: to investigate (1) the validity of the MyotonPRO as a method of estimating muscle stiffness, (2) the reliability of shear wave elastography (SWE) in assessing MSK tissue stiffness, (3) the occurrence of tissue- and region-specific differences in MSK tissue stiffness, and (4) the impact of exercise interventions on acute changes in MSK tissue stiffness. To achieve these objectives and gain this novel knowledge, this thesis contains five distinct phases: the exploratory phase (Chapters 1 and 2); the methodological phase (Chapters 3 and 4); the mechanistic phase (Chapter 5); the experimental phase (Chapter 6); and the synthesis phase (Chapter 7).

The exploratory phase involved an introduction to the topic and sets out the objectives of the thesis (Chapter 1), and a narrative review outlining the importance of understanding muscle stiffness and the methods of measuring it; and evaluating the broad literature surrounding the applications of SWE as a method of estimating muscle stiffness in the clinical and athletic settings (Chapter 2). The methodological phase involved assessing the validity of the MyotonPRO as a method of estimating MSK tissue stiffness compared with SWE (Chapter 3), and assessing the reliability of SWE for estimating the stiffness of rectus femoris (RF) muscle and overlying skin and fascial tissue (Chapter 4). The mechanistic phase involved the use of SWE to determine tissue- and region-specific differences in RF muscle and fascial tissue stiffness (Chapter 5). The experimental phase involved a randomised crossover intervention study to assess the impact of real-world training interventions on MSK tissue stiffness and examine the relationship between MSK tissue stiffness and joint range of motion/flexibility

(Chapter 6). Finally, the synthesis phase discusses the findings of the thesis, placing them in the context of the existing literature and providing practical recommendations for the measurement of muscle stiffness applied in the clinical and athletic settings, as well as describing the future directions of research in the field (Chapter 7).

Keywords: Stiffness, Shear wave elastography, Muscle, Fascia

Word count: 38,837

Acknowledgements

Firstly, I must extend my gratitude to my supervisors Professor Barry Drust and Dr Eduardo Martinez-Valdes for their guidance and support throughout my PhD journey. Barry – thank you for challenging my way of thinking and developing me into a more rounded, independent researcher. Ed – thank you for your feedback and putting up with my unscheduled drop-ins to your office to discuss data and stats.

I would also like to thank my funders at Liverpool Football Club for trusting me with this project, funding the work within this thesis, and allowing me to be involved in interesting projects outside of my PhD to engage my brain on things other than shear wave elastography.

To my parents, thank you for always believing in me, my choices, and my abilities. This belief has been a constant source of strength throughout the challenging times along my academic journey. Throughout my life you have consistently set the best examples of perseverance and resilience that have undoubtedly taught me the importance of hard work and instilled the drive which has got me to this stage. You have been the most incredible role models, and you inspire me every single day. Spencer, thank you for taking me to the pub and supporting me when times were tough (but you do now have to exclusively refer to me as the only, and likely first, Dr Ley).

Miriam, you have been with me for this entire PhD journey and for that, I can only apologise. Thank you for always reminding me that there is far more to life outside of work and this PhD; thank you for always being by my side during the tough times; thank you for always believing in me and being my biggest supporter; thank you for being my best friend; and above all else,

thank you for going to Spain so I could knuckle down for a year (and have some great holidays). Huge shoutout to the Sutton Trust.

To my PGR family past and present, thank you for all the support and strength you have given me over the past 3 years, it has been a pleasure to share my PhD journey with you all. Ally (co-founding member of coffee club), thank you for the mini eggs, daily 8am(ish) walks into campus and, above all, your friendship. Eddie, Elsa, Georgia, Joe (occasionally Matt), Lucy, Marie, Megan, Morgan, Roz, and the rest of the PGR office crew thank you for the laughs, coffee runs, pub trips, Tesco snack runs, mental health loops of campus, and mezz lunches that have got me through this PhD. Without your advice, support, and sense of humour this would have been a far tougher 3 years. I know you will all go on to achieve incredible things.

To my friends outside of PhD life, whether we have lived together or played hockey together (or both), thank you for distracting me from the occasional hell that this PhD has thrown at me. Thanks to you all, but Alice, Ben, Mike, Neve, and Thorney, to name a few. Thank you also to the rest of the amazing friends I have made throughout my 7 year journey here at the University of Birmingham, it is one I will never forget and be eternally grateful for.

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List of Abbreviations

AKE	Active knee extension
ANOVA	Analysis of Variance
B-RPE	Breathlessness rating of perceived exertion
BB	Biceps brachii
BF	Biceps femoris
BFlh	Biceps femoris long head
BMI	Body mass index
Ca^{2+}	Calcium ion
CG	Compression garments
CMJ	Countermovement jump
CP	Cerebral palsy
CT	Computed tomography
CUR	Countermovement utilisation ratio
CWI	Cold water immersion
DEEP	Deep muscle tissue
DIST	Distal

DMD	Duchenne muscular dystrophy
dRPE	Differential rating of perceived exertion
ECM	Extracellular matrix
EIMD	Exercise-induced muscle damage
EMG	Electromyography
FAS	Fascia
FR	Foam rolling
GPS	Global positioning system
HR	Heart rate
ICC	Intraclass correlation coefficient
IKD	Isokinetic dynamometer
LE-RPE	Leg exertion rating of perceived exertion
LG	Lateral gastrocnemius
MAS	Modified Ashworth scale
MED	Medial
MG	Medial gastrocnemius

MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
MSK	Musculoskeletal
MVIC	Maximal voluntary isometric contraction
NEU	Neutral condition
PAST	Passively stretched condition
PD	Parkinson’s disease
PROX	Proximal
PUL	Performance of upper limb module
REL	Relaxed condition
RF	Rectus femoris
ROI	Region of interest
ROM	Range of motion
RSA	Repeat sprint ability
SAT	Subcutaneous adipose tissue thickness
SD	Standard deviation

SEM	Standard error of measurement
SKIN	Skin tissue
SM	Semimembranosus
SOL	Soleus
SPSS	Statistical Package for the Social Sciences
ST	Semitendinosus
SUP	Superficial muscle tissue
SWE	Shea wave elastography
SWV	Shear wave velocity
TA	Tibialis anterior
TB	Triceps brachii
TT	Thomas test
UPDRS	Unified Parkinson's Disease Rating Scale
VAS	Visual analogue scale
VI	Vastus intermedius
VL	Vastus lateralis

VM	Vastus medialis

CHAPTER 1

General Introduction

1.1. Background

The biomechanical properties of skeletal muscle and its surrounding structures are fundamental components supporting human movement and locomotion, with consequences in disease and injury. Stiffness is a mechanical property which is related to a material's ability to resist deformation in response to the application of an external load (Kelc et al., 2013). In the MSK context, muscle tissue lengthens in response to tension from connecting tendons. Stiffer muscle tissue lengthens less in response to a given tension than less stiff muscle tissue. The gold standard measure of material stiffness involves application of known force to a material and measuring its resultant change in shape (Kodesho et al., 2021b). In MSK tissue, this involves muscle dissection, so non-invasive proxy measures were developed to overcome this limitation (Nakao et al., 2023). Performance tests, such as unilateral hopping on a force plate, have been used to quantify muscle stiffness (Pruyn et al., 2012). These, however, do not specifically assess a muscle in isolation, as results are also dependent on the stiffness of connecting and surrounding tissue, meaning they instead quantify stiffness of a muscle or limb unit (Pruyn et al., 2016). Magnetic resonance imaging (MRI) and ultrasound-based techniques have been developed to overcome the lack of specificity of performance tests, with SWE providing a relatively cheap, practical, valid and reliable measure of muscle stiffness compared to the gold standard tensile testing (Brinker and Klatt, 2016, Davis et al., 2019). Following this, the MyotonPRO was developed as an easier, handheld, portable alternative to imaging techniques, but doubts remain over its validity in assessing muscle mechanical properties (Bravo-Sánchez et al., 2021).

SWE may prove to be an important tool in both athletic and clinical populations. In the athletic context, it is important to optimise levels of muscle stiffness, as it has been associated with

enhanced MSK performance, but also with a greater injury risk (Pickering Rodriguez et al., 2017, Ando et al., 2021b). Furthermore, muscle stiffness has been shown to be differentially altered by different exercise modalities (Sadeghi et al., 2018, Heales et al., 2018). Within this research, elevations in muscle stiffness have been associated with muscle damage (Lacourpaille et al., 2017b, Xu et al., 2019). SWE may prove useful in the identification of MSK injury, assessing athlete muscle condition, recovery status and readiness to play, as well as longitudinally monitoring injury risk, rehabilitation progress and adaptation to training interventions.

In clinical populations, elevated muscle stiffness has been observed in an array of conditions, including cerebral palsy, Parkinson's disease, and Duchenne muscular dystrophy, whilst low levels of muscle stiffness have been associated with sarcopenia (Lee et al., 2016, Marusiak et al., 2010, Lacourpaille et al., 2017a, Okyar Baş et al., 2023). SWE, therefore, has the potential to aid the diagnosis of disorders related to excessive levels of muscle stiffness, such as cerebral palsy, Parkinson's disease, and Duchenne muscular dystrophy. It could also prove useful in monitoring disease progression and severity, as well as in assessing the effectiveness of treatments (Oppold et al., 2023). The possible consequences of excessively low or high levels of muscle stiffness may therefore be substantial in both athletic and clinical populations.

Multiple factors including intermuscular differences; intramuscular/regional differences; and the effects of muscle length, and tissue type and depth must be considered when assessing MSK mechanical properties, as all have been shown to alter stiffness (Itsuda et al., 2024). A better understanding of passive MSK properties must be understood to allow the reliable measurement using SWE and accurate interpretation of data in these applied athletic and clinical settings.

This involves further development and standardisation of MSK SWE measurement procedures (Cipriano et al., 2022).

Whilst these are promising potential applications of SWE, there is still more research required to develop the methodological protocols for use of SWE, and further validation required for the MyotonPRO before it can be used as an alternative or supplementary tool for the estimation of muscle stiffness. Research must also focus on growing understanding of passive muscle stiffness, in addition to the effects of intra-muscular differences, tension and muscle length. Furthermore, future research must build on the existing literature to understand how the exercise-induced changes in muscle stiffness apply to the elite sport setting and determine if real-world exercise interventions influence MSK stiffness as laboratory-based interventions have been shown to.

1.2. Thesis objectives

The thesis has the following objectives to achieve its aim of investigating the use of SWE to understand MSK tissue stiffness and its response to exercise.

1. To assess the validity and comparability of the MyotonPRO to SWE as a method of estimating muscle tissue stiffness.
2. To assess the reliability of SWE in assessing skin, deep fascia and muscle tissue stiffness.
3. To determine the effects of tissue type, measurement depth, tissue region, and muscle length on stiffness estimated by SWE.
4. To determine the impact of exercise interventions on lower limb muscle stiffness, and the associations it has with exercise-related variables.

CHAPTER 2

Evaluating the Functional Consequences of Muscle Stiffness and the Utilisation of Shear Wave Elastography and the MyotonPRO in its Measurement for Application in Athletic and Clinical Settings: A Narrative Literature Review

2.1. Introduction

This review covers the background information relevant to this thesis, explaining what muscle stiffness is, why it is important, and how it is measured. Since estimation of MSK tissue stiffness using SWE is a relatively new research area, there is not an abundance of literature detailing the functional consequences of and factors affecting MSK tissue stiffness. This review, therefore, aims to provide a broad overview of the research interests in the field of MSK SWE and identify gaps in the literature for future topics of research. As such, this review will critically evaluate the literature on the factors affecting MSK stiffness; considerations in its measurement and interpretation of data; the role of MSK stiffness in clinical outcomes; the impact of MSK stiffness on physical performance; the acute effects of exercise on MSK stiffness; the associations between MSK stiffness and muscle damage and ROM; the effect of recovery strategies on MSK stiffness; the contribution of MSK stiffness to injury risk; potential underlying physiological mechanisms determining MSK stiffness; and current limitations with estimation of MSK stiffness using SWE and the MyotonPRO.

2.2. Material mechanical properties

Mechanical properties are a result of a material's response to stress and strain. Mechanical stress refers to the equal and opposite reaction force exerted by a material in response to an applied external force. It is therefore, simply referred to and quantified as the external force applied to a material. It is widely accepted that there are five types of mechanical stress, describing the different directions of forces which can be applied to a material: tension; compression; shear; torsion; and bending (Figure 2.1).

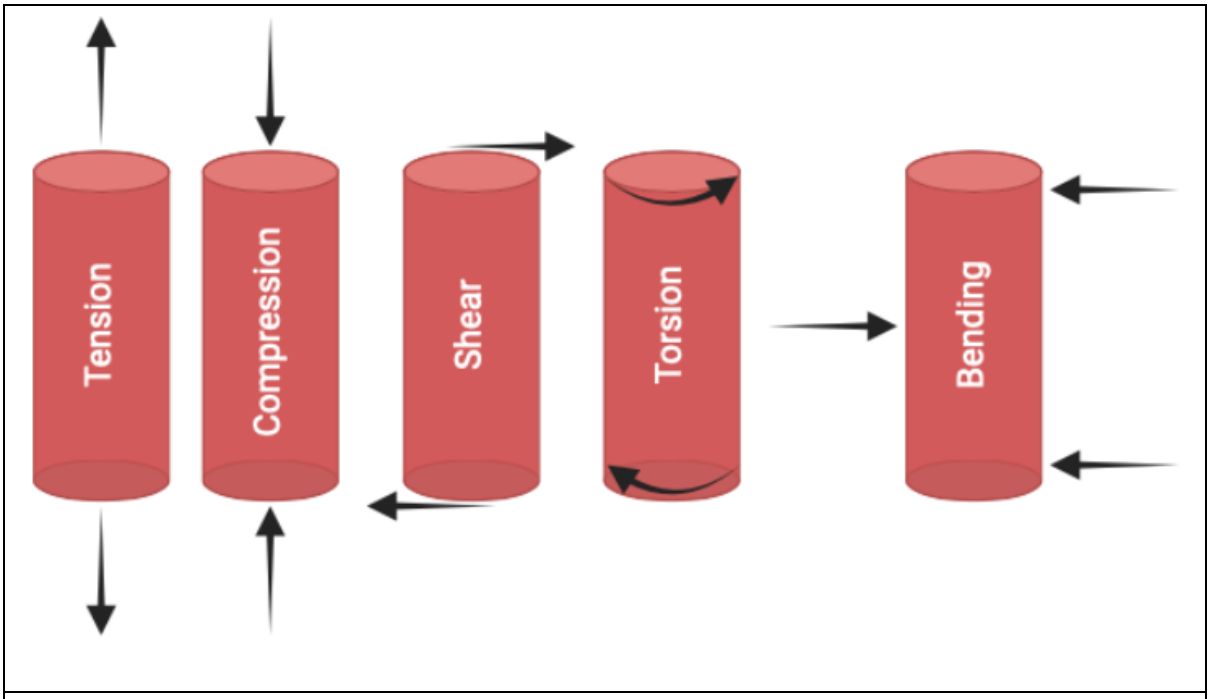

Figure 2.1. Schematic demonstrating the five types of mechanical stress which can be exerted on a material due to the direction of the external forces applied to it.

As a result of these forces, the material changes shape. The amount of this deformation is referred to and quantified as the mechanical strain. Since strain is a direct consequence of stress, the two can be plotted against each other to produce a stress/strain curve (Figure 2.2). The ratio between stress and strain determines the mechanical properties of a material. Stiffness is based on Hooke's law, which describes stress and strain as directly proportional to one another, occurring in the linear region of the stress/strain curve. The slope of this linear region represents Young's modulus, which is a measure of how much a material resists deformation under stress, and quantifies the material's stiffness (Figure 2.2).

2.2.1 Muscle stiffness

Human skeletal muscle comprises bundles of muscle fibres surrounded by a network of connective tissue. Each individual muscle fibre is surrounded by endomysium connective

tissue, these fibres are bundled together to form fascicles which are covered by a layer of perimysium connective tissue. Groups of fascicles are encased by the outermost layer of connective tissue, the epimysium (Frontera and Ochala, 2015). The collective material properties of these tissues all contribute to the overall mechanical function of skeletal muscle. This inhomogeneity makes the quantification of stiffness in muscle more complex than in other materials, as it is impacted by tissues with different mechanical properties. This also means that skeletal muscle is anisotropic and nonlinearly viscoelastic, so its mechanical properties vary based on the direction of measurement (Koo and Hug, 2015). It is less complex to measure the stiffness of homogenous materials, such as steel, as they are uniform in their composition, thus the whole unit responds in the same way to stress.

Muscle tissue passive mechanical properties refer to the physical properties of muscle tissue that influence its response to mechanical stress and strain in the absence of active muscle contraction (Lieber and Binder-Markey, 2021). Muscle stiffness differs to muscle tone in that it is a property related just to the mechanics of the muscle tissue itself. Tone, however, is the baseline level of muscle contraction which is regulated by muscle spindles and involves regulation by the nervous system (Sanger et al., 2003). This is investigated most in the literature as it is easier and more reliable than estimating the stiffness of actively contracting muscle tissue.

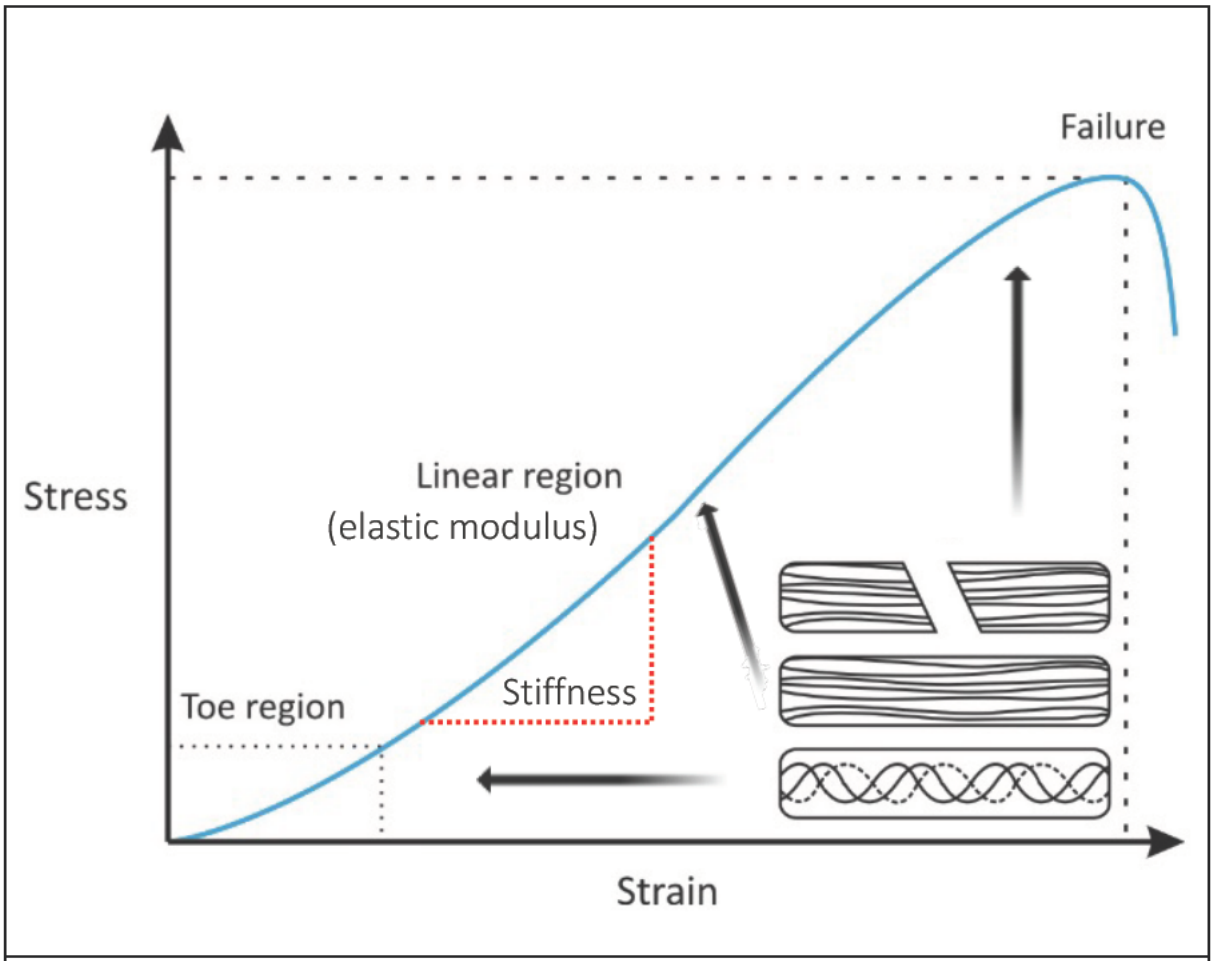

Figure 2.2. The stress/strain curve of muscle tissue, adapted from Kelc et al. (2013).
The stress/strain curve comprises three distinct regions: the toe region, the linear region, and the failure region. The toe region occurs at low levels of strain and involves the straightening of crimpled muscle fibrils. In the linear region, muscle fibres respond linearly to load (Hooke's law), until the strain is too great for the muscle fibres and macroscopic tears occur, the third and final region, failure. The slope of the linear region quantifies Young's modulus and the muscle's stiffness (kPa).

The mechanical properties of a specific muscle are influenced by several factors, including the structure and composition of the muscle tissue, the level of muscle activation, and the presence of any injuries or damage to the tissue. Additionally, the relationship between stress and strain is influenced by temperature and the rate at which load is applied to the material. It is, therefore,

pertinent to understand the behaviour of muscle tissue under these different conditions for developing strategies to prevent or treat muscle injuries and disorders.

2.3. The importance of quantifying muscle stiffness

Muscle stiffness is thought to be a regulator of performance, particularly in explosive actions such as jumping and sprinting, whereby higher levels of stiffness appear advantageous due to improved efficiency in transmitting forces to the tendon (discussed in Section 2.9) (Kalkhoven and Watsford, 2018). It has been reported, however, that an excessively high level of muscle stiffness is a risk factor for injury (discussed in Section 2.14) (Pickering Rodriguez et al., 2017). Considering these two polarised consequences of muscle stiffness, it is likely that there is an optimal level of muscle stiffness at which musculoskeletal performance is enhanced, whilst injury risk is minimised (Figure 2.3) (Butler et al., 2003).

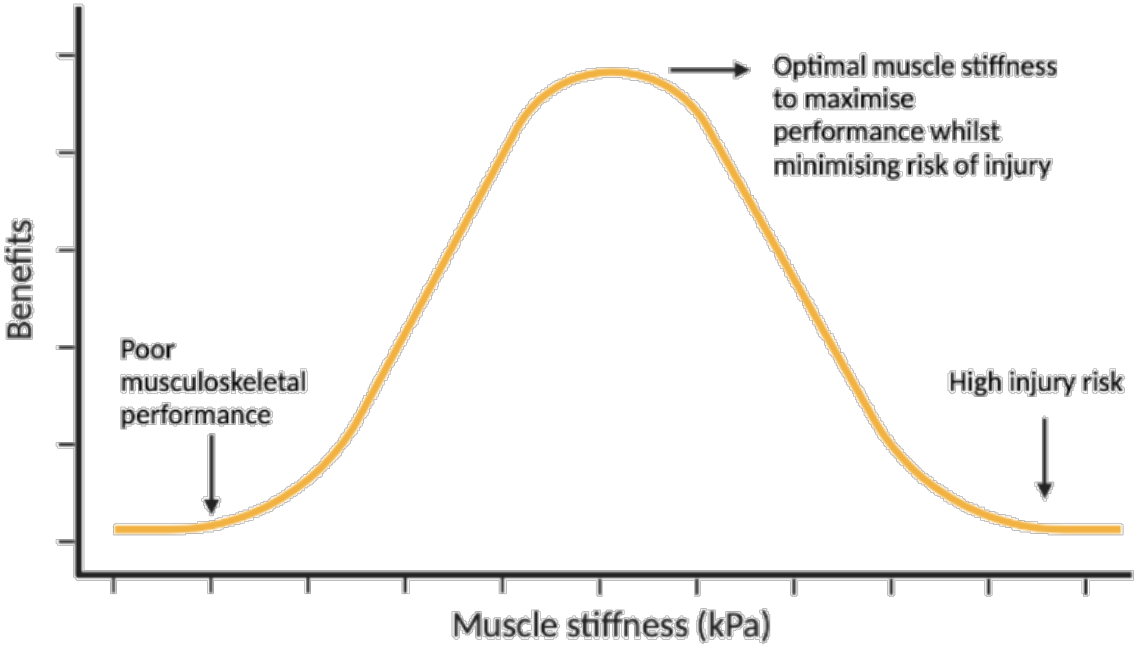

Figure 2.3. The relationship between muscle stiffness, musculoskeletal performance, and injury risk.

Low levels of muscle stiffness are associated with poor musculoskeletal performance, but a low injury risk. High levels of muscle stiffness are associated with enhanced performance, but this is outweighed by a high injury risk. There is a trade-off between the two and an optimal level of muscle stiffness at which performance is enhanced, with minimal injury risk.

2.4. Methods of quantifying muscle stiffness

The gold standard mechanical method of quantifying material stiffness is called tensile testing. With application to skeletal muscle, this involves its dissection, whereafter the tendons at each end of the muscle are clamped. One end is fixed, and the other is subjected to a controlled uniaxial force at a constant rate (stress). This causes elongation of the muscle, which is measured as change in muscle length (strain) (Figure 2.4) (Kodesho et al., 2021b, Nakao et al., 2023). Knowing the force applied to the material (stress) and the change in muscle length in

response to the force (strain) enables the plotting of a stress-strain curve (Figure 2.2), from which muscle stiffness can be calculated as the slope of the linear region (Kelc et al., 2013). This gold standard method, however, has its obvious limitations in practicality as it can only be performed on cadavers.

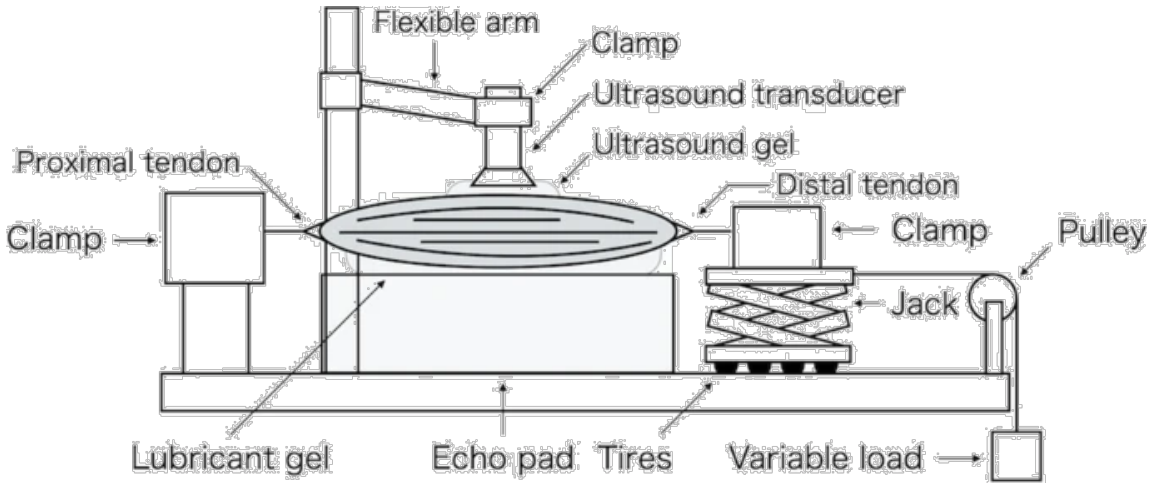

Figure 2.4. A representation of the setup for the comparison between tensile testing and shear wave elastography in muscle, taken from Kodesho et al. (2021b).
The muscle is clamped at both tendons and a known load is applied to the muscle at one end (stress), with the strain measured as change in muscle length. This figure is taken from a study which validated shear wave elastography against tensile testing, hence the ultrasonography.

2.4.1. Performance tests as a method of quantifying muscle stiffness

Performance tests were developed to estimate stiffness in a practical and accessible way. Unilateral hopping tests and free-oscillation techniques have been utilised as methods of estimating stiffness of the musculature (Pruyn et al., 2016). Unilateral hopping tests are used to calculate vertical or whole leg stiffness. Subjects are required to continuously hop on one leg

whilst motion capture technology, such as a 3-dimensional camera, measures the frequency and amplitude of the hops. From this data, leg stiffness is calculated as the ratio of peak ground reaction force to the maximum vertical displacement of the centre of mass during the ground-contact phase, divided by body mass (Pruyn et al., 2012). Oscillation tests are also performed on a force plate or utilising a load cell, but involve the application of a controlled force or displacement to the leg, causing perturbation resulting in oscillations of the leg. The frequency and amplitude of these oscillations are measured by the force plate or load cell, with the natural frequency at which the leg oscillates in response to perturbation being related to leg stiffness (Pruyn et al., 2016).

Despite these techniques providing useful insights into muscle stiffness and being non-invasive, simple tests to carry out, they are not specific to muscle as they are sensitive to other biological tissue, such as tendons and ligaments. Moreover, the unilateral hopping test is limited since factors other than stiffness, including muscle strength and power, influence hop frequency and amplitude. Consequently, other methods need to be developed to overcome these limitations.

2.4.2. Plotting force-fascicle length curves to quantify muscle stiffness

To more accurately estimate muscle passive stiffness without contribution or interference of active properties such as strength, a method of measuring passive length-tension properties was developed (Hoang et al., 2005). This method involves passively stretching the muscle through a set range of motion (ROM) using an isokinetic dynamometer (IKD) to measure passive force, and measuring change in muscle fascicle length using B-mode ultrasonography. Synchronisation of passive force measurements from the IKD with fascicle length measurements from ultrasound images allows plotting of a force-fascicle length curve from

which stiffness can be calculated (Hoang et al., 2005). This method has sound theoretical underpinnings, with the quantification based on plotting of a muscle fascicle-specific stress-strain curve, and has been shown to be a reliable method of assessing muscle stiffness (Carvalhais et al., 2011). However, the methodology requires access to ultrasound, an IKD, and electromyography (EMG), making it a broadly inaccessible technique in clinical practice. Furthermore, the stretching of the muscle around a joint means that there are passive structures other than the muscle fascicles, i.e., skin, tendons, the extracellular matrix (ECM) and joint resistance, which can contribute to the measured passive force. In addition, there are methodological concerns with the measurement of muscle fascicle length using ultrasound, particularly considering muscle fascicle curvature (Heieis et al., 2023). This methodology also requires time-consuming post-testing analyses to plot the curve and calculate stiffness. There is, therefore, a need for development of further techniques which can overcome these limitations and provide real-time feedback on muscle mechanical properties.

2.4.3. Magnetic resonance elastography as a method of quantifying muscle stiffness

Magnetic resonance elastography (MRE) is a non-invasive MRI-based technique which estimates stiffness of biological tissues. MRE works by mechanically inducing shear waves which cause an oscillatory motion as the mechanical waves pass through the tissue. These minute displacements in tissue are captured by MRI, enabling calculation of the velocity at which the shear waves are travelling through the tissue (Brinker and Klatt, 2016). Shear wave velocity (SWV) is directly related to tissue stiffness. Shear waves travel faster through stiffer tissue (Taljanovic et al., 2017). This technique has been validated against tensile testing, with agreement between MRE and tensile stress (Brinker and Klatt, 2016). Furthermore, MRE has been shown to be a reliable method of estimating stiffness both in ex vivo phantoms and in vivo

in muscle tissue (Hsieh et al., 2022, Ito et al., 2020). Whilst MRI is a high quality imaging technique, it is not the most practical due to the high demand for use of the limited equipment, as well as the high financial costs associated with its use (Giussani et al., 2022). Additional techniques which are more convenient and cost-effective are therefore required for measurement of muscle stiffness on a larger scale in research, and in athletic and clinical practice.

2.4.4. Tensiomyography as a method of quantifying muscle stiffness

Tensiomyography (TMG) is a non-invasive electromechanical method of estimating muscle stiffness by measuring muscle displacement in response to an electrically evoked contraction (Lohr et al., 2019). Electrical stimulation is delivered by electrodes, triggering a muscle contraction, with a high precision sensor measuring muscle displacement. This method measures contraction time to indicate muscle fibre type and as a proxy for muscle fatigue and muscle damage (Hunter et al., 2012). The extent of muscle displacement is used to estimate muscle stiffness, with a lower displacement representative of higher stiffness. Unlike MRE, SWE and the MyotonPRO, however, TMG provides a measure of whole-muscle stiffness and does not allow for the measurement of specific regions of interest (ROIs). Results from the literature indicate that there is no correlation between SWE and TMG, likely because TMG, unlike SWE, is reliant on electrophysiological properties of muscle and measures whole-muscle stiffness (Bravo-Sánchez et al., 2021, Alfuraih et al., 2022).

2.4.5. Shear wave elastography as a method of quantifying muscle stiffness

Ultrasound imaging has been used for decades to assess skeletal muscle anatomical and architectural properties such as cross-sectional area, muscle thickness, pennation angle, and

fascicle length (Narici, 1999, Kwah et al., 2013). These properties have biomechanical implications with regards to contractile force generation and force-velocity relationships based on the arrangement of muscle fibres (Narici, 1999). Ultrasonography has proven a useful, reliable tool in researching MSK anatomy and physiology with the provision of real-time visual feedback of muscle condition and properties through non-invasive methodology, which is quick, easy and cheap relative to Computed Tomography (CT) or MRI techniques.

SWE is an ultrasound-based imaging technique used to estimate the stiffness of tissues. It has a similar methodology to MRE, inducing a shear wave, or a wave of mechanical displacement, within the tissue being examined. To create the shear wave, an ultrasound probe sends a burst of focussed sound waves into a specific ROI within the tissue. This creates a small area of compression, which rapidly expands and contracts, creating a wave that propagates through the tissue perpendicular to the ultrasound beam. Again, the speed of this shear wave is related to the stiffness of the tissue, with stiffer tissues propagating the wave faster than softer tissues (Taljanovic et al., 2017). The ultrasound transducer tracks the shear wave travelling through the tissue using ultra-high frame rate ultrasonic imaging and measures
its velocity, from which shear modulus, or stiffness, is calculated (kPa), producing an elastogram (Figure 2.5) (Davis et al., 2019).

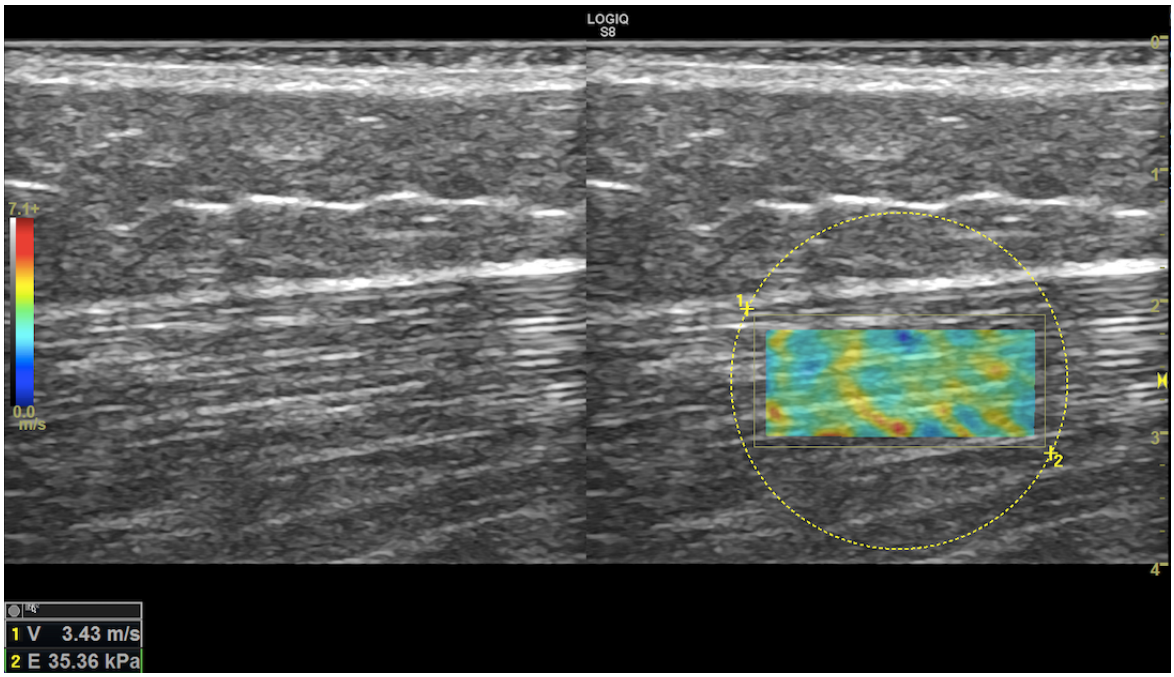

Figure 2.5. An example of shear wave elastography in the biceps femoris muscle.
On the left is the B-mode ultrasound image, repeated on the right where an elastogram is superimposed over the B-mode image and shear wave velocity is estimated within that region of interest. The yellow circle represents the measuring tool used to quantify shear wave velocity and shear modulus (shown in the bottom left corner) within the selected area.

The Young's modulus equation used to calculate muscle shear modulus assesses the stress/strain ratio of a material, assuming the material is isotropic and homogenous (Figure 2.2). For this reason, SWE has been successfully used in the clinical field for assessment of homogenous tissue, with applications in the evaluation and diagnosis of liver cirrhosis and in the examination of breast masses (Frulio and Trillaud, 2013, Cosgrove et al., 2012). As a result, it was thought that this technique could be applied to the musculoskeletal system to non-invasively and locally estimate stiffness of specific tissues. Multiple studies have shown SWE to provide reliable and repeatable measurements of muscle stiffness, helping validate the effectiveness of the technique and demonstrating its potential for use in both research and applied settings (Lacourpaille et al., 2012, Dubois et al., 2015, MacDonald et al., 2016, Feng et

al., 2018, Wang et al., 2022, Varol et al., 2023, Yi et al., 2023). Within these studies, SWE has demonstrated moderate to very high repeatability both intra- and inter-session, as well as intra- and inter-rater. Similar to TMG, SWE has been shown to be sensitive to exercise and contraction-induced changes in muscle stiffness (Lacourpaille et al., 2017b, Yoshitake et al., 2014, Hunter et al., 2012). In addition to displaying high levels of reliability, SWE has been validated against MRE (Koga et al., 2021, Oudry et al., 2009). More importantly, SWE has been compared to the gold standard tensile testing using cadaver dissections, wherein shear modulus correlated well with passive force (Kodesho et al., 2021b, Nakao et al., 2023). Moreover, high levels of measurement repeatability have been demonstrated regardless of demographic features and body composition factors, such as fat mass and body mass index (BMI) (Varol et al., 2023). SWE is therefore a viable and cheaper alternative to MRE in the estimation of muscle stiffness. Despite this, SWE is not without its limitations (discussed in Section 2.16.1). However, the ability of SWE to provide an internal marker of localised tissue stiffness using non-invasive ultrasound methodology, and with real-time visual feedback make it a useful monitoring tool for muscle stiffness in athletes and clinical populations (Eby et al., 2013).

2.4.6. The MyotonPRO as a method of quantifying muscle stiffness

Myotonometry is a novel method of quantifying muscle tissue properties using similar mathematical principles as the free-oscillation technique. The method involves precise application of a mechanical compression force by a small probe (Figure 2.6), inducing tissue deformation and a decaying natural oscillation within the displaced muscle tissue, from which biomechanical properties are calculated (Feng et al., 2018). These properties are coined tone, dynamic stiffness, elasticity, relaxation, and creep by Myoton® (Myoton®). This thesis will

focus on the dynamic stiffness measurement provided by the MyotonPRO. It is important to note that Myoton® define dynamic stiffness as the resistance of biological soft tissues to a force of deformation, which is quantified in N/m (Myoton®). Details of this method, along with MRE, TMG and SWE are summarised in Table 2.1.

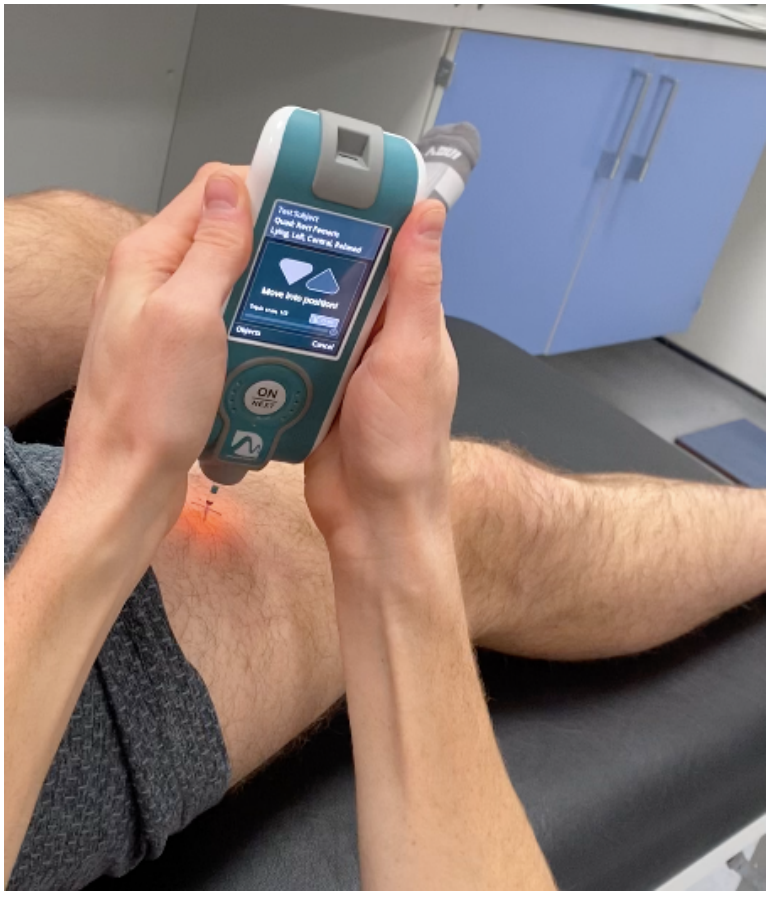

Figure 2.6. Image of the MyotonPRO being used on the rectus femoris muscle of a supine participant.
The device is lowered until the light is green and the device automatically applies a mechanical compression force at the skin's surface, measuring the oscillation of the below tissue.

Significant correlations have been found between SWE and MyotonPRO measurements of both muscle and tendon stiffness, as well as in gel phantoms of a known stiffness (Feng et al., 2018,

Kurashina et al., 2023). This agreement between the two measurement devices provides promising evidence in support of the MyotonPRO's use in assessing muscle and tendon mechanical properties. As a result, interest amongst sports science practitioners in the MyotonPRO has risen in recent years due to its ease of use, since it is a wireless handheld device, thus can be used anywhere, and measurements take a matter of seconds. Moreover, multiple studies have reported excellent reliability of measurement using the MyotonPRO, both intra- and inter-rater, and intra- and inter-day (Agyapong-Badu et al., 2013, Aird et al., 2012, Bravo-Sánchez et al., 2021). Previous versions of the MyotonPRO (Myoton-3) has also been shown to be sensitive to changes in passive mechanical properties of the BF caused by changes in knee joint angle (Ditroilo et al., 2011). The MyotonPRO does, however, have multiple limitations, most notably its lack of tissue specificity and limited depth of measurement (explored in Chapter 3).

Table 2.1

Feature	**MRE**	**TMG**	**SWE**	**MyotonPRO**
Modality	MRI	Electromechanical probe	Ultrasound	Handheld mechanical device
Force Application	External vibration	Electrical stimulation of muscle	Acoustic radiation force	Mechanical tap from probe
Measurement Method	Tracks mechanical wave propagation	Measures muscle belly displacement after contraction	Measures shear wave speed (m/s)	Measures damped oscillations after mechanical impulse

Output & unit of measurement	Colour elastogram, stiffness in kPa	Muscle displacement value in mm	Colour elastogram, stiffness in kPa or shear wave velocity m/s	Dynamic stiffness value in N/m
Depth Limitations	None (deep and superficial tissues)	Surface muscles only	Limited by ultrasound depth (~4–6 cm optimal)	Surface muscles and tendons only covered by <2cm subcutaneous adipose tissue

Table 2.1. Summary of the different methods of estimating muscle stiffness.
MRE, magnetic resonance elastography; SWE, shear wave elastography; TMG, tensiomyography; MRI magnetic resonance imaging.

2.5. Passive muscle stiffness properties

The substantial spatial specificity of measurement provided by SWE allows comprehensive estimation of biological soft tissue stiffness. This research has provided some insight into intermuscular differences; intramuscular/regional differences; and the effects of sex, muscle length, and tissue depth on SWV. Intermuscular differences have been established both between and within muscle groups (Lee et al., 2021, Xu et al., 2018). These findings are perhaps unsurprising given the different functional roles of individual muscles. The varying mechanical properties are likely evolutionary in aiding the specific functional contractile roles of the different muscles in human movement. Moreso, intramuscular differences have been observed among different muscle regions, with deeper muscle tissue and that closer to tendons appearing to exhibit a greater SWV than more superficial or medial tissue. Sex differences have also been observed in skeletal muscle tissue stiffness measured by SWE, showing elevated muscle stiffness in males, with these findings likely reflecting enhanced MSK performance (Lee et al.,

2021, McPherson et al., 2020). Increases in muscle length are known to augment muscle stiffness (Berrigan et al., 2020, Le Sant et al., 2015, Itsuda et al., 2024). This is to be expected, since an increase in muscle length reflects an increase in muscle strain due to tension stress (Figure 2.1) exerted on the muscle. Changes in muscle length are also likely to alter the distribution of strain along the length of a muscle as stiffness is affected non-uniformly in response to stress, particularly in biarticular muscles (discussed in Section 2.7 and explored further in Chapter 5). These factors may all influence the spatial occurrence of muscle injury and specific mechanisms by which injury occurs in different athletic scenarios.

2.6. Application to the elite sporting environment

The physical demands of elite team sports, such as football, require athletes to train and compete at the highest level of human performance day-in-day-out over the course of a competitive season. In some circumstances, fixture periods become congested with minimal time to recover between training and matches, increasing the risk of injury (Carling et al., 2016). The primary roles of sports science practitioners are to maximise athlete performance, and minimise injury risk. As such, several objective and subjective markers are collected by sports scientists to gain an insight into the players' recovery status and readiness to perform (Mohr et al., 2016). Examples of the data collected for such purposes include global positioning system (GPS) metrics, counter-movement jump (CMJ) height, repeat sprint ability (RSA) and strength assessments pre- and post-match play to assess readiness and fatigue; and player subjective wellness questionnaires including variables such as muscle soreness, sleep quality, fatigue, and stress to evaluate player status. While such markers give an indication of a player's acute response to a game and assess their wellness and physical condition in the lead up to a game, they do not provide an objective internal indicator of the structural properties and condition of

muscle tissue. As team sport-related activities are associated with induction of muscle damage and inflammatory responses, the ability to accurately quantify these properties could significantly enhance the monitoring process used within elite sport by providing a better reflection of the authentic physiological consequences of the total physical load experienced by athletes (Romagnoli et al., 2016). Accurate quantification of these physiological consequences provides another layer to the monitoring process of elite athletes and enables greater insight into their physical state to estimate readiness to play, recovery status and risk of injury.

Current techniques used to measure physiological state, however, require invasive methods which are uncomfortable for athletes, impractical for regular use, and cause physiological disturbance through sampling of blood and muscle tissue for biomarkers such as lactate, C-reactive protein and markers of oxidative stress. ROM tests are commonly used in professional football to assess flexibility and muscle extensibility, as restricted ROM has been linked with muscle damage and is a risk factor for injury and cause of pain (Cejudo, 2022, Cejudo et al., 2020). These tests include the modified Thomas test to assess quadriceps and hip flexor ROM; the sit and reach test and passive or active knee extension tests to assess hamstring ROM; the bent knee fall out test to assess adductor ROM; and the knee to wall test to assess ROM of the plantar flexors. These tests are commonly used to assess imbalances in flexibility and detect large deficiency in ROM or changes to ROM over time. Small changes or inter-limb differences, however, are likely due to testing variability and do not provide practitioners with detailed useable information on the condition of muscle tissue. These measurements are, therefore, unable to provide a detailed insight into authentic muscle mechanical properties so cannot be used as standalone monitoring tools and, instead, supplement an athlete monitoring programme alongside other proxy indicators of recovery status and readiness to perform.

Previous methods used in an attempt to estimate muscle stiffness, such as unilateral hopping tests, are relatively poor predictors of actual muscle stiffness, since they are also influenced by non-muscular tissue (i.e., tendons, ligaments, and the joint capsule) and can only be used to assess a whole muscle-group (Ličen and Kozinc, 2022). Moreover, performance of these tests may, in themselves, cause injury. As such, the non-invasive SWE and MyotonPRO techniques could be useful tools for objectively quantifying muscle tissue properties, providing immediate feedback for practitioners. Utilised alongside markers of load, such as GPS metrics, muscle stiffness quantification can provide insight into injury risk since increasing muscle stiffness alongside load may indicate that the muscle is struggling to cope with the demand (Lacourpaille et al., 2017b). The mechanism behind the association between elevated levels of muscle stiffness and injury risk is, at present, poorly understood and requires further investigation.

In elite football, explosive actions, such as sprinting and jumping, often result in creating goal scoring opportunities and in crucial defensive actions (Faude et al., 2012). As a result, it may be beneficial for practitioners to implement practices which may increase muscle stiffness and improve performance in sprinting and jumping to improve players' ability to support both attacking and defensive phases of play.

MSK injuries can cause substantial reductions in training and performance capacity. This comes with financial costs to elite sports clubs as a result of rehabilitative treatment and wages of injured athletes, but also impacts athletes' careers, livelihoods and wellbeing (Bangert et al., 2024, Haugen, 2022). Exercise has been shown to influence muscle stiffness measured by SWE, with the literature highlighting that different exercise modalities result in contrasting outcomes (discussed in Section 2.10) (Andonian et al., 2016, Lacourpaille et al., 2017b). There is also

some evidence to suggest that acute alterations in muscle stiffness in response to an exercise stimulus can be compounded by exercising on the following day (Morin et al., 2023). Further research needs to focus on these more chronic changes in muscle stiffness in response to training programmes and provide longer term insights into adaptive alterations in stiffness to determine if SWE may be used to longitudinally monitor athletes' MSK mechanical properties.

2.7. Regional differences in muscle stiffness

Since SWE records the shear modulus of only a small ROI, it must be considered that results are not necessarily representative of the whole muscle, due to the inhomogeneity of muscle tissue. It is likely that shear modulus of a ROI varies based on its location, with muscle tissue closer to a tendon likely to record larger shear modulus values due to tension from the connecting tendon. As discussed below, this assumption appears, to some extent, to be true, with results varying between muscles. Further research is required to elucidate the regional differences in muscle stiffness with confidence, as well as to understand the contrasting effects of exercise on muscle stiffness regionally. It is important to understand the spatial inconsistencies in stiffness of a muscle as this may be related to injury risk as a muscle experiences different levels of stress across regions. A pertinent example of this can be observed in injuries to the hamstring muscles, which occur more frequently at proximal locations than at distal locations, with a meta-analysis of 713 hamstring injuries reporting 62.8% occurring proximally (Grange et al., 2022). It is known that MSK tissue stiffness is elevated in the affected region post-injury, but it is yet to be determined if higher levels of MSK tissue stiffness regionally precede and contribute to injury (Kawai et al., 2021a, Kawai et al., 2021b). Investigating the exercise-induced changes in hamstring muscle stiffness regionally may elucidate a potential source for this imbalance in hamstring muscle injury location.

In the hamstring muscles, four studies have found greater shear modulus or SWV at distal locations than at medial or proximal sites (Miyamoto et al., 2020, Vaz et al., 2021, Cornelson et al., 2022, Miyamoto and Hirata, 2021). These results represent a comprehensive analysis of hamstring regional stiffness, as SWE measurements are taken in all three muscles, at different knee and hip angles, and under conditions of both rest and contraction, with results still supporting the same conclusion. This lower proximal stiffness may contribute towards the observed greater prevalence of injury, since the proximal region may be less reliant on its mechanical stiffness as a method of force generation and therefore requires greater muscle fibre recruitment and contraction force, placing the tissue under greater stress.

Results are less conclusive, however, in the triceps surae and quadriceps muscles. Findings from one study in the medial gastrocnemius (MG) appear to show a greater shear modulus in muscle tissue located distally than that at medial or proximal locations, i.e., muscle tissue closer to the Achilles tendon is stiffer (Le Sant et al., 2017). In contrast, however, results from Zhou and colleagues display a trend in which muscle tissue shear modulus is higher proximally, decreased medially and recovered slightly distally in the MG and lateral gastrocnemius (LG), although the significance of these results was not reported (Zhou et al., 2019, Zhou et al., 2020). These results appear to align with predictions that shear modulus is greater closer to a tendon and further studies are required which analyse for or report statistical significance.

Studies investigating differences in regional quadriceps muscle stiffness are less prevalent and report conflicting findings. Kodesho and colleagues recorded greater shear moduli at proximal locations than at central or distal locations of the RF (Kodesho et al., 2021a). Contrastingly,

Freitas and colleagues found no significant differences between proximal and distal regions of the RF, vastus lateralis (VL) or vastus medialis (VM) (Freitas et al., 2019). These findings, however, may be a result of methodological flaws, since it is reported that proximal and distal SWE measurements were recorded at ~20-40% and ~60-80% of muscle length, respectively (Freitas et al., 2019). The lacking standardisation of measurement location is a substantial limitation and contrasts the comprehensive standardisation of the seven locations measured only in the RF in the study by Kodesho and colleagues (Kodesho et al., 2021a).

It appears, however, that quantifying regional differences in muscle stiffness is not this straightforward, with joint angles differentially influencing muscle length and localised regional stress in biarticular muscles. It is known that increasing muscle length increases muscle stiffness, indeed, this is the very premise by which tensile testing quantifies material stiffness (Le Sant et al., 2015). Results indicate, however, that the manipulation of joint angles to alter muscle length does not result in uniform increases in muscle stiffness. In biarticular muscles, regions closest to the joint causing its stretch display higher levels of stiffness than when they are not stretched, and compared with other regions of the muscle. For example, higher levels of proximal MG stiffness are observed when participants are prone, i.e., the knee is extended, stretching the muscle proximally (Zhou et al., 2019, Zhou et al., 2020). Additionally, research conducted on the RF found greater stiffness proximally than distally, but in this study, participants were positioned supine (with the hip extended), stretching the muscle in this proximal region (Kodesho et al., 2021a).

More comprehensive research is required within different muscles to understand the influence of regional stretching and exercise stimuli and contraction modalities on regional MSK tissue stiffness.

2.8. The influence of muscle stiffness on clinical outcomes

Abnormal levels of muscle stiffness have been identified in diseased states and associated with their severity. Assessment of muscle biomechanical properties in these patient groups are often too difficult to complete in the clinical setting (e.g., force-fascicle length curve plotting with dynamometry and ultrasonography, discussed in section 2.4.2), or rely on qualitative assessments such as manual palpation and the modified Ashworth scale (MAS) (Harb and Kishner, 2024). The ability to quantitatively measure muscle mechanical properties and the development of techniques and protocols to do so may, therefore, prove useful in the diagnosis and severity monitoring of disease, and in assessing the effectiveness of treatments. MSK SWE has therefore been utilised for research in clinical populations. This section will discuss the current state of the literature investigating the clinical utility of SWE in patients with cerebral palsy, Parkinson's disease, Duchenne muscular dystrophy, and sarcopenia.

2.8.1. Muscle stiffness in cerebral palsy

Cerebral palsy (CP) is a condition caused by abnormal development of or damage to the brain before, during or shortly after birth, resulting in movement disorders such as spasticity (Vitrikas et al., 2020). Thus far, research has been conducted using SWE to compare lower leg muscle stiffness values between limbs in CP patients, and healthy controls; assess the relationship between muscle stiffness and current diagnostic practice; and evaluate the effectiveness of interventions aimed to relieve symptoms of CP and reduce muscle spasticity.

Cross-sectional studies have found elevated levels of MG, LG, and soleus (SOL) muscle stiffness, assessed by SWE in patients with CP compared with healthy age-matched controls (Lallemant-Dudek et al., 2021, Brandenburg et al., 2016, Vola et al., 2018). Furthermore,

stiffness has been assessed in the more- and less-affected limbs of patients with hemiplegic CP who experience unilateral symptoms, finding higher levels of MG and tibialis anterior (TA) stiffness in the more-affected limb (Lallemant-Dudek et al., 2021, Lee et al., 2016, Boulard et al., 2021). CP patients often suffer with pes equinus deformity which severely limits ankle dorsiflexion ROM, preventing normal gait. Analan and Aslan (2023) found higher SWV in the gastrocnemius than in the TA of CP patients and hypothesised that this elevated plantarflexor stiffness may be contribute to the pes equinus deformity observed in CP.

Stiffness of the SOL, MG and LG muscles in CP patients have been shown to have significant positive correlations with MAS score, which rates the severity of spasticity (Vola et al., 2018, Dağ et al., 2020, Bertan et al., 2020). SWE has also been utilised as a method of assessing the efficacy of interventions aimed at alleviating muscle spasticity. Injection of botulinum toxin A (Botox) has recently been proposed as a treatment for muscle spasticity and contractures in CP patients by reducing muscle activity (Multani et al., 2019). It has been shown that MG, LG and TA muscle stiffness significantly reduce 1 month after Botox injection, and that these reductions are significantly correlated with improvements in MAS score and passive ankle ROM following the intervention (Dağ et al., 2020, Bertan et al., 2020).

Based on this discussed literature, it is evident that SWE is a useful quantitative tool in the diagnosis and assessment of severity of muscle spasticity and assessing the effectiveness of treatments/interventions in CP patients. The literature has, thus far, focussed on the lower limb due to the severity of muscle spasticity experienced in these muscles, and the prevalence of pes equinus deformities in CP patients, limiting normal gait. Future research should, however, assess other muscle groups in CP patients to determine if further differences exist to typically

developing children and, if so, aid development of treatments in these muscles also. Furthermore, longitudinal studies should be conducted to investigate if and how muscle stiffness changes over time in CP patients, and if this is associated with progression or worsening of spasticity-related symptoms.

2.8.2. Muscle stiffness in Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease with motor symptoms including tremor, slow movement, and poor balance (Tolosa et al., 2021). Rigidity is one of the most prevalent symptoms associated with PD, but its clinical assessment relies upon subjective measures such as the semi-quantitative Unified Parkinson's disease Rating Scale (UPDRS) which can lead to error in diagnosis (Patrick et al., 2001, Ferreira-Sánchez et al., 2020). As a result, research has recently been conducted to evaluate the utility of SWE in quantitatively assessing skeletal muscle stiffness as a proxy for rigidity. This research has found differences between PD patients and healthy age-matched controls; relationships between muscle stiffness assessed by SWE and rigidity by the UPDRS; distinguished between PD symptom phenotypes; and assessed the efficacy of interventions aimed at treating symptoms of rigidity-dominant PD.

Cross-sectional studies have revealed elevated levels of muscle stiffness in the biceps brachii (BB) and brachioradialis muscles of PD patients compared to healthy age-matched controls (Ding et al., 2021, Oppold et al., 2023). Du et al. (2016) found similar differences in BB muscle stiffness between PD patients and healthy-age matched controls but revealed no significant differences between 'remarkably' and 'mildly' symptomatic arms in PD patients. Whilst this reveals SWE may not be sensitive to symptom severity between limbs in PD patients, this study did find a significant positive correlation between rigidity scores from the UPDRS-III and shear

modulus of the BB (r=0.646) (Du et al., 2016). In support of this, Oppold et al. (2023) also found a significant positive correlation between SWV of the BB and UPDRS-III rigidity score. These results support the utility of SWE in assessing rigidity in PD patients by revealing a strong positive relationship between muscle shear modulus and current clinical diagnosis practices. Whilst Du et al. (2016) failed to report a difference between limb severity, Ding et al. (2024) showed that SWE is sensitive to different PD symptom phenotypes. Here, passive LG shear modulus was found to be significantly higher in PD patients with a tremor dominance than in patients with postural instability gait difficulty, perhaps revealing that SWE may be useful in researching the biomechanical properties of and mechanisms underlying the different symptom phenotypes of PD (Ding et al., 2024).

Similarly to CP research, SWE has been utilised in assessing the efficacy of treatments in PD patients. Li et al. (2022) investigated the effects of Qihuang needle therapy in patients with PD on stiffness of the BB, brachioradialis, RF and LG, finding reduced muscle stiffness 2, 3 and 4 weeks after the 4-week treatment period, compared to the sham acupuncture control group. These positive results were accompanied by significantly larger reductions in UPDRS-III rigidity scores in the Qihuang needle therapy group than in the sham acupuncture control group, highlighting the clinical relevance of the findings (Li et al., 2022). Deep brain stimulation and levodopa medication, a dopamine precursor, are well-established treatments used for alleviating motor symptoms in PD patients. Oppold et al. (2023) found significant reductions in both rigidity measured by the UPDRS-III and stiffness measured by SWE in response to deep brain stimulation, which were compounded by combined treatment with levodopa medication.

Based on the available literature, SWE appears to be a useful quantitative tool in the assessment of muscle rigidity, and evaluating the efficacy of treatments for motor symptoms in PD patients. Future research should establish if differences exist between more- and less-affected limbs to help clinicians objectively assess symptom severity. In addition, longitudinal studies should be conducted to investigate if changes to SWV and UPDRS-III scores are related over time to examine the efficacy of SWE as a method of monitoring progression of rigidity symptoms in PD patients.

2.8.3. Muscle stiffness in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a genetic disorder categorised by progressive muscle wasting and weakening resulting in movement difficulties (Duan et al., 2021). Along the same lines of enquiry as in CP and PD, SWE has been utilised in patients with DMD to compare their muscle stiffness to typically developing children, finding conflicting results, and to longitudinally monitor symptom progression. However, studies have not investigated the influence of treatments on muscle stiffness in DMD patients.

In the lower limb muscles (RF, MG, VL, adductor magnus, gluteus maximus, and TA) the literature comprehensively shows DMD patients to have significantly greater muscle stiffness, as measured by SWE than typically developing children (Lin et al., 2021, Pichiecchio et al., 2018, Yu et al., 2022, Lacourpaille et al., 2015). In the upper limb muscles, however, the literature shows conflicting results. Lacourpaille et al. (2015) report significantly higher levels of muscle stiffness in the BB and triceps brachii (TB), but not in the abductor digit minimi in DMD patients than healthy age-matched controls. A cross-sectional study by Lin et al. (2023), however, found significantly lower SWV in the BB of DMD patients than in typically

developing controls, as well as a decreasing SWV with age (3-18yrs). In this study, the authors also found SWV of the BB and deltoid muscles to significantly positively correlate with results in the performance of upper limb module (PUL), used to assess muscle function (Lin et al., 2023). These lower levels of upper limb muscle stiffness observed in the DMD patients may be related to strength losses, and the relationship with PUL score highlights the clinical significance of the findings in assessing the severity of muscle wastage. Further support for the clinical utilisation of SWE in DMD patients comes from research by Lacourpaille et al. (2017a) finding significantly greater shear modulus in TA, GM and TB muscles at a 12-month follow up compared to baseline, whilst no such increases were found in the healthy control group.

These findings indicate that SWE is a useful quantitative monitoring tool for determining differences in muscle stiffness between DMD patients and healthy controls, and assessing the degree of muscle wastage in DMD patients. Future research should focus on clarifying the muscle stiffness properties of the upper limb in DMD patients compared to typically developing children, and investigate the utility of SWE in evaluating the efficacy of treatments and interventions to alleviate symptoms in patients with DMD.

2.8.4. Muscle stiffness in sarcopenia

Sarcopenia is a progressive disease most commonly seen in the elderly, characterised by the loss of muscle strength, quality, quantity, and physical performance (Ardeljan and Hurezeanu, 2024). Muscle stiffness estimation using SWE has been used by researchers to investigate changes associated with ageing, compare those with sarcopenia to healthy age-matched controls, and correlate muscle stiffness values with sarcopenia-related clinical outcome measures.

Şendur et al. (2020) assessed the MG using SWE in 57 participants aged 18-74, finding a significant negative correlation between muscle stiffness and age. Moreover, SWE has been utilised in cross-sectional studies to compare patients with sarcopenia to healthy age-matched controls, or controls with the same comorbidity, as sarcopenia is often concomitant with other diseases in the elderly, such as type 2 diabetes. These studies have comprehensively found muscle stiffness to be significantly lower in older participants with sarcopenia in the TA, MG, SOL, RF, VL, VM, vastus intermedius (VI), biceps femoris (BF), semitendinosus (ST), semimembranosus (SM), and BB muscles (Wang et al., 2023, Okyar Baş et al., 2023, Chen et al., 2023, Alfuraih et al., 2019). Furthermore, muscle stiffness of the TA, MG, SOL, RF, VL, VM, VI, BF, ST, SM and BB assessed by SWE have all been significantly positively correlated with grip strength (Wang et al., 2023, Alfuraih et al., 2019). TA, MG and SOL stiffness were significantly positively correlated to gait speed (Wang et al., 2023). Moreover, the TA, MG, SOL, RF, VL, VM, VI, BF, ST, SM and BB muscles have all been shown to significantly positively correlate with muscle mass, number of chair stands and isometric knee flexion and extension strength, and significantly negatively correlate with walking time (Alfuraih et al., 2019).

These findings suggest that SWE is useful in detecting sarcopenia and those at risk of developing sarcopenia with age, and correlates well with multiple clinical outcomes associated with sarcopenia. It is known that resistance training can induce acute increases in muscle stiffness assessed by SWE (discussed in section 2.10.2), therefore future research should focus on testing the chronic effects of strength training interventions on muscle stiffness in patients with sarcopenia.

2.9. The influence of muscle stiffness on musculoskeletal performance

A certain degree of muscle stiffness is thought to be beneficial for exercise performance as it contributes to the muscle's ability to efficiently store and release elastic energy. Considering the biomechanical properties of muscle through Hill's 3-element model, force transmission is greater through a stiffer material than a more compliant material as its recovery from deformation is faster (Hill, 1938). This would suggest that stiffer muscle is beneficial for performance of explosive actions.

Multiple studies have explored this hypothesis, examining the relationship between muscle stiffness and performance of sprinting, jumping, and rapid changes of direction. These studies utilised different methods of estimating the stiffness of different lower limb muscles, all drawing the same conclusion that stiffer muscle is beneficial for performance of explosive actions (Kalkhoven and Watsford, 2018, Pruyn et al., 2014, Miyamoto et al., 2019, Ando et al., 2021b). These cross-sectional studies provide only low-level evidence in support of this hypothesis. Therefore, more recent studies sought to understand the relationship between passive muscle stiffness and performance of explosive actions mechanistically. In one study, researchers measured squat jump height, CMJ height and stiffness of the quadriceps muscles (Djurić et al., 2023). Countermovement utilisation ratio (CUR) was expressed as the ratio between squat jump and CMJ height, with a greater CUR indicating a larger difference between the two and a greater contribution of passive elastic forces. Results revealed significant positive relationships between quadriceps stiffness and CUR, indicating that greater passive stiffness of these muscles benefits performance of explosive actions by increasing the passive elastic contribution of muscle (Djurić et al., 2023, Van Hooren and Zolotarjova, 2017). Similarly, drop jump performance seems to be enhanced by greater levels of passive muscle stiffness allowing

for a greater release of stored elastic energy. Drop jump index is the ratio between jump height and contact time, wherein a greater drop jump index reflects a shorter contact time for the same jump height. One study found a significant correlation between MG stiffness and drop jump index, proposing that this is due to greater release of elastic energy from stiffer muscle tissue at the contact phase (Ando et al., 2021b). Moreover, the same researcher reported an association between rate of torque development and MG muscle stiffness (Ando, 2024). Given that the mechanism behind this hypothesis is that stiffer muscle has a greater store of elastic energy, muscle stiffness is likely also beneficial to running performance by increasing running economy and lowering oxygen cost. There is some evidence for this in the form of a cross-sectional study in which long distance runners exhibited higher VL stiffness than sprinters, however, more research is required to support this (Miyamoto et al., 2019).

Contrastingly, excessively high levels of muscle stiffness may impair exercise performance by reducing ROM, reducing movement efficiency (Hirata et al., 2020). Additionally, chronic drop jump training has been shown to decrease MG muscle stiffness, despite improvements in drop jump performance (Ando et al., 2021a). Training-induced improvement in drop jump performance, thus, cannot be attributed to increases in passive muscle stiffness in the MG. This brings into question the efficacy of using interventions to enhance muscle stiffness as a mode of improving performance in explosive actions, suggesting the mechanism of improved performance lies elsewhere. These findings, however, are specific to the MG and further research should be done in other lower limb muscles contributing to drop jump performance. Although, preliminary findings in other muscles appear to show a level of agreement, such that acute drop jump performance decreases stiffness of the VL and LG (Hill et al., 2022). Overall,

these findings suggest that, although passive muscle stiffness may be beneficial for performance of explosive actions, it is not the main driver of training-induced performance enhancement.

Despite these suggestive findings and a sound theoretical basis behind the positive relationship between muscle stiffness and performance, through Hill's 3-element model of muscle and the release of stored elastic energy, there is a need for more extensive research in this field. Specifically, research is required to confirm these findings and build on this knowledge through testing of more lower limb muscles with both SWE and the MyotonPRO in broader population samples.

2.10. The effect of different exercise modalities on muscle stiffness

Research thus far indicates that alterations in muscle stiffness following exercise are dependent on the contraction type, exercise modality, and the volume and intensity of the exercise bout. It is important to understand how these factors influence muscle stiffness to understand the muscular response to different training modalities and aid the development of interventions and training plans to alter muscle stiffness to reach optimal levels for performance and injury prevention. This section will discuss the literature investigating the effects of endurance exercise, resistance, plyometric and sprint training, and eccentric and isometric contractions on muscle stiffness.

2.10.1. The effect of endurance exercise on muscle stiffness

The bulk of the literature indicates that endurance or aerobic-based exercise bouts lead to a decrease in muscle stiffness measured by SWE (Andonian et al., 2016, Morales-Artacho et al., 2017, Sadeghi et al., 2018, Fouré et al., 2022). These results were observed in multiple lower

limb muscles including those belonging to the quadriceps, hamstrings, and triceps surae muscle groups following exercise ranging from 15min steady-state cycling, and 6.4km running, up to a mountainous 330km ultra-marathon. As is to be expected in research, however, there are some results which contrast this, finding increases in muscle stiffness of lower limb muscles following exercise comprising 30min treadmill running and ultra-marathon races of <60km (Ohya et al., 2017, Fouré et al., 2022). Findings from these studies are summarised in Table 2.2.

Whilst results following 30min steady state treadmill running appear to contradict the literature, finding increases in muscle stiffness post-exercise, these findings were in the flexor digitorum longus and tibialis posterior muscles (Ohya et al., 2017). These smaller muscles have not been subject to extensive research and these results may be due to their specific contraction profile in their contribution to running. Other muscles investigated in this study, including the medial and lateral gastrocnemii, and the peroneus longus and peroneus brevis muscles appear to conform to the literature, displaying a trend of decreased stiffness after the running intervention, however, these findings were not statistically significant. This highlights the intermuscular differences in response to exercise, emphasising the complexity of the field and the large amount of research which is still to be done. Similarly, contrasting results were found by Fouré and colleagues, with increases in stiffness of the triceps surae muscles observed following the shorter races, but decreases observed in the same muscles following the longer races (Fouré et al., 2022). This reinforces the role of volume in muscular mechanical response and adaptation to exercise. Moreover, results were obtained following various mountainous ultra-marathon events, making it complex to draw detailed and confident conclusions since the exercise intervention was not standardised between participants. Although research by Andonian and

colleagues, finding decreases in muscle stiffness, was also centred around a mountainous ultra-marathon race, these results were observed in the quadriceps muscles, again stressing the intermuscular differences (Andonian et al., 2016). These intermuscular differences are likely a result of the muscles' individual roles and contraction profiles whilst performing the exercise.

2.10.2. The effect of resistance training on muscle stiffness

In direct contrast to these findings in endurance exercise, the literature shows that resistance exercise induces increases in muscle stiffness (Kato et al., 2018, Akagi et al., 2015, Pournot et al., 2016, Ustabaşıoğlu et al., 2022). These studies examined the effects of resistance exercise on the GM, TB, and BB, all drawing the same conclusion that muscle stiffness is significantly augmented following resistance exercise. The methodology and findings of these studies are summarised in Table 2.2.

One study, however, found no significant differences in muscle stiffness following resistance exercise. This was the control group of a study conducted by Lacourpaille and colleagues, in which participants completed 3 sets of 10 maximal bicep curls, only including the concentric elbow flexion phase (Lacourpaille et al., 2017b). Since the other protocols involved both flexion and extension phases, this implicates the eccentric component with the observed increases in muscle stiffness. This is well accepted in the literature, as is to be discussed in Section 2.10.6, and more research is needed to understand how isolated concentric contractions impact muscle mechanical properties. It is worthy of note, however, that this control group completed one less set of bicep curls compared with participants in the study by Pournot et al. (2016), and stiffness was measured 30min post-exercise, unlike the latter study, in which significant differences were observed immediately post-exercise and after 15min passive recovery (Lacourpaille et al.,

2017b). This reduced exercise volume and greater recovery time may, therefore, explain the absence of a significant increase in muscle stiffness, which may have acutely risen in the 30min post-exercise period, and not the lack of eccentric contractions. It is this heterogeneity in study design which makes it complex to draw absolute conclusions from the literature and caution must be taken when extrapolating findings to other contexts.

To summarise, resistance exercise causes an increase in muscle stiffness which is likely influenced by the inclusion of eccentric contractions and the volume and intensity of the exercise.

2.10.3. The effect of plyometric training on muscle stiffness

Interest in the mechanical adaptations of muscle to plyometric training gained interest as popularity of the training technique grew in the athletic conditioning field. This is because of the known benefits of passive muscle force on performance of explosive actions, including drop jump performance (discussed in Section 2.9) (Djurić et al., 2023). Increases in both passive and active muscle stiffness have been observed following chronic plyometric training studies of at least 6 weeks, utilising the force-fascicle length methodology (discussed in Section 2.4.2) (Fouré et al., 2012, Kubo et al., 2017, Kubo et al., 2021). These findings are perhaps unsurprising, as they are accompanied by improved jumping performance and stretch-shortening cycle muscle function (Markovic and Mikulic, 2010). Increased muscle stiffness appears to be a beneficial adaptation to plyometric training which results in improved performance, much in the same way that improved maximal oxygen uptake is an adaptive response to endurance training that improves performance (Huang et al., 2005). Whilst this evidence for chronic training adaptations is strong, it has not been replicated using SWE or in

other muscle groups. Consequently, future research should focus on utilising MRE and SWE to detect both acute and chronic changes in muscle stiffness in response to plyometric training.

2.10.4. The effect of sprint training on muscle stiffness

Sprinting is an important component of many team sports, with Faude et al. (2012) finding it to be the most frequent action in the lead to up goal situations in professional football. However, it is also the most common mechanism of hamstring injury due to the explosive nature of the acceleration and maximal velocity phases (Woods et al., 2004). As such, two recent studies have investigated the acute effects of repeated sprint exposure on stiffness of the hamstring muscles using SWE (Pimenta et al., 2023, Freitas et al., 2023). Both studies utilised a repeated sprint protocol comprising 10 x 30m sprints and found increased active biceps femoris long head (BFlh) shear modulus immediately after. These results were obtained with SWE utilised during an isometric contraction of 20% maximal voluntary isometric contraction (MVIC). Pimenta et al. (2023) found no changes in passive hamstring muscle stiffness following the exercise protocol, but Freitas et al. (2023) observed significant reductions in semitendinosus (ST) and BFlh passive muscle stiffness. Due to the rapid contractile force generation and strain placed on the hamstring muscles during sprinting, linking it to an increased injury risk, the increases in active BFlh muscle stiffness are to be expected. It is unexpected that Freitas et al. (2023) observed reductions in passive hamstring muscle stiffness following repeated sprint bouts. There are several limitations with the study, however, that may explain these unexpected findings. Firstly, the authors reported very poor reliability in the estimation of passive BFlh shear modulus, presenting an intraclass correlation coefficient (ICC) of -0.01, indicating no level of agreement or repeatability between the measures and, in doing so, invalidating these findings. Secondly, previous studies have reported stretching muscles beyond their slack length

to be best practice when estimating stiffness using SWE (Koo and Hug, 2015). Freitas et al. (2023), however, assessed passive hamstring muscle stiffness with participants prone and the knees flexed at 30º. Lacourpaille et al. (2017b), for example, reported significant increases in passive muscle stiffness measured at long, but not short muscle lengths following eccentric muscle damage-inducing exercise bouts. Finally, the design of the sprint protocol meant that participants were not consistently running at maximum velocity. This was confirmed by the reporting of significant reductions in maximum velocity across the 10 repetitions. The reasons for this are twofold: (1) participants were only given 30s rest between sprint bouts, which is not sufficient for recovery (Haugen et al., 2019), and (2) it is well-established that humans reach maximum velocity at ~40m, but the sprint repetitions in the present study were only 30m (Healy et al., 2022). This study is, therefore, limited in that it is not assessing the effects of maximal velocity on hamstring muscle mechanical properties, but of acceleration. Consequently, whilst the evidence suggests an acute increase in active BFlh muscle stiffness measured by SWE, further research is required to determine the effects of maximal velocity sprinting on passive hamstring muscle stiffness which uses different exercise and SWE measurement protocols to overcome the limitations of the existing literature.

2.10.5. The effect of isometric contractions on muscle stiffness

Based on the findings in endurance, resistance, plyometric and sprint exercise, it is likely that changes in stiffness are not a direct consequence of exercise modality, but instead a function of the muscle's dominant form of contraction during the exercise.

Research into the effect of isometric contractions on muscle stiffness is extremely limited, with the tested exercise protocols contrasting starkly. Two studies tested a series of MVICs on the

knee extensors, finding reductions in VL stiffness post-exercise (Siracusa et al., 2019, Chalchat et al., 2020). Another tested exercise protocol involved sustained contraction of the plantar flexors for 1h at 10% MVIC and found an increase in GM stiffness post-exercise (Akagi et al., 2017). Further details of these studies can be found in Table 2.2. The contrasting results are likely due to the heterogeneity in study design, both in assessing different muscles and in the difference in volume and intensity of the contractions. It is, therefore, evident that more research is required into the influence of isometric contractions on alterations in muscle passive mechanical properties to draw proper conclusions into their effect, but also to understand the influence of contraction volume and intensity.

2.10.6. The effect of eccentric contractions on muscle stiffness

There is a growing body of evidence within the literature indicating that eccentric resistance exercise causes an increase in muscle stiffness (Heales et al., 2018, Guilhem et al., 2016, Jones et al., 2021, Tsuchiya et al., 2019, Leung et al., 2017, Goreau et al., 2022, Voglar et al., 2022, Chalchat et al., 2022, Chalchat et al., 2023, Xu et al., 2019, Ema et al., 2021, Lall et al., 2022, Inami et al., 2022, Janecki et al., 2011, Kawczyński et al., 2018). In these studies, similar results have been observed in different muscle groups with exercise interventions involving maximal and sub-maximal eccentric contractions of the knee extensors and flexors, elbow flexors, and plantar flexors, resulting in augmented muscle stiffness measured by SWE. Furthermore, it has been found that the augmented muscle stiffness response to eccentric exercise accumulates with exercise completed on consecutive days (Morin et al., 2023). More research is needed to support these findings. Details of the exercise protocols used in these studies and the muscles evaluated can be found in Table 2.2.

Despite this body of evidence strongly suggesting that eccentric exercise increases muscle stiffness, there are, again, some studies which have found contrasting results (Xu et al., 2019, Hotfiel et al., 2018, Heiss et al., 2018, Kawama et al., 2022, Kisilewicz et al., 2020). Xu et al. (2019) used an exercise stimulus of 75 maximal eccentric knee extensions and found a significant increase in rectus femoris stiffness, a significant decrease in vastus lateralis stiffness, and no change in vastus medialis stiffness, compared to baseline. This further reinforces the intermuscular variability in response to the same exercise stimulus.

Table 2.2

Paper	**Population (N) Sex Age ± SD (years)**	**Exercise intervention**	**Muscles evaluated**	**Effect on stiffness**	**Time of measurement**
Endurance exercise					
Andonian et al., 2016	Endurance runners (27) M 43 ± 8.6	Tor des Geants® mountainous ultra-marathon (330km, elevation gain 24,000m)	RF, VM, VL (combined as quadriceps)	↓	Immediately and 48h post-race
Morales-Artacho et al., 2017	Physically active (14) M 26.6 ± 4.5	15min cycling warm-up (5min @ 40% & 5min @ 60% maximal power (W) & 5 6s all-out sprints every min for 6min)	BF, ST, SM (combined as hamstrings)	↓	5min and 30min post-cycling
Sadeghi et al., 2018	Well-trained runners (9) 7 M, 2 F 20 ± 2	4 mile running race	SOL, RF	↓	24h post-race
Sadeghi et al., 2018	Well-trained runners (10) 6 M, 4 F 26 ± 6	13.1 mile running race	ST	↓	24h & 1wk post-race

Fouré et al., 2022	Endurance runners (26) 18 M, 8 F 38 ± 8	Ultra-marathon >100km (Courmayeur-Champex-Chamonix, sur les Traces des Ducs de Savoie, or Ultra-Trail du Mont-Blanc)	GM, GL, SOL (combined as triceps surae)	↓	Within 1h post-race
Ohya et al., 2017	Healthy (20) M 20.9 ± 0.6	30min treadmill running @ 12km/h, 0º incline	Flexor digitorum longus, tibialis posterior	↑	Immediately post-exercise
Fouré et al., 2022	Endurance runners (23) 12 M, 11 F 36 ± 9	Ultra-marathon <60km (de Martigny-Combe à Chamonix or Orsières-Champex-Chamonix)	GM, GL, SOL (individually and combined as triceps surae)	↑	Within 1h post-race
Eccentric resistance exercise					
Heales et al., 2018	Healthy, untrained (13) 6 M, 7 F 22.4 ± 3.5	5 x 20 maximal eccentric non-dominant knee extensions (2min between sets)	RF	↑	Immediately post-exercise
Lacourpaille et al., 2017	Healthy (21) M & F 25.6 ± 2.7 (low load, n=10) 23.3 ± 4.7 (high load, n=11)	5 x 15 or 30 (low & high load groups) maximal eccentric knee extensions (90s between sets)	RF, VM, VL (combined as quadriceps)	↑	30min post-exercise
Lacourpaille et al., 2017	Healthy (24) M & F 23.8 ± 3.2 (low load, n=11) 26.4 ± 2.5 (high load, n=13)	3 or 6 (low & high load groups) x 10 maximal eccentric elbow flexions (90s between sets)	BB, BA (combined as elbow flexors)	↑	30min post-exercise

Lacourpaille et al., 2014	Healthy (16) 11 M, 5 F 23.6 ± 3.2	3 x 10 maximal eccentric elbow flexions (90s between sets)	BB, BA (combined as elbow flexors)	↑	1h post-exercise
Guilhem et al., 2016	Healthy (17) 9 M, 8 F 25.0 ± 3.7	10 x 30 maximal eccentric plantarflexions	GM	↑	5min post-exercise
Jones et al., 2021	Healthy, untrained (10) M 23.7 ± 3.2	10 x 10 bicep curls (1s concentric phase, 3s eccentric phase)	BB	↑	24h & 48h post-exercise
Tsuchiya et al., 2019	Healthy, untrained (8) M 20.9 ± 0.4	6 x 10 maximal eccentric bicep curls @ 100% MVC (90s between sets)	BB	↑	Immediately post-exercise
Inami et al., 2022	Healthy (17) M 22.0 ± 4.7	5 x 10 eccentric bicep curls @ 50% MVC (2min between sets)	BB	↑	Immediately, 1h, 24h, 48h, 72h & 96h post-exercise
Leung et al., 2017	Healthy, untrained (45) 36 M, 9 F 21.2 ± 1.3	10 x 15 eccentric heel raises (2s lowering phase, 30s between sets)	GM, GL	↑	Immediately post-exercise
Goreau et al., 2022	Healthy, untrained (24) 14 M (26.4 ± 1.9), 10 F (25.5 ± 2.3)	5 x 15 maximal eccentric knee flexions (2min between sets)	BF, ST	↑	30min post-exercise
Voglar et al., 2022	Healthy (14) 7 M, 7 F 25.5 ± 4.7	3 x 10 maximal eccentric knee flexions (2min between sets) 3 x 6 Nordic hamstring exercise (2min between sets)	BF, ST	↑	Immediately post-exercise – BF Immediately, 1h & 24h post exercise – ST
Chalchat et al., 2022	Healthy, untrained (16) M	45min downhill walking on a treadmill, carrying 30%	RF, VL	↑	Immediately, 4h, 24h, 48h, 72h, 7d post-exercise – RF

	31.5 ± 5.9	body mass, gradient -25%, velocity 4.5km/h			Immediately, 4h, 24h post-exercise – VL
Chalchat et al., 2023	Healthy, untrained (15) M 32.2 ± 5.8	45min downhill walking on a treadmill, carrying 30% body mass, gradient -25%, velocity 4.5km/h	RF, VL	↑	Immediately, 4h, 24h, 48h, 72h, 7d post-exercise – RF Immediately, 4h, 24h, 48h post-exercise – VL
Ema et al., 2021	Healthy, untrained (28) M 22 ± 1	10 x 10 maximal eccentric knee extensions (3.5s between reps, 2min between sets)	RF	↑	24h, 48h & 72h post-exercise
Lall et al., 2022	Healthy, untrained (35) 20 M, 15 F	3 x 50 eccentric calf raises @ body weight on a step	MG, LG	↑	Immediately, 24h & 48h post-exercise
Morin et al., 2023	Healthy, physically active (15) 9 M, 6 F 21 ± 1	2 x 15 maximal eccentric knee extensions (2min between sets) with additional sets completed until MVC loss of >20%	RF, VL, VM	↑	30min post-exercise
Xu et al., 2019	Healthy, untrained (26) 15 M, 11 F 23.8 ± 3.3	75 maximal eccentric knee extensions (6s between reps)	RF, VL	↑/↓	Immediately & 48h post-exercise – RF ↑ Immediately post-exercise – VL ↓
Agten et al., 2017	Healthy (10) 5 M, 5 F 39.6 ± 4.6 (M) 30.6 ± 13.5 (F)	3 x 12 eccentric elbow flexions @ 90% 1RM	BA	×	15min, 12h, 24h, 48h, 72h & 1wk post-exercise
Hotfiel et al., 2018	Healthy (15) 8 M, 7 F 24 ± 4	5 x 30 eccentric heel raises @ bodyweight + 25%	GM, SOL	↓/×	60h post-exercise

Heiss et al., 2018	Healthy, untrained (14) 8 M, 6 F 24 ± 4	5 x 30 eccentric heel raises @ bodyweight + 25%	GM		60h post-exercise
Kawama et al., 2022	Healthy (13) M 24.1 ± 2.9	3 x 10 stiff-leg deadlifts (eccentric phase) @ 60% body mass with large ROM	SM		3min post-exercise
Kisilewicz et al., 2020	Healthy, untrained (14) 11 M, 3 F 23.2 ± 3.0	5 x 10 maximal eccentric shoulder raises (2min between sets)	Trapezius		24h post-exercise
Concentric resistance exercise					
Kato et al., 2018	Healthy, active (16) 9 M, 7 F 33.3 ± 5.4	3 x 20 heel raises	GM		Immediately post-exercise
Akagi et al., 2015	Healthy, untrained (18) M 22.4 ± 2.6	5 x 8 elbow extensions (2s concentric and eccentric phases, 90s between sets)	TB		Within 15min post-exercise
Pournot et al., 2016	Healthy, untrained (11) 6 M, 5 F 38 ± 9	4 x 10 bilateral bicep curls @70% 1RM (60s between sets)	BB		Immediately & 15min post-exercise
Ustabaşıoğlu et al., 2022	Healthy, untrained (60) M 28.9	3 x 8 bicep curls @ 70-80% 1RM (2min between sets) High velocity group = 2s concentric, 1s eccentric, Low velocity group = 8s concentric, 4s eccentric	BB		Immediately post-exercise
Lacourpaille et al., 2017	Healthy (8) M & F 24.1 ± 1.9	3 x 10 maximal bicep curls (90s between sets)	BB, BA (combined as elbow flexors)		30min post-exercise
Isometric exercise					

Akagi et al., 2017	Healthy, sedentary (19) M 22 ± 1	Sustained contraction of the plantar flexors for 1h @ 10% MVC	GM	↑	Immediately post-exercise
Siracusa et al., 2019	Healthy French Army soldiers (15) M 29 ± 2.5	6 x 10 isometric MVC of knee extensors (5s between reps, 15s between sets)	VL	↓	Immediately after each set
Chalchat et al., 2020	Healthy, trained (12) M 29.0 ± 4.5	6 x 10 isometric MVC of knee extensors (5s between reps, 10s between sets)	VL	↓	Immediately & 20min post-exercise

Table 2.2. The effect of exercise modality on muscle stiffness measured by shear wave elastography.

This table shows the weight of evidence for changes in muscle stiffness induced by different exercise modalities and contraction types.

SD = standard deviation, M = male, F = female, RF = rectus femoris, VM = vastus medialis, VL = vastus lateralis, BF = biceps femoris, ST = semitendinosus, SM = semimembranosus, SOL = soleus, BB = biceps brachii, BA = brachialis, GM = gastrocnemius medialis, GL = gastrocnemius lateralis, TB = triceps brachii, ROM = range of motion, ↑ = increase, ↓ = decrease, × = no significant change.

Since the literature shows clear changes in muscle stiffness in response to laboratory-based exercise interventions, research should now be conducted in more applied settings utilising exercise interventions which are typically performed in recreational and elite sport, as well as establishing the effects of sport-specific performance on muscle stiffness.

2.11. The relationship between muscle stiffness and muscle damage

Muscle damage is caused by strenuous or unaccustomed exercise, particularly when involving a high volume of eccentric contractions, resulting in microscopic tears in muscle fibres (Proske and Morgan, 2001). Muscle damage causes muscle soreness, weakness and swelling, and is quantified through microscopic analysis of muscle fibres obtained from biopsies (Clarkson and Hubal, 2002). Due to the invasive nature of muscle biopsies, simpler methods are often used as proxy measures to estimate the severity of muscle damage. These include blood biomarkers, such as creatine kinase, peak torque during MVIC, pain pressure threshold, limb circumference, and subjective ratings of soreness on a visual analogue scale (VAS) often rated 1-10 (Clarkson and Hubal, 2002).

As discussed in Section 2.10.6, many studies have found a relationship between eccentric, muscle damage-inducing exercise and muscle stiffness measured by SWE (Heales et al., 2018, Guilhem et al., 2016, Jones et al., 2021, Tsuchiya et al., 2019, Leung et al., 2017, Goreau et al., 2022, Voglar et al., 2022, Chalchat et al., 2022, Chalchat et al., 2023, Xu et al., 2019, Ema et al., 2021, Lall et al., 2022, Inami et al., 2022, Janecki et al., 2011, Kawczyński et al., 2018). Specific interest in the relationship between muscle stiffness and muscle damage was sparked by the publication of research conducted by Lacourpaille and colleagues (Lacourpaille et al., 2017b, Lacourpaille et al., 2014). The study involved eccentric exercise of the elbow flexors or the knee extensors with pre- and post-exercise measurements of MVIC and muscle stiffness, via SWE. SWE measurements were taken 30mins post-exercise and peak torque during MVIC was recorded 48h post-exercise. Results revealed significant negative relationships between the relative increase in shear modulus and the relative decrease in peak torque in both the elbow flexors ($r = -0.80$) and knee extensors ($r = -0.82$) (Lacourpaille et al., 2017b). These correlational analyses indicate that the greater the increase in muscle stiffness in response to an

eccentric exercise bout, the greater the decrease in peak torque 48h post-exercise, indicating a greater degree of muscle damage. The authors claim that these results imply SWE can be used as a predictor of the degree of muscle damage sustained from an exercise bout (Lacourpaille et al., 2017b). If correct, this could prove useful as an early, non-invasive marker of muscle damage, which may be superior to other proxy measures, enhancing practitioners' ability to accurately monitor recovery status and readiness to perform in elite athletes.

The findings that eccentric exercise increases muscle stiffness are logical, since increasing elongation and mechanical load cause an increase in material stiffness, and that is exactly what happens during eccentric contractions: muscle length is increased under tension. It is therefore possible that the increases in stiffness are reflective of the amount of elongation and load incurred by the muscle fibres during the exercise bout, which is also directly linked to the amount of damage incurred. A theory behind this being that myofibrillar disruptions caused by damage-inducing exercise disturb calcium ion (Ca^{2+}) homeostasis, described in more detail in Section 2.15.

To understand this relationship further, similar studies are needed that utilise Z-band streaming from muscle biopsies as the gold standard method of quantifying muscle damage, correlated with alterations in muscle stiffness following muscle damage-inducing eccentric exercise. Moreover, it is unclear how limb positioning, muscle length during contractions and consecutive exercise bouts may differentially impact muscle damage and stiffness in localised regions over time. Further experimental studies are required to understand the mechanism behind these observed changes in stiffness and to understand what they mean in relation to muscle damage.

2.12. The relationship between muscle stiffness and range of motion

Since muscle lengthening is affected by its biomechanical properties, it is likely that a relationship exists between muscle stiffness and flexibility. There is a breadth of evidence in the literature to support this theory, but more research is required to establish the extent of this relationship in different muscle groups.

The literature indicates that acute stretching-based interventions, are effective at both increasing ROM and decreasing muscle stiffness. These observations are consistent between a variety of stretching-based interventions in different lower limb muscle groups. Interventions involved static and dynamic stretching, proprioceptive neuromuscular facilitation, and foam rolling with and without vibration, all resulting in reduced stiffness of the GM, GL, RF, BF, ST and SM (Reiner et al., 2021a, Akagi and Takahashi, 2013, Ikeda et al., 2021, Reiner et al., 2021b, Reiner et al., 2022, Miyamoto et al., 2017, Umegaki et al., 2015, Nakao et al., 2018, Caliskan et al., 2019).

These reductions in muscle stiffness and increases in ROM are also present in response to chronic stretching interventions lasting 4-12 weeks, targeting the plantar flexors and knee flexors/hip extensors (Andrade et al., 2020, Akagi and Takahashi, 2014, Ichihashi et al., 2016). To our knowledge, only one study has reported a relationship between muscle stiffness and ROM (Reiner et al., 2024b). Stiffness was assessed in the GM, GL, RF, ST, SM, and BFlh, and ROM tests were performed for these muscle groups (standing wall push test for the triceps surae muscles, sit-and-reach test for the hamstring muscles, and modified Thomas test for the RF). No relationships were observed between plantarflexor ROM and muscle stiffness, or the modified Thomas test and RF stiffness. For the hamstrings, however, significant negative

correlations were observed between ROM in the sit-and-reach test and SM, BFlh and overall hamstring stiffness, but not for the ST alone (Reiner et al., 2024b). These findings provide evidence for a relationship between ROM and muscle stiffness and warrant further investigation. Presence of a relationship between the two has ramifications in injury prevention, with reducing muscle stiffness and increasing ROM both individually associated with lower risk of injury. Furthermore, stretching interventions may be useful in a rehabilitative setting, since muscle stiffness has been shown to remain elevated for years following injury (Kawai et al., 2021a, Kawai et al., 2021b, Yagiz et al., 2024). This would lead to enhanced recovery from injury and reduced risk of reinjury.

2.13. The effect of recovery strategies on musculoskeletal tissue stiffness

As discussed previously in this literature review, muscle stiffness has been shown to be altered by exercise (with increases in muscle stiffness related to incurrence of muscle damage); impede flexibility; and reduce following acute and chronic stretching interventions. For these reasons, muscle stiffness has been targeted by recovery strategy interventions aimed at reducing or preventing rises in muscle stiffness observed after exercise-induced muscle damage (EIMD). To this end, the literature has investigated the effects of massage, foam rolling, compression garments, cold water immersion, cryotherapy, local high frequency percussive massage, and whole-body vibration on muscle stiffness.

2.13.1. The effect of massage on muscle stiffness

Massage has been used for thousands of years for relaxation and rehabilitation purposes with subsequent increases in joint ROM thought to be a consequence of the mechanical pressure inducing reductions in passive and active muscle stiffness and increased blood flow

(Weerapong et al., 2005). Until recently, however, this has not been easy to study but, with the development of SWE, there is now an abundance of research being carried out to test this hypothesis. The literature appears to show consistent but very acute reductions in stiffness of the erector spinae, upper trapezius, masseter, BB, and MG muscles following massage (Jelen et al., 2024, Bethers et al., 2021, Olchowy et al., 2020, Inami et al., 2024, Eriksson Crommert et al., 2015). These results were found with a variety of different types of massage, including conventional therapeutic massage, sports massage, and positional release therapy, whilst investigations were also performed into the effects of massage pressure on efficacy of massage to reduce muscle stiffness. Results were similar across massage types, with studies finding no differences between the effects of conventional therapeutic massage compared with sports massage and positional release therapy in the erector spinae and upper trapezius muscles, respectively (Jelen et al., 2024, Bethers et al., 2021). Contradicting common assumptions, Inami et al. (2024) found significant negative correlations between % change in BB and MG stiffness and massage pressure measured by force plate sensors, indicating that lighter massages elicit greater reductions in muscle stiffness. Results were similar across massage durations, with positional release therapy comprising 3 x 90s treatments and conventional therapeutic massages lasting 5min and 30min all finding reductions in muscle stiffness to a similar magnitude. With the exception of one of these studies, changes in muscle stiffness persisted for no longer than 10min post-massage, highlighting the acute effects of massage on muscle stiffness. Bethers et al. (2021), however, found significant reductions in upper trapezius muscle stiffness following both positional release therapy and conventional therapeutic massage to be sustained significantly below baseline, but significantly above immediately post, at 48h post-treatment. These observations were made by examining specific trigger points in individual participants, which may explain the greater longevity of the effect of massage. This is supported by Olchowy

et al. (2020) who found a significant strong positive correlation between stiffness pre-massage and the difference between pre- and post-massage stiffness (r=0.79), indicating that massage has its greatest effects on areas/muscles of a higher baseline stiffness.

In conclusion, massage is an effective intervention for acutely reducing muscle stiffness, regardless of type or duration, which may explain the mechanism by which massage acts as a beneficial recovery strategy from EIMD. Future research should focus on investigating the use of massage in the post-exercise period to determine if it prevents or reduces the observed increases in muscle stiffness following EIMD (Hunter et al., 2006).

2.13.2. The effect of foam rolling on muscle stiffness

Foam rolling (FR) is a popular method of self-myofascial release used as both warm-up and recovery strategies. Pre-exercise FR has been shown to induce slight improvements in physical performance markers, whilst post-exercise FR exerts a small protective effect against reductions in performance, but similarly to massage, its biggest effects are in perceptions of muscle pain and recovery (Wiewelhove et al., 2019). Due to its mechanism of action, it was thought that FR may alleviate symptoms of muscle damage and reduce muscle stiffness in the same way as massage, whilst providing a cheaper and more convenient alternative. However, the literature has shown conflicting effects of FR on muscle stiffness both individually and following EIMD (Reiner et al., 2022, Reiner et al., 2021b, Mayer et al., 2020, Reiner et al., 2023, Vatovec et al., 2024, Macgregor et al., 2018). Reiner et al. (2022) found that 2min of vibration FR of the hamstrings led to immediate reductions in ST and SM shear modulus, but found no significant changes in BFlh shear modulus. Similarly, Reiner et al. (2021b) found that 3 x 1min bouts of either conventional or vibration FR of the quadriceps induced similar

reductions in RF, but not VM or VL shear modulus. Contrastingly, Reiner et al. (2023) found no effect of 2min ball FR on pectoralis major muscle shear modulus, and Mayer et al. (2020) found no effect of lateral thigh FR on VL or VI muscle stiffness at 0h, 30min, 6h, or 24h post-FR.

One study investigated the effects of FR alongside exercise (Vatovec et al., 2024). Participants completed a bilateral hamstring EIMD protocol, with 2min FR unilaterally pre-, 24h post- and 48h post-exercise, with the other leg completing passive rest and serving as the control. Shear modulus of the hamstring muscles was measured before and after the pre-exercise FR bout, 1h post exercise, then after the subsequent recovery FR or passive rest bouts. Conflicting the rest of the literature, Vatovec et al. (2024) found significant increases in BF and ST shear modulus immediately after the pre-exercise FR bout. Furthermore, the authors observed a significant increase in BF and ST shear modulus 1h post-exercise, which was sustained at 24h post-exercise (post-FR) in the ST, with no difference between limbs. These findings indicate that FR had no effect on muscle damage-induced increases in muscle stiffness in the hamstring muscles.

Future studies should be designed such that they include post-exercise FR to see if it prevents or attenuates the exercise-induced augmentation in muscle shear modulus. Further research is required to determine the effects of FR on muscle stiffness after exercise and establish optimal FR protocols for reducing muscle stiffness.

2.13.3. The effect of compression garments on muscle stiffness

Compression garments (CG) are worn both during and after exercise, with evidence suggesting they can enhance recovery of strength and endurance performance (Brown et al., 2017).

Although not much research has been conducted into the impact of CG on post-exercise muscle stiffness outcomes, there are two studies which have investigated their impact on MG stiffness having been worn both during and post-exercise (Kato et al., 2018, Heiss et al., 2018). In the study by Kato et al. (2018), participants conducted heel rise exercise with and without CG. MG stiffness increased significantly post-exercise under both conditions, but post-exercise stiffness was significantly lower with CG than without. In the study by Heiss et al. (2018), participants completed bilateral eccentric heel rise exercise without CG, and unilaterally wore CG for 60h post-exercise, with the other leg acting as the control. 60h post-exercise, CG prevented the muscle damage-induced changes in MG stiffness that were found in the control leg (Table 2.2). These results indicate a protective effect of CG on MG muscle stiffness augmentation when worn during or after exercise and indicate that this field of research warrants further study in other muscle groups to determine the mechanism by and extent to which CG enhances recovery from exercise.

2.13.4. The effect of cold water immersion on muscle stiffness

Cold water immersion (CWI) and cryotherapy are used as methods to enhance and accelerate muscle recovery after EIMD by reducing inflammation and oedema, however, little research has been conducted to investigate the effects they have on muscle mechanical properties (Moore et al., 2023). In the absence of exercise, Hüttel et al. (2020) found CWI to reduce VI muscle SWV, but Pinto et al. (2020) found no significant differences in muscle stiffness between control and CWI limbs after a plyometric exercise bout. Point et al. (2018) found that bouts of cryotherapy lead to an increase in muscle stiffness for up to 40min, in line with reductions in muscle temperature. These findings are logical, since material stiffness increases as its temperature decreases. Since other strategies appear to exert beneficial effects on recovery by

reducing muscle stiffness, further research is required to determine if cold exposure is beneficial for biomechanical recovery of muscle tissue and how this is linked to physiological and performance markers, and perceptions of recovery.

2.13.5. The effect of massage guns on muscle stiffness

Use of local high frequency percussive massage (i.e., massage guns) is becoming increasingly popular in recreational and elite sport to aid recovery and alleviate muscle soreness, showing improvements in some markers of recovery, including flexibility (Ferreira et al., 2023). Research into the effects of massage guns on muscle mechanical properties is scarce, with the current literature indicating no effect of their use on muscle stiffness (Liu et al., 2024, Pournot et al., 2016). Liu et al. (2024) utilised 5min of treatment to the MG, finding no change in muscle stiffness, but a significant increase in ankle dorsiflexion ROM. Interestingly, the authors did, however, report a significant decrease in MG deep fascia stiffness following the treatment, indicating that perhaps the improvements in ROM and beneficial effects of the intervention were driven by reductions in deep fascia stiffness as opposed to muscle stiffness (Liu et al., 2024). In the study by Pournot et al. (2016), participants completed bilateral biceps curls, then received a randomised unilateral 10min treatment with a massage gun, with the contralateral arm acting as the control. BB muscle stiffness was elevated above baseline 5min post-exercise and 5min post-recovery in both limbs, with no effect of treatment (Pournot et al., 2016). Another study investigated the effects of whole-body vibration on the muscle stiffness response to EIMD using a vibration plate (Jones et al., 2021). The EIMD bicep curl protocol significantly increased BB muscle stiffness in both the control and experimental groups, but after 1 week BB stiffness in the group who underwent whole body vibration recovered to baseline, whilst the control group had not (Jones et al., 2021).

Future research should focus on investigating the efficacy of local high frequency percussive massage and whole-body vibration techniques as strategies to reduce muscle and fascial tissue stiffness following EIMD, as well as investigate the relationship between deep fascia stiffness and ROM.

2.14. The influence of muscle stiffness on injury susceptibility

The mechanism by which muscle stiffness properties increase injury susceptibility is multifactorial, with contributions from altered material properties and susceptibility to strain, limb imbalances, and restricting ROM.

Material stiffness increases with stress and strain until failure, wherein the material ruptures; muscle tissue is not immune to this material principle (Figure 2.2) (Kelc et al., 2013). Having a higher level of passive stiffness means the tissue is at a closer starting point to its physiological maximum and requires a smaller increase in stress to cause injury. This explains findings in the literature which report a greater rate of injury occurrence among team sport athletes with greater passive muscle stiffness (Pickering Rodriguez et al., 2017, Watsford et al., 2010). These results were consistent between different methods of quantifying stiffness of the soleus, hamstrings and whole leg and indicate that greater levels of muscle stiffness increase the risk of injury. Further evidence in support of the influence of mechanical properties on injury exists in the post-injury period. Research utilising both the MyotonPRO and SWE to measure hamstring muscle and fascial tissue stiffness at sites of previous injury show that stiffness at these locations is elevated for years following injury (Kawai et al., 2021a, Kawai et al., 2021b, Yagiz et al., 2024).

This chronic elevation of stiffness may be a cause of re-injury, therefore interventions such as static stretching programmes which reduce passive stiffness may be beneficial to injured athletes even after the traditional recovery period and return to play (Ichihashi et al., 2016, Andrade et al., 2020, Umegaki et al., 2015, Akagi and Takahashi, 2014, Ikeda et al., 2021, Nakao et al., 2018). This is supported by Miyamoto et al. (2024) who found that BFlh muscle stiffness increases in sprinters after 2 weeks of training cessation, and hypothesised that this increase in muscle stiffness following time off contributes to the increased injury risk associated with return to training. This evidence of higher levels of muscle stiffness preceding injury followed by localised elevated stiffness following injury shows that the two are, indeed, inexplicably linked. Furthermore, both muscle stiffness and injury occurrence are known to increase with age and it is entirely possible that this increase in muscle stiffness and the subsequent altered tissue mechanical properties may partially explain the increased risk of injury as we get older (Watsford et al., 2010, Agyapong-Badu et al., 2016).

With regards to the acute moment of injury event, a framework produced by Kalkhoven et al. (2020) highlights that injury is usually caused by increases in strain, not stress. The authors also propose that muscle is more vulnerable to injury when it is under tension, actively contracting. This is because contracting makes the muscle stiffer and therefore more resistant to stress-induced strain. Rapid increases in muscle length in these circumstances are therefore more likely to cause muscle tears than when the muscle is in a relaxed state (Kalkhoven and Watsford, 2020). This model explains the high hamstring muscle injury rate during sharp deceleration from sprinting, since the hamstring is rapidly changing length under the high levels of tension involved in braking. Kalkhoven and Watsford (2020) propose that the mechanism by which

eccentric training (e.g., Nordic hamstring curls) is protective of injury is through increasing muscle fascicle length. This is because longer muscle fascicles have a greater ability to tolerate increases in absolute strain, as there are more sarcomeres in series across which the strain is distributed (Timmins et al., 2015).

Imbalances in muscle stiffness are another way in which tissue mechanical properties impact injury. One study linking higher levels of muscle stiffness with injury susceptibility found hamstring stiffness to be significantly higher in the non-injured limb than the injured limb (Watsford et al., 2010). This appears to contradict the hypothesis that stiffer muscle tissue is at greater risk of injury, however, it may be indicative of inferior strength or explosive power, particularly relevant in the hamstring muscles of the team sports players from this study, which play an important role in sprinting (Morin et al., 2015). This may imply muscular strength imbalances between the injured and non-injured limb prior to injury onset, which is a known risk factor for injury (Helme et al., 2021).

The third mechanism by which muscle stiffness influences injury risk is through its impact on ROM. Greater levels of muscle stiffness have been shown to impede hamstring ROM and reduce flexibility, which has long been considered a risk factor for muscular injury in team sports (Reiner et al., 2024a, Witvrouw et al., 2003). Elevated levels of muscle stiffness can therefore be attributed to increasing injury susceptibility through reducing ROM.

To summarise, muscle stiffness influences injury risk via three mechanisms: increased muscle stiffness leaves the tissue closer to its physiological maximum level of strain; imbalances in stiffness and their associations with strength; and reductions in ROM and flexibility. More

research is required, however, to more conclusively establish the mechanism by which elevated levels of muscle stiffness increase injury risk.

2.15. Potential mechanisms of muscle stiffness

At present, the aetiology of muscle stiffness is not fully understood. One way to try and understand the factors influencing muscle stiffness is through observations made in conditions which alter muscle stiffness, and those in which abnormal levels of stiffness are exhibited. These conditions include EIMD, muscle disorders, and injury, which all influence muscle architecture in different ways.

Looking through the lens of EIMD, it is evident that muscle damage causes an increase in muscle stiffness, but little is understood about the exact mechanism by which damage to muscle fibres induces structural changes that increase stiffness of the tissue. One mechanism, proposed by Lacourpaille and colleagues, hypothesises that changes in muscle stiffness post-exercise are mediated by disruptions in Ca^{2+} homeostasis (Lacourpaille et al., 2017b). The theory behind this being that perturbed Ca^{2+} homeostasis caused by myofibrillar disruptions because of damaging exercise increases stable actin-myosin cross-bridge formation and the binding of actin and titin (Whitehead et al., 2001, Herzog, 2014). This is supported by the observation of greater increases in shear modulus at longer muscle lengths, above the muscle's slack length, since elongated muscle fibres are known to have a greater sensitivity to Ca^{2+} (Lacourpaille et al., 2017b, Le Sant et al., 2015, Balnave and Allen, 1996). This is further supported since it is known that cross-bridge formation increases muscle stiffness, as humans are able to actively adjust muscle stiffness in anticipation of, or in response to, high levels of stress by muscle activation and the stretch reflex (Kalkhoven and Watsford, 2020). This hypothesis should be

tested more stringently using invasive methodology to examine the relationship between muscle fibre damage, via Z-band streaming from muscle biopsies, and sarcoplasmic Ca^{2+} concentration. This mechanism alters skeletal muscle architecture by increasing the formation of stable cross-bridges increasing muscle stiffness as is observed during isometric contractions, i.e., active, not passive, muscle stiffness.

Z-band streaming is the misalignment of Z-bands within sarcomeres and its quantification by electron microscopy is considered as the gold standard method of quantifying muscle damage. It may be the case that the accumulation of abnormally arranged Z-bands within a muscle, seen acutely in response to eccentric exercise, is the cause of augmented muscle stiffness. This is because uniform arrangement of Z-bands will allow adjacent sarcomeres within a myofibril to lengthen in a consistent manner, whereas misalignment of sarcomeres disrupts this consistency in myofibril lengthening. This would reduce the efficiency of muscle lengthening, increasing stiffness at any given length, and reducing range of motion. In this case, muscle architecture is altered by a misaligned organisation of Z-bands.

Considering the influence of muscle disorders on muscle stiffness allows us to learn from observations made in these conditions and apply the physiological and biomechanical underpinnings in disordered states to healthy populations. Immobilisation occurs in diseased states, such as in cerebral palsy, a condition in which patients suffer from muscle contractures caused by excessive muscle stiffness, as measured by SWE (Lee et al., 2016). Sarcomere length has been shown to decrease with immobilisation (Okita et al., 2004). This increases the total number of sarcomeres, since shorter sarcomeres are more densely packed into muscle fibres with a greater number of sarcomeres in series (de Souza Leite and Rassier, 2020). This alters

muscle architecture through increasing the quantity of sarcomeres and Z-bands within a muscle. One study on extracted rat soleus muscle used atomic force microscopy to measure Young's modulus and found the Z-disc to be the area of greatest stiffness within a sarcomere (Xiao et al., 2020, Ogneva et al., 2010). This is likely due to the densely packed α-actinin molecules which form a solid lattice-like structure to stabilise the contractile protein filaments, actin and myosin, allowing the Z-disc to anchor the sarcomere (Sjöblom et al., 2008). Z-bands are therefore likely to influence muscle stiffness by altering muscle architecture through either their organisation and alignment, or their total quantity within a muscle increasing net stiffness.

Further knowledge from diseased states can be gained from the role of the fascia and ECM in muscle mechanical properties. Smith and colleagues conducted a cross-sectional analysis of ECM components in children with cerebral palsy and typically developing healthy controls (Smith et al., 2021). Muscle bundle stiffness (kPa), measured from biopsy samples by loading fibres with strain, recording their stress response, and using this data to plot a relaxed stress versus sarcomere length curve, was ~50% higher in children with CP than in the healthy age-matched controls, aligning with earlier SWE results reported by Lee and colleagues (Smith et al., 2021, Lee et al., 2016). Accompanying the observed augmented stiffness in muscle tissue of CP patients are differences in ECM composition. Biopsy samples of CP patients comprised greater quantities of all types of collagen, more pyridinoline crosslinks in collagen, higher proteoglycan and uronic acid content, a greater number of fibroblasts, and a lower quantity of biglycan, which was a negative predictor of stiffness (Smith et al., 2021). These results display clear differences between the composition of muscular ECM in patients with cerebral palsy compared to healthy controls, implicating these differences with augmented stiffness. Correlational analysis, however, reported a weak relationship between collagen content and

muscle stiffness, highlighting the complex nature of physiological systems and emphasising the importance of further research into the underpinning mechanisms behind muscle stiffness. A likely explanation behind these findings is that the greater amount of collagen is accompanied by a greater quantity of cross-linking between collagen fibres (Smith et al., 2021). The deep fascia is arranged into two or three layers of parallel collagen bundles which glide over each other, with the help of the lubricating hyaluronic acid (Fede et al., 2021b). Collagen cross-linking between layers, therefore, reduces their ability to glide and increases rigidity of the fascial layers, increasing stiffness (Lubin and Zidi, 2024). This results in altered muscle architecture through modified quantities of various proteins within the ECM and collagen cross-linking in fibrous structures.

Evidence from injured tissue also points towards the fascia and increased collagen cross-linking. Kawai and colleagues reported elevated fascial tissue stiffness following hamstring injury which was sustained for years after the injury and subsequent recovery (Kawai et al., 2021a). It is feasible that muscle injury causes increases in collagen cross-linking at the site of the injury, as observed in scar tissue of the skin, which would lead to greater stiffness in that region and further implicates collagen cross-linking, even in the absence of injury, with elevations in muscle stiffness (Forrest and Jackson, 1971). More research needs to be conducted to uncover the role of the fascia in muscle stiffness using SWE which has been shown to reliably estimate fascia stiffness (Zhou et al., 2022b).

To conclude, differences in muscle stiffness are likely a result of a muscle's architectural properties, which can be studied through EIMD, muscle disorders, and injury. EIMD alters muscle architecture by increasing stable actin-myosin cross-bridge formation and Z-band

streaming causing misalignment and modified organisation of Z-bands. Muscle disorders also impact Z-band organisation, through reduction in sarcomere length and a resultant increase in the total quantity of Z-bands within a muscle. Moreover, both muscle disorders and injury result in altered structure of the fascia and ECM, with altered muscle architecture through increased collagen cross-linking the likely culprit for their influence on muscle stiffness (Figure 2.7).

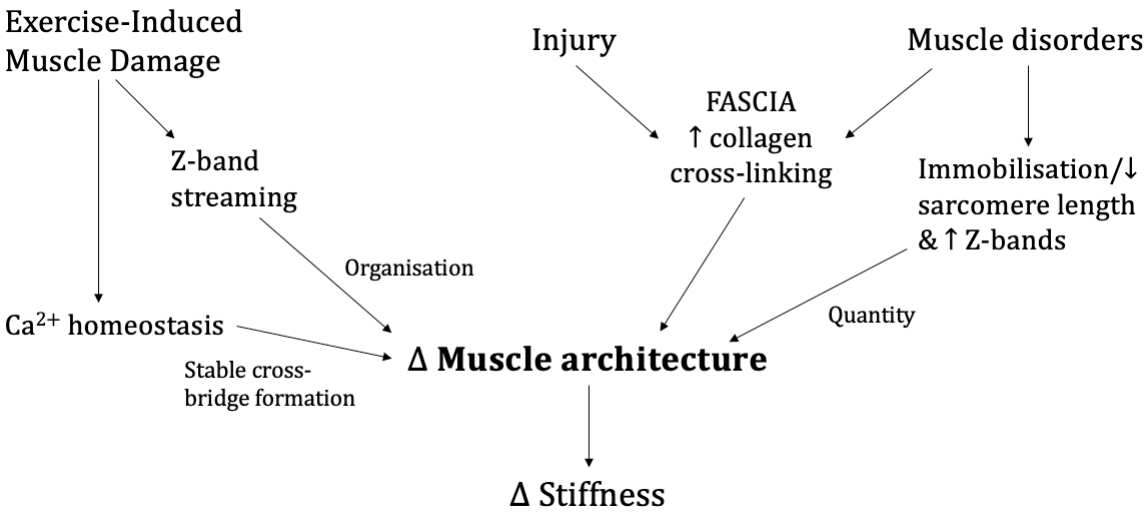

Figure 2.7. Mechanisms which may contribute to muscle stiffness.
EIMD, injury and muscle disorders are all known to change muscle stiffness and likely act through different mechanisms to do so, all of which alter the muscle architecture.

2.16. Limitations in the estimation of muscle stiffness

Despite building on the use of performance tests to estimate muscle stiffness, by allowing localised tissue-specific measurements, SWE and the MyotonPRO are not without their own limitations, with both techniques being operator-dependent and estimation of muscle stiffness being sensitive to changes in temperature.

2.16.1. Limitations of shear wave elastography for use in muscle tissue

Ultrasound-based techniques in general have their limitations. Image resolution can be limited by penetration depth of sound waves, and by issues such as shadowing and speckling caused by interference to the sound waves. Furthermore, ultrasound is an operator-dependant technique influenced by skill, experience, and technique (Wijntjes and van Alfen, 2021).

In specific relation to SWE, muscle is anisotropic, displaying heterogeneity both within and between musculoskeletal structures (Davis et al., 2019). As a result, there have been difficulties translating the success of its use in the homogenous liver and breast tissues to muscle tissue. Consequently, the commonly used shear modulus measurement may not be the best method of quantifying muscle stiffness and, instead, the initial SWV value may be more reflective of musculoskeletal mechanical properties.

Nevertheless, there are still many details which need refining when it comes to SWE measurement protocol and limitations which practitioners should be aware of when using the technology and evaluating the results. A very poignant example of this is the effect of compression of the muscle on SWV and shear modulus results. Compression of the muscle tissue by pressure from the ultrasound probe onto the skin causes huge increases in SWV and shear modulus values and introduces greater variance into the results (Vachutka et al., 2018). To avoid this, practitioners should be experienced in the use of ultrasound and ensure the visibility of a layer of coupling gel over the skin on the B-mode image (Wang et al., 2022). Ideally, an ultrasound probe holder should be used to prevent movement or compression of the probe and human error during measurements (Cipriano et al., 2022). Muscle contraction also affects muscle stiffness, if this is not monitored or controlled, estimation of stiffness by SWE

may be overestimated (Yoshitake et al., 2014). Furthermore, whilst it can be beneficial that SWE allows assessment of specific regions of interest, this does mean that estimations of a ROI are not representative of the whole muscle. Moreover, different angles of the ultrasound probe during shear wave elastogram acquisition have been shown to produce different SWV values, the probe plane should be at 0º, parallel to the muscle surface, and orientated along the direction of the muscle fibres (Wang et al., 2022).

SWE measurements are also affected by temperature, as it has been reported that higher muscle temperatures increase the speed of acoustic waves travelling through muscle tissue, but decreases reported active muscle stiffness (Bernabei et al., 2020, Marsh, 2016). This must, therefore, be considered when measurements are taken following exercise in higher ambient temperatures and compared to pre-exercise baselines where muscle temperature would have been substantially lower (Saltin et al., 1968). This is particularly relevant to professional football, where players often compete in unfamiliarly hot and humid ambient conditions, particularly in important competitions such as at the Qatar World Cup 2022 (Chodor et al., 2021).

SWE measurements can be affected by greater subcutaneous adipose tissue thickness and the subsequent increase in measurement depth (Cho et al., 2019, Wang et al., 2020). The layer of adipose tissue above the muscle can affect the propagation speed of the shear wave, making measurements less reliable (Cho et al., 2019). Furthermore, intramuscular adipose tissue content can influence SWV, with a study in the MG reporting that greater intramuscular adipose content is associated with lower stiffness measurements (Yoshiko et al., 2023). In addition, intramuscular fat infiltration impacts the measurement of muscle stiffness by increasing tissue inhomogeneity, with a greater amount of fat associated with lower shear modulus (Wang et al.,

2024). Appropriate mathematical correction methods should be developed to account for the impact of subcutaneous adipose tissue thickness and intramuscular fat infiltration on SWE measurements.

Another methodological consideration for practitioners involves the timing of SWE measurements for investigating the effect of an exercise bout on muscle stiffness. There is yet to be published a comprehensive study investigating the time-course response of muscle stiffness to different exercise modality and intensity stimuli. Many studies have reported acute effects of exercise on muscle stiffness lasting up to an hour post-exercise and recovering to baseline hereafter (Morales-Artacho et al., 2017, Ohya et al., 2017, Fouré et al., 2022, Heales et al., 2018, Lacourpaille et al., 2017b, Lacourpaille et al., 2014, Guilhem et al., 2016, Tsuchiya et al., 2019, Leung et al., 2017, Goreau et al., 2022, Kawama et al., 2022, Kato et al., 2018, Akagi et al., 2015, Pournot et al., 2016, Ustabaşıoğlu et al., 2022, Akagi et al., 2017, Siracusa et al., 2019, Chalchat et al., 2020). Multiple other studies, however, have reported more enduring changes in muscle stiffness, with differences from the pre-exercise baseline persisting longer than an hour and, in some cases, up to 7 days post-exercise (Andonian et al., 2016, Sadeghi et al., 2018, Jones et al., 2021, Voglar et al., 2022, Chalchat et al., 2022, Chalchat et al., 2023, Ema et al., 2021, Lall et al., 2022, Inami et al., 2022, Xu et al., 2019, Hotfiel et al., 2018, Heiss et al., 2018, Kisilewicz et al., 2020). The discrepancy between these findings is likely due to the heterogeneity in exercise interventions between studies. The length of time that muscle stiffness is altered after exercise cessation is likely related to the magnitude of the exercise stimulus and the amount of muscle damage and fatigue incurred by the bout, with more intense or longer exercise bouts inducing changes in muscle stiffness which are sustained for a longer period of time. For example, research by Andonian and colleagues found that immediate

reductions in quadriceps muscle stiffness following a 330km mountainous ultra-marathon were sustained at 48h post-race (Andonian et al., 2016). These extended alterations in stiffness may be due to the hugely damaging and fatiguing exercise stimulus, demonstrated by the significant increases in plasma creatine kinase, myoglobin, lactate, and C-reactive protein (Andonian et al., 2016). It is, therefore, best practice to take SWE measurements within an hour post-exercise to capture changes in muscle stiffness which may have occurred in response to exercise. Further research should be conducted to establish whether longer-lasting changes in muscle stiffness are related to volume and intensity of the exercise stimulus.

2.16.2. Limitations of the MyotonPRO for use in muscle tissue

While the MyotonPRO shows promise in quantifying muscle stiffness, like any measurement technique, it is not without limitations and further research is required to inform the details surrounding its use.

Similar to SWE, there are questions around the applicability of measuring muscle stiffness after exercise which is accompanied by changes in muscle temperature, as any changes may be temperature-induced, as opposed to exercise-induced (Bernabei et al., 2020, Marsh, 2016). To establish this, a study is required which records muscle stiffness using a variety of methods at different muscle temperatures, induced in the absence of exercise.

The MyotonPRO cannot measure the stiffness of muscle covered by >2cm of subcutaneous adipose tissue (Myoton®). This means that the results obtained are from very superficial areas of muscle. SWE, however, allows operators to measure stiffness within a specific ROI which can be superimposed anywhere over the B-mode ultrasound image, allowing depth resolution

and a level of specificity which is not possible with the MyotonPRO. This allows researchers to compare superficial and deep areas within a muscle, insight which the MyotonPRO is not able to provide (Murillo et al., 2019). Rather interestingly, this could prove useful in the rehabilitation setting, where the specific site of muscular injury can be identified using B-mode ultrasound, and its stiffness can be measured throughout the rehabilitation process to monitor recovery (Kawai et al., 2021a). Furthermore, results obtained from the MyotonPRO are affected by overlying fascial, adipose, and skin tissue, because the measurement result describes the property as a weighted average of all layers under the probe (Bravo-Sánchez et al., 2021). This also means that the results are not fully representative of deeper areas of muscle tissue.

In addition, measurements using the MyotonPRO are limited to isometric conditions, either relaxed at rest or during isometric contractions. SWE, however, is able to provide information on active muscle stiffness, during contraction or passive muscle movement, by providing real-time feedback on tissue stiffness.

As with SWE, there is a lack of standardisation of the measurement protocol, both in position of participants and the probe itself (Agyapong-Badu et al., 2016, Kalkhoven and Watsford, 2018, Kong et al., 2018). Moreover, there is less research using the MyotonPRO in an exercise setting, compared with SWE, so even less is known about the time-course of its sensitivity to exercise-induced changes in muscle stiffness (Janecki et al., 2011, Lall et al., 2022).

2.17. Conclusions and future directions

There appears to be an individualised optimal level of muscle stiffness at which MSK performance is enhanced, and injury risk is minimised. It is, therefore, important to further SWE research and develop standardised measuring protocols in order to implement monitoring of

muscle stiffness by SWE in athletes. Shear modulus of a ROI is different throughout a muscle, and this may have relevance in being a risk factor for injury in specific muscular regions and is likely a result of proximity to the muscle's attaching tendons, as well as localised stretching of a muscle as a consequence of joint angles. Further research is required to understand how these regional differences are influenced by movement, particularly in biarticular muscles which may be affected locally based on angles of two joints. More research is required to validate the MyotonPRO against SWE in a variety of muscles under a variety of conditions, as well as understand the dampening effect of non-target tissue on the measurement, to examine its effectiveness as a tool for quickly estimating muscle stiffness. In addition, SWE appears to be a useful tool in researching, diagnosing and monitoring a variety of MSK and motor-related diseases. If the MyotonPRO is proven to be a valid alternative or supplement to SWE in assessing muscle mechanical properties, it too could be a valuable tool in the clinical setting due to its portability and ease of use.

Whilst endurance exercise has been shown to decrease muscle stiffness and resistance exercise causes an augmentation of muscle stiffness, these changes in muscle stiffness are likely a consequence of the type of contractions performed, with eccentric contractions increasing stiffness, whilst the jury is still out on the effect that isometric contractions have on muscle stiffness. Further research is required to understand how training performed in applied sport settings, such as sprint training, impacts muscle stiffness. The relationship between muscle stiffness and muscle damage is, at present, poorly understood but the current body of evidence suggests that a relationship does exist. More research is required to better understand this relationship as it has implications in muscle injury, recovery, and athlete readiness to perform. Moreover, further research is required to understand how strategies aimed at improving

recovery from EIMD have an effect on reducing muscle stiffness. The time-course of changes in muscle stiffness following an exercise bout has not been comprehensively assessed, thus inferences have had to be made from different studies recording post-exercise measurements at different time-points. This makes drawing conclusions complex, due to inhomogeneity of study design and exercise protocol. However, it has revealed that the duration of altered muscle stiffness is dependent on the intensity and duration of the exercise bout. Furthermore, research is required to establish the underpinning physiological mechanisms behind muscle stiffness and its alteration following exercise, with likely contribution coming from the structural changes to the muscle architecture and ECM, as well as Ca^{2+} disturbances post-exercise.

2.18. Approach of the thesis

To fulfil its aim of investigating MSK tissue stiffness and its response to exercise, this thesis consists of three distinct phases of research (Figure 2.8). The objective of the methodological phase is to develop the methodological protocols used for the estimation of MSK tissue stiffness by assessing the validity of the MyotonPRO and the reliability of SWE in measuring different tissues under different conditions. The objective of the mechanistic phase is to investigate regional characteristics of biarticular MSK tissue to better understand its mechanical properties. The objective of the experimental phase is to explore the effect of real-world exercise interventions on MSK tissue stiffness and determine its relationship with flexibility.

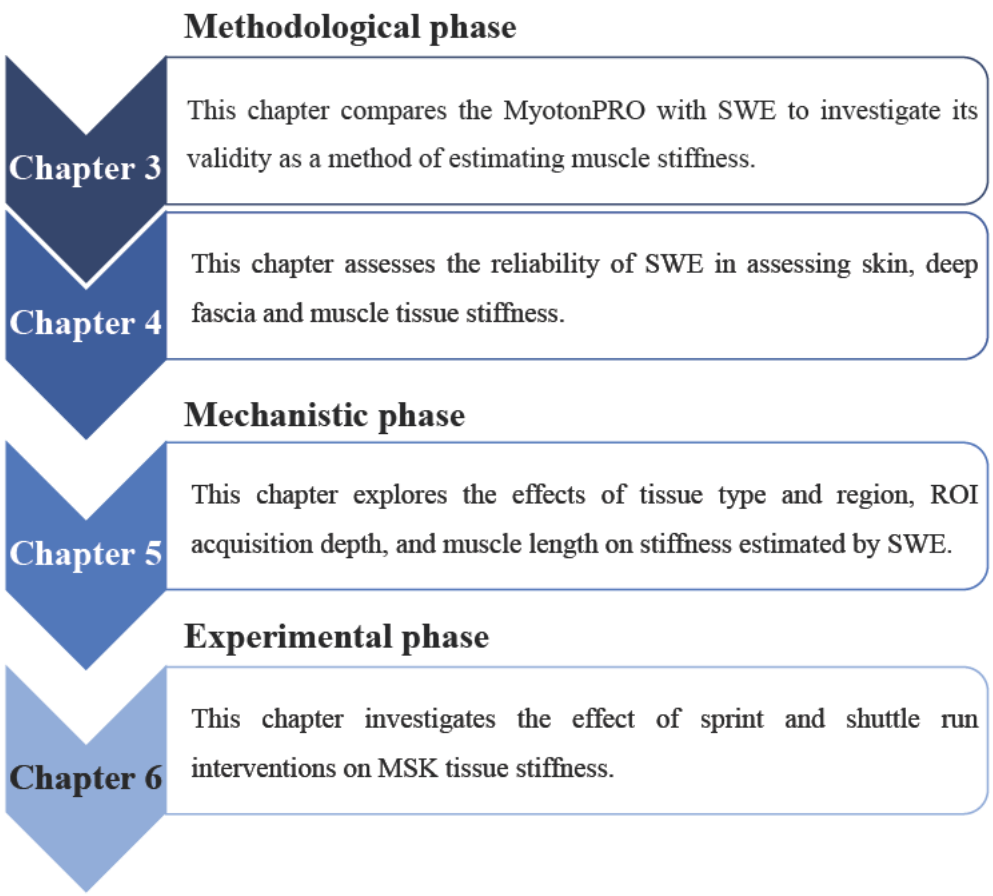

Figure 2.8. The systematic approach of the thesis.

2.19. Hypotheses

1. Estimation of muscle stiffness by the MyotonPRO will not exhibit strong positive correlations with measurements using SWE, but will have a better agreement at more superficial tissue.
2. SWE will be a repeatable method of estimating skin, fascia and muscle tissue in both superficial and deep regions.
3. Increasing muscle length will increase fascia and muscle stiffness estimated by SWE, preferentially increasing the stiffness of regions adjacent to the joint which moves to stretch it.

4. Both sprint and shuttle run interventions will acutely increase RF and BFlh muscle stiffness, with a cumulative effect of exercising on consecutive days.

CHAPTER 3

MyotonPRO is not Comparable to Shear Wave Elastography in the Measurement of Rectus Femoris Muscle Stiffness due to Interference of Subcutaneous Adipose Tissue

A version of this chapter is under review in the Scandinavian Journal of Medicine & Science in Sports

Rationale

As discussed in Section 2.4.5, the MyotonPRO is a relatively new tool for assessing the mechanical properties of biological soft tissue, which was developed to overcome the limitations of elastography-based techniques (MRE and SWE), most notably their inaccessibility. If the MyotonPRO is validated against existing techniques for estimating MSK stiffness, it could prove a useful tool in clinical and athletic contexts due to its ease of use, portability and low relative cost. This chapter aims to assess the comparability of the MyotonPRO to SWE in the estimation of muscle stiffness.

3.1. Abstract

Tissue biomechanical properties are important for optimising MSK performance and reducing injury risk. The MyotonPRO was developed as a simple, portable, non-invasive, and highly reliable method for the estimation of muscle stiffness. It has not, however, been comprehensively validated against SWE, a validated ultrasound-based method for the assessment of tissue stiffness. To assess this, 20 participants completed two visits in which RF stiffness was measured twice with the MyotonPRO device and SWE at three distinct muscle regions (proximal, medial, and distal), three muscle lengths (relaxed - REL, neutral - NEU, and passively stretched - PAST), and four depths (skin - SKIN, fascia - FAS, superficial muscle - SUP, and deep muscle - DEEP). Additionally, subcutaneous adipose tissue thickness (SAT) was recorded at each location under every condition using B-mode ultrasound imaging. When pooling the regions in REL, the MyotonPRO exhibited a weak significant negative correlation with SWV in DEEP ($r=-.27$, $P=.020$), but not at SKIN, FAS, or SUP. In NEU and PAST, stiffness estimated by the MyotonPRO had a moderate positive relationship with SWV of the SKIN ($r=.66$, $P<.001$; $r=.60$, $P<.001$), but weaker relationships with deeper tissue (FAS, $r=.26$,

P=.023; r=.20, P=.069; SUP, r=.24, P=.036; r=.26, P=.023; DEEP, r=.315, P=.008; r=.454, P<.001, respectively). Stiffness measured by the MyotonPRO exhibited significant moderate to strong negative correlations with SAT in every region under every condition, except for medially in REL. These results suggest that the two methods cannot be used interchangeably to estimate stiffness of the RF muscle.

3.2. Introduction

As discussed in Section 2.2.1, stiffness is a mechanical property of biological tissue quantified by the extent of tissue deformation (strain) in response to an applied force (stress) (Lieber and Binder-Markey, 2021). It is calculated as the slope of a material's stress/strain curve (Kelc et al., 2013). Changes in muscle stiffness can impact athletic performance, with higher levels of stiffness associated with improved performance in explosive actions such as sprinting and jumping, but also with a greater risk of injury (Kalkhoven and Watsford, 2018, Miyamoto et al., 2019, Pickering Rodriguez et al., 2017, Watsford et al., 2010). Measurement of muscle stiffness is also relevant in clinical settings, with augmented muscle stiffness aligning with muscle contractures and rigidity observed in patients with CP and PD (Brandenburg et al., 2016, Lee et al., 2016, Marusiak et al., 2010). Tensile testing is the gold standard for assessing material stiffness. In muscle, this method involves dissecting the muscle, clamping one tendon, applying increasing force to the other tendon, and measuring the resulting change in muscle length (Kodesho et al., 2021; Nakao et al., 2023). However, this approach has clear limitations when applied to athletic and clinical populations, necessitating the development and validation of non-invasive techniques for estimating tissue stiffness.

SWE is an ultrasound-based technique in which shear waves are induced in muscle with the speed of their propagation through the tissue tracked by the transducer. A faster SWV indicates greater tissue stiffness (Davis et al., 2019). This technique has already been implemented in homogenous, isotropic tissue in clinical settings, with the quantification of stiffness being utilised in the diagnosis of liver cirrhosis and identification of cancerous breast masses (Frulio and Trillaud, 2013, Cosgrove et al., 2012). SWE has been strongly correlated with tensile testing of muscle tissue in cadavers, validating its use in non-homogenous, anisotropic tissue (Kodesho et al., 2021b, Nakao et al., 2023). MSK SWE has been performed in clinical research settings, detecting muscular differences between children with CP and healthy age-matched controls (Brandenburg et al., 2016). It has also been applied in athletic settings, assessing changes in muscle stiffness following damaging exercise (Lacourpaille et al., 2017b, Guilhem et al., 2016). SWE is therefore considered a valid method for the non-invasive estimation of muscle stiffness, with applications in clinical and athletic research settings.

The MyotonPRO is a novel handheld device developed as a portable method for assessing muscle mechanical properties as a simpler alternative to SWE. This is because SWE is expensive, requires more extensive operator training, and is not easily portable. The MyotonPRO estimates dynamic stiffness based on tissue deformation and oscillation in response to application of a mechanical force at the skin (Feng et al., 2018). Myoton® define dynamic stiffness as the resistance of biological soft tissues to a force of deformation (Myoton®). Research has compared the MyotonPRO with SWE in muscles and gel phantom models, finding significant positive correlations between the two (Feng et al., 2018, Kurashina et al., 2023, Lee et al., 2021). This provides some evidence validating the MyotonPRO, indicating that it may be a viable alternative to SWE. Moreover, it may also have clinical

relevance, as research has shown an association with rigidity in patients with PD (Marusiak et al., 2010), and could be potentially useful in athletic settings as it has proven to be sensitive to the eccentric exercise-induced increase in muscle stiffness (Kawczyński et al., 2018).

The RF is the central superficial quadriceps muscle, crossing both the hip and knee joints. The biarticular anatomy of the RF means it contributes to both hip flexion and knee extension. This means it has roles in locomotion, with hip flexion in the toe off phase of gait, and is particularly important in actions requiring explosive knee extension, such as kicking a ball. Consequently, RF muscle injuries are highly prevalent in football and has been reported to have the highest injury occurrence of the quadriceps muscle group (Cross et al., 2004, Mendiguchia et al., 2013), accounting for 29% of injuries in a preseason period prior to the commencement of the English Premier League season (Woods et al., 2002). It is, therefore, important to understand the biomechanical properties of the RF in the context of movement, injury and disease. The literature shows SWE to be a reliable method of estimating RF muscle stiffness at a variety of muscle lengths manipulated by all combinations of lengthening and shortening of the muscle by hip and knee flexion and extension (Kodesho et al., 2021a, Lee et al., 2021, Coombes et al., 2018, Lacourpaille et al., 2012, Otsuka et al., 2019).

The RF is a muscle of high functional importance and, within the existing literature, its assessment via SWE has been validated against the gold standard tensile testing, accompanied by strong evidence of its reliability (Kodesho et al., 2021b). As a result, the subsequent chapters focus on the estimation of RF muscle tissue stiffness.

Despite some evidence supporting the validity of the MyotonPRO, there is a known dampening effect of the mechanical impulse caused by interference of non-target biological soft tissue.

Significant negative correlations have been found between dynamic stiffness of the RF and VL muscles, measured by the MyotonPRO, and subcutaneous adipose tissue thickness (SAT) measured by skinfold callipers and ultrasound, respectively (Mencel et al., 2021, Bravo-Sánchez et al., 2021). Consequently, further research is required to establish the efficacy of the MyotonPRO in estimating muscle stiffness. Therefore, the aim of this study was to examine the relationship between stiffness of the RF muscle, and its supra-adjacent tissue estimated by SWE and the MyotonPRO under different muscle lengths. We also attempted to assess the impact of SAT on dynamic stiffness values recorded by the MyotonPRO. Muscle length was manipulated to alter tissue stiffness and determine sensitivity of the MyotonPRO to these changes, which SWE is known to detect.

Based on previous research, we hypothesise there will be significant positive correlations between stiffness estimated by SWE and the MyotonPRO, but for this relationship to be impacted by SAT and, therefore negative relationships between SAT and stiffness estimated by the MyotonPRO (Bravo-Sánchez et al., 2021).

3.3. Materials and methods

3.3.1. Participants

A total of 20 healthy active participants (10 males and 10 females; age: 26.3 ± 4.0 years; height: 1.70 ± 0.10 m; weight: 67.7 ± 12.7 kg; BMI: 23.2 ± 2.8 kg/m^2) volunteered for this study. Participants had no history of musculoskeletal injury in the examined limb. Ethical approval for the study was approved by the Science, Technology, Engineering and Mathematics Committee at the University of Birmingham (ERN_0432-Apr2023) and all procedures

conformed to the Declaration of Helsinki. Participants provided written informed consent prior to measurements. Sample size was determined via a power analysis (G* Power software) based on previous research, with statistical power set at 0.80, an effect size of 0.50 and the alpha level set at 0.05 (Taş and Salkın, 2019).

3.3.2. Experimental design

Participants attended a familiarisation session and two experimental visits, conducted on separate days within a seven-day period. Both experimental visits were completed at the same time of day to avoid effects of routine and circadian rhythm on measurements. Participants were asked to refrain from lower body exercise, and consumption of alcohol and recreational drugs 24h prior to each experimental visit. Measurements were repeated twice per experimental session, with a 30min rest period between measurements, during which participants remained seated. This allowed the calculation of both intra- and inter-day repeatability of measurements.

3.3.3. Experimental procedures

Before starting the experiment, participant height (SECA, Hamburg Germany) and weight (Ohaus ChampII, New Jersey USA) were measured, following which, participants rested in the laboratory, set at a temperature of 21ºC, for 10min on the examination table to stabilise body conditions, as per previous research (Zhou et al., 2022a, Lall et al., 2022). Muscle stiffness was estimated at three sites along the RF muscle: proximal (PROX), medial (MED), and distal (DIST). These sites were 15%, 50% and 85% of muscle length, respectively. Muscle length was determined using B-mode ultrasound with 16-linear array probe (50mm, 4-15MHz) (LOGIQ S8 GE Healthcare, Milwaukee, USA) to identify the proximal and distal musculotendinous junctions of the RF, which were marked on the skin using a permanent

marker, and muscle length (cm) measured using a measuring tape. At these three sites, muscle stiffness was estimated in three different conditions to manipulate muscle length: relaxed, neutral, and passively stretched. In the relaxed (REL) condition, participants laid supine on an examination table (Figure 3.1A). In the neutral (NEU) condition, participants were seated in an IKD (Biodex System 3 Dynamometer, Biodex Medical Systems) with the hip joint fixed at 110º relative to full hip flexion and the knee joint fixed at 90º of flexion (Figure 3.1B). In the passively stretched (PAST) condition, participants were laid down in the IKD, with the hip joint fixed at 155º relative to full hip flexion and the knee joint fixed at 90º (Figure 3.1C). To ensure consistency of measurement location between measurements, the location of the ultrasound and MyotonPRO probes were marked on the skin in permanent marker and anatomical landmarks were identified using B-mode ultrasound. During measurements, participants were asked to remain still and relaxed, avoiding muscle contractions. The dominant leg, defined as the preferred leg used to kick a football, was selected for stiffness measurement. SAT was determined using the measuring tool on the ultrasound device, and was measured from the centre of the image, corresponding with the location at which MyotonPRO dynamic stiffness measurements were taken.

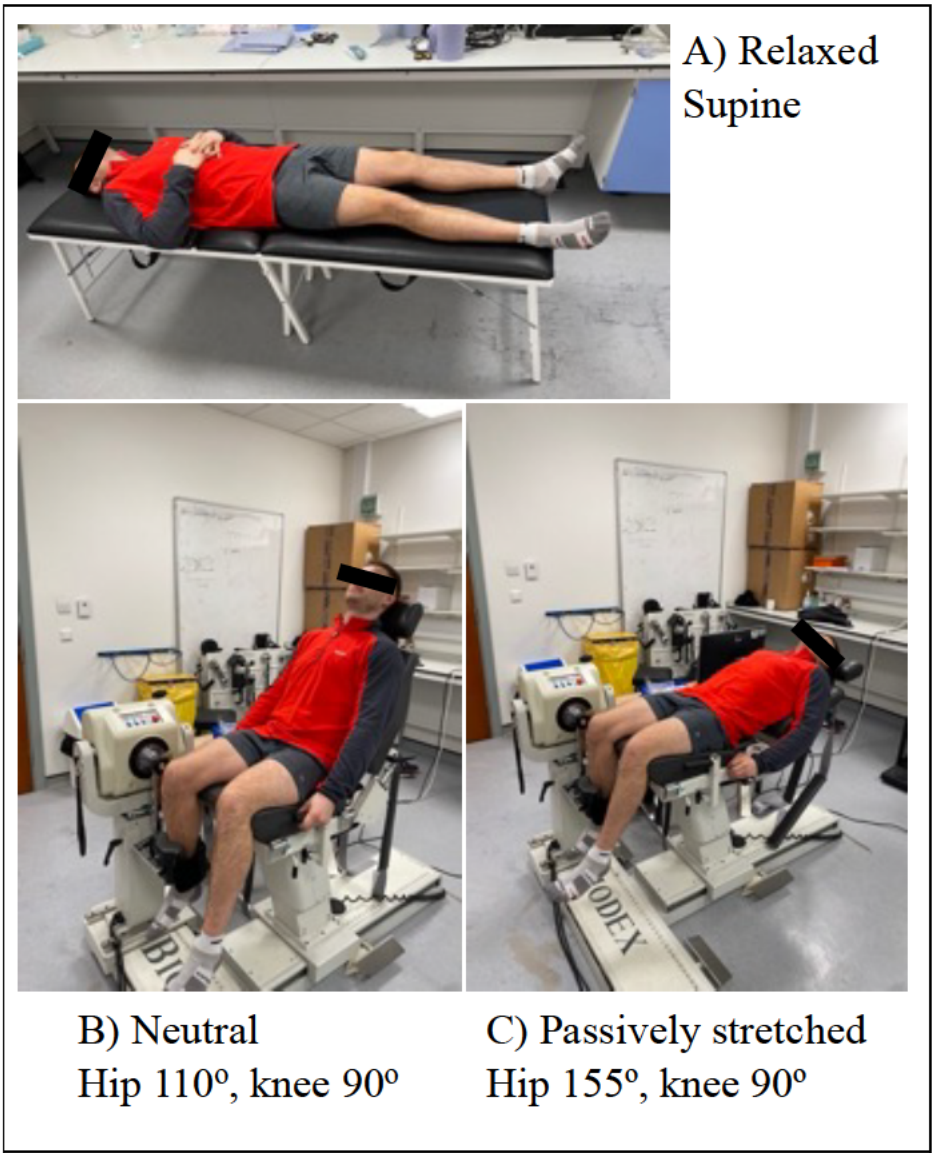

Figure 3.1. Participant positioning in the three different conditions to manipulate rectus femoris muscle length A) relaxed, B) neutral, and C) passively stretched.

3.3.4. Shear wave elastography

Stiffness was estimated via SWE using an ultrasound device equipped with SWE on a 9-linear array probe (44mm, 2-8MHz) (LOGIQ S8 GE Healthcare, Milwaukee, USA) which was positioned longitudinally to the muscle fascicles to ensure propagation of shear waves along the fascicles. All SWV measurements and selection and interpretation of elastograms were

performed by the same PhD student, with one year of MSK ultrasonography and SWE experience. At each measurement site (PROX, MED and DIST) SWE was measured at four different tissue sites: skin (SKIN), deep fascia (FAS), superficial muscle (SUP), and deep muscle (DEEP) (Figure 3.2). For measurements of SKIN, FAS, and distal SUP and DEEP, a 2.2cmx0.5cm region of interest (ROI) was selected. For proximal and medial SUP and DEEP, a 2.2cmx1.0cm ROI was selected to capture more of the muscle. To ensure consistency of repeated measures, anatomical landmarks were identified on the B-mode ultrasound image of the first measurement, ensuring they were in the same location in the frame during subsequent measurements. Depth of ROI positioning varied between participants based on the thickness of SAT, and other overlying fibrous structures. This ROI displayed a real-time shear wave elastogram colour map, which was recorded for 15-20s. The three most visually similar consecutive frames were used for image processing, wherein mean SWV (m/s) was determined. The mean values from these three consecutive images were used for further analysis. During measurements, a probe holder (Manfrotto, Italy) was used to ensure stability of the transducer during measurements and avoid compression of underlying tissue (Contreras-Hernandez et al., 2024). Furthermore, to avoid compression, a thick layer of acoustic coupling gel (>1mm) was used, ensuring it could be visualised on the B-mode image. If there were voids or saturation in the shear wave elastogram, the ROI and transducer positions were adjusted to optimise image quality and consistency. In instances where voids could not be avoided, frames with the least amount of saturation were selected for analysis. These SWE measurement protocols were conducted to align with best practice and are reported as per guidelines produced by Cipriano et al. (2022).

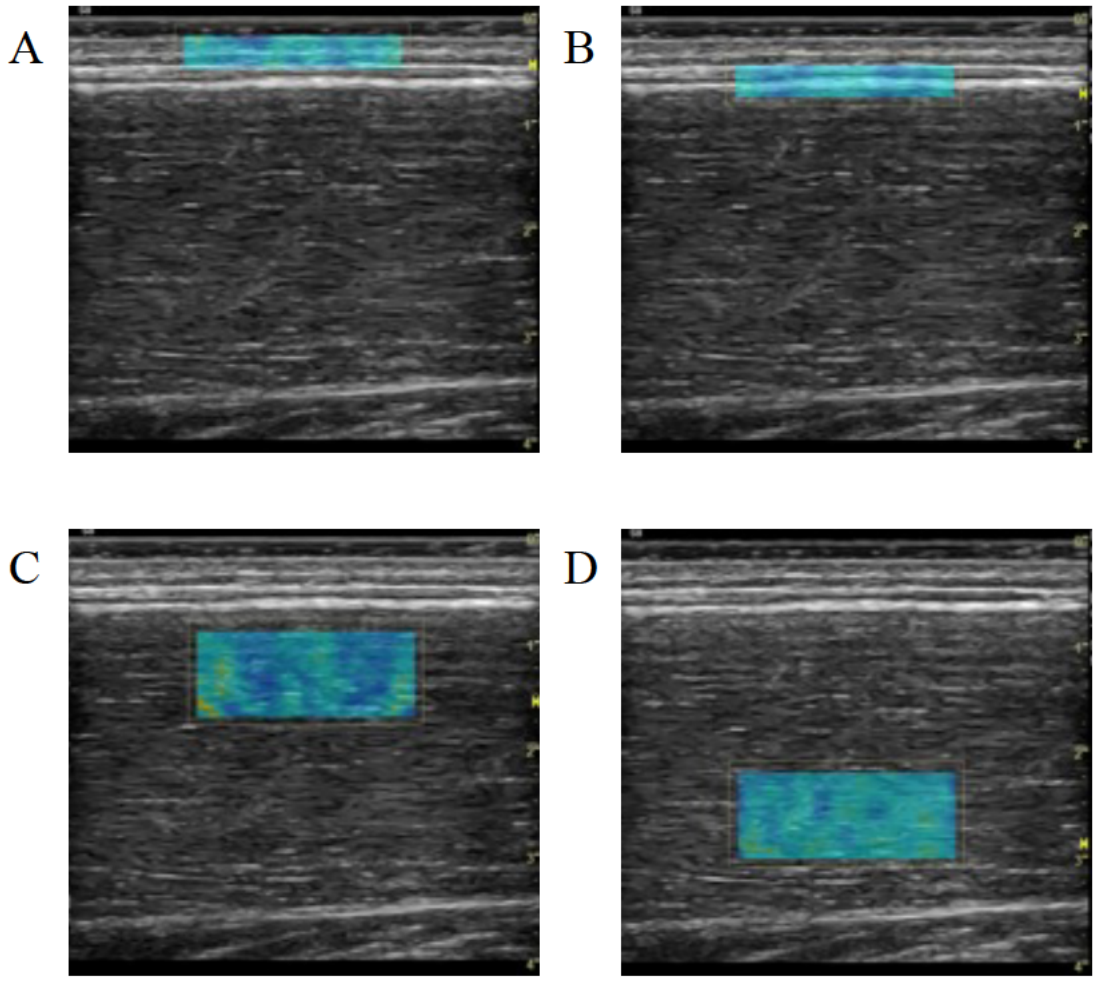

Figure 3.2. Example images of the regions of interest used to estimate A) skin, B) fascia, C) superficial muscle, and D) deep muscle stiffness using shear wave elastography

3.3.5. Myotonometry

A MyotonPRO (Myoton AS, Tallinn, Estonia) device was used to quantify muscle tissue dynamic stiffness. Measurements with the MyotonPRO were taken at the same sites as with SWE, from the centre of the box drawn around the ultrasound transducer. The device was held with two hands, with the arms resting on the examination table or IKD to ensure maximum stability and minimise movement of the device. The MyotonPRO recorded three measurements at each site, from which the mean was used for analysis. The MyotonPRO applies a precise

mechanical compression force of 0.6N for 15ms, inducing a decaying natural oscillation within the displaced muscle tissue which is measured by the device and used to calculate biomechanical properties of tissue.

The MyotonPRO calculates dynamic stiffness as per the following equation (Myoton®):

$$S = a_{max} \cdot m_{probe} / \Delta l$$

S, dynamic stiffness (N/m); a_{max}, maximal acceleration of the damped oscillation (mG); m_{probe}, mass of the measurement mechanism (kg); Δl, maximum displacement of tissue (m).

3.3.6. Statistical analysis

All statistical analysis was conducted using IBM SPSS software (version 29). Intra- and inter-day repeatability of SWE measurements were calculated for all three muscle regions (PROX, MED and DIST) at all four ROI depths (SKIN, FAS, SUP and DEEP) in all three conditions (REL, NEU and PAST), as well as for the MyotonPRO, in all three regions and conditions, using intraclass correlation coefficients (ICC). It is generally accepted that ICC values less than 0.50 indicate poor reliability, values between 0.50 and 0.75 are indicative of moderate reliability, values between 0.75 and 0.90 indicate good reliability and values above 0.90 represent excellent reliability (Koo and Li, 2016). Simple linear regressions with Pearson's correlations were performed to assess the level of agreement between stiffness measured by the MyotonPRO and at the four ROI depths with SWE, repeated for each condition. These analyses were conducted at each region separately, as well as with the regions pooled under each condition. Simple linear regressions with Pearson's correlations were also performed to assess the relationship between SAT and stiffness measured by the MyotonPRO, individually at each region under each condition. For these measurements, data was excluded where SAT exceeded 2cm because Myoton® state in the user manual that the device is unable to measure tissue

covered by >2cm adipose tissue (Myoton®). This involved exclusion of measurements from PROX in only 1 participant. Strength of Pearson's correlations are reported as negligible (0.00-0.09), weak (0.10-0.39), moderate (0.40-0.69), strong (0.70-0.89), and very strong (0.90-1.00) (Schober et al., 2018). To determine regional differences in SAT, a 3 condition (REL, NEU, PAST) by 3 location (PROX, MED, DIST) two-way repeated measures analysis of variance (ANOVA) was performed. Post hoc pairwise comparisons were conducted using the Bonferroni correction to investigate significant differences in detail. All values reported in text and graphs are mean ± standard deviation (SD) unless otherwise specified. For all analyses, statistical significance was set at $\alpha < 0.05$.

3.4. Results

3.4.1. Repeatability of shear wave elastography and the MyotonPRO

SWV exhibited moderate intra-session repeatability (ICC 0.69), and good inter-day repeatability (ICC 0.80). The MyotonPRO exhibited excellent intra-session repeatability (ICC 0.97) and inter-day repeatability (ICC 0.97).

3.4.2. Relationship between shear wave elastography and the MyotonPRO

With regards to the pooled data, in REL, Pearson's correlation and simple linear regressions revealed a weak significant negative correlation between stiffness estimated by the MyotonPRO and SWV at DEEP (r=-.27, P=.020, R^2=.07), but no significant correlations were observed at SKIN (r=-.23, P=.430, R^2=.00), FAS (r=-.21, P=.052, R^2=.05) or SUP (r=-.18, P=.084, R^2=.03) (Figure 3.3).

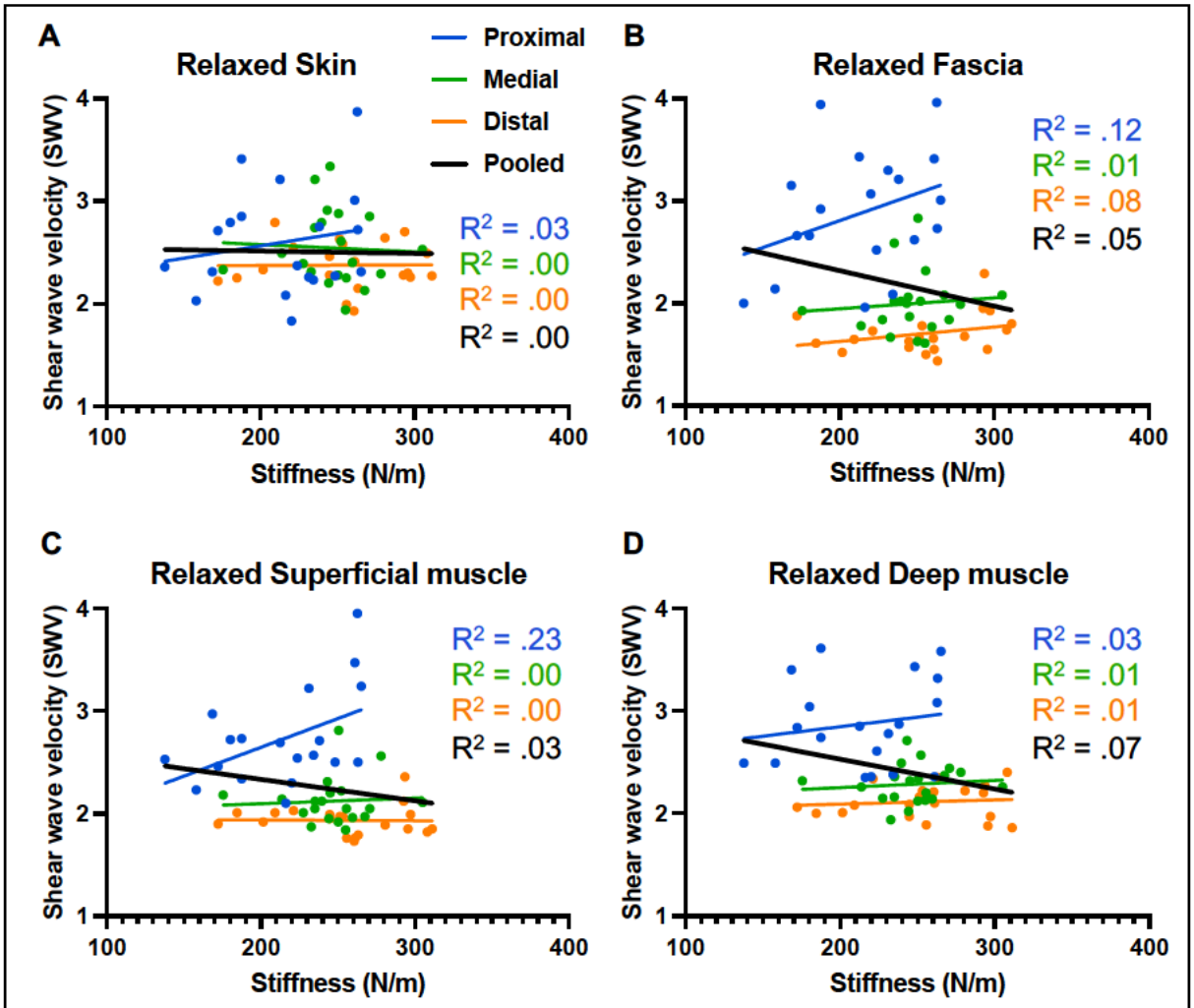

Figure 3.3. The relationship between shear wave velocity, measured by shear wave elastography at the (A) skin, (B) fascia, (C) superficial muscle, and (D) deep muscle and dynamic stiffness, measured the MyotonPRO, including the simple linear regression R^2 values for each region separately and pooled, in the relaxed condition.

Pearson's correlation and simple linear regressions of pooled data in NEU, however, reveal significant positive correlations between stiffness estimated by the MyotonPRO and SWV at all measurement depths (Figure 3.4). A moderate correlation was found at the SKIN ($r=.66$, $P<.001$, $R^2=.44$), but weak correlations observed at FAS ($r=.26$, $P=.023$, $R^2=.07$), SUP ($r=.24$, $P=.036$, $R^2=.06$), and DEEP ($r=.32$, $P=.008$, $R^2=.10$).

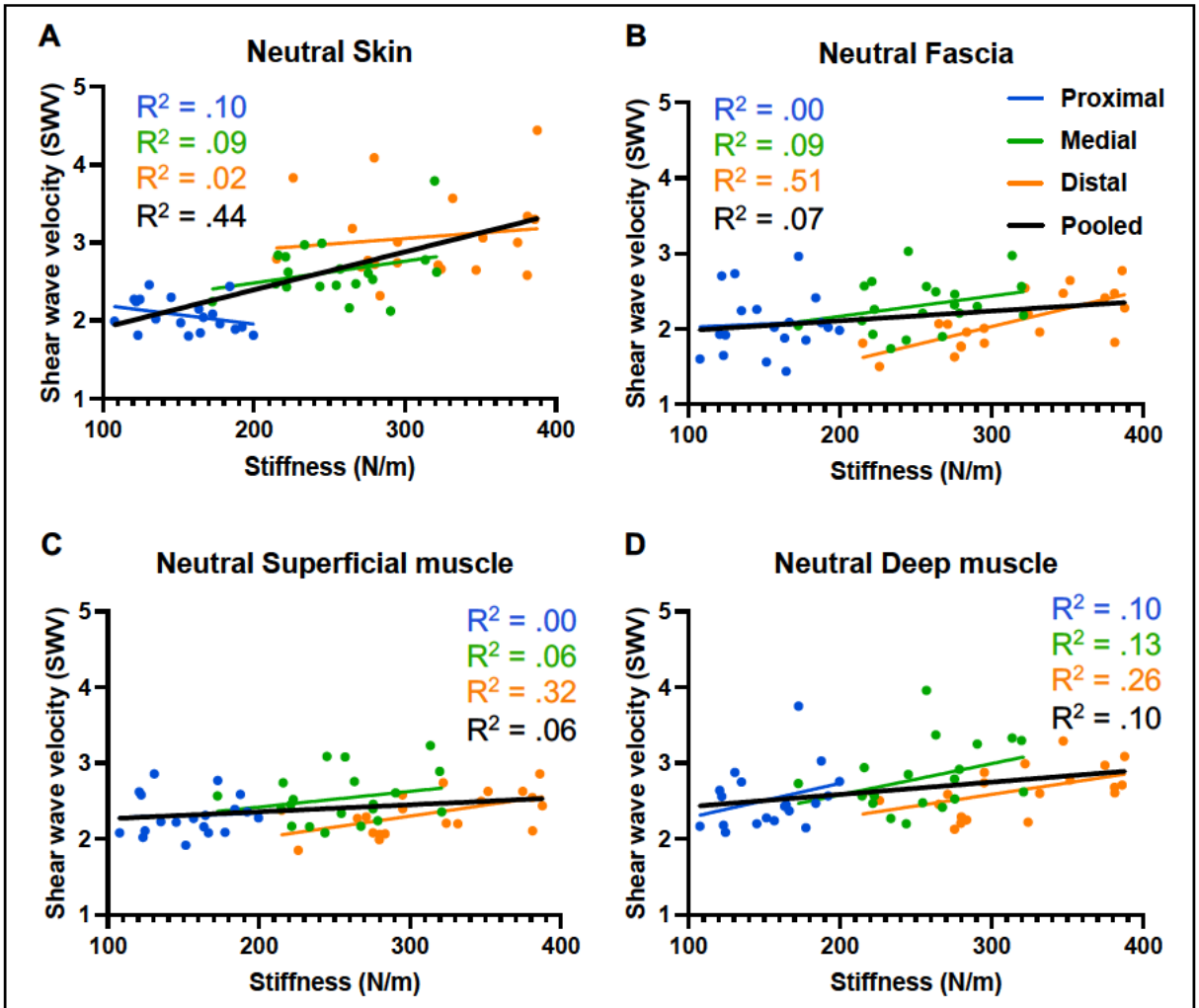

Figure 3.4. The relationship between shear wave velocity, measured by shear wave elastography at the (A) skin, (B) fascia, (C) superficial muscle, and (D) deep muscle and dynamic stiffness, measured the MyotonPRO, including the simple linear regression R^2 values for each region separately and pooled, in the neutral condition.

For pooled data in PAST, significant moderate positive correlations between stiffness estimated by the MyotonPRO and SWV were observed at SKIN (r=.60, P<.001, R^2=.36) and DEEP (r=.45, P<.001, R^2=.21), a significant weak correlation at SUP (r=.26, P=.023, R^2=.07), but no significant relationship at FAS (r=.20, P=.069, R^2=.04) (Figure 3.5). Full data with Pearson's correlations for each region is provided in Appendix 1.

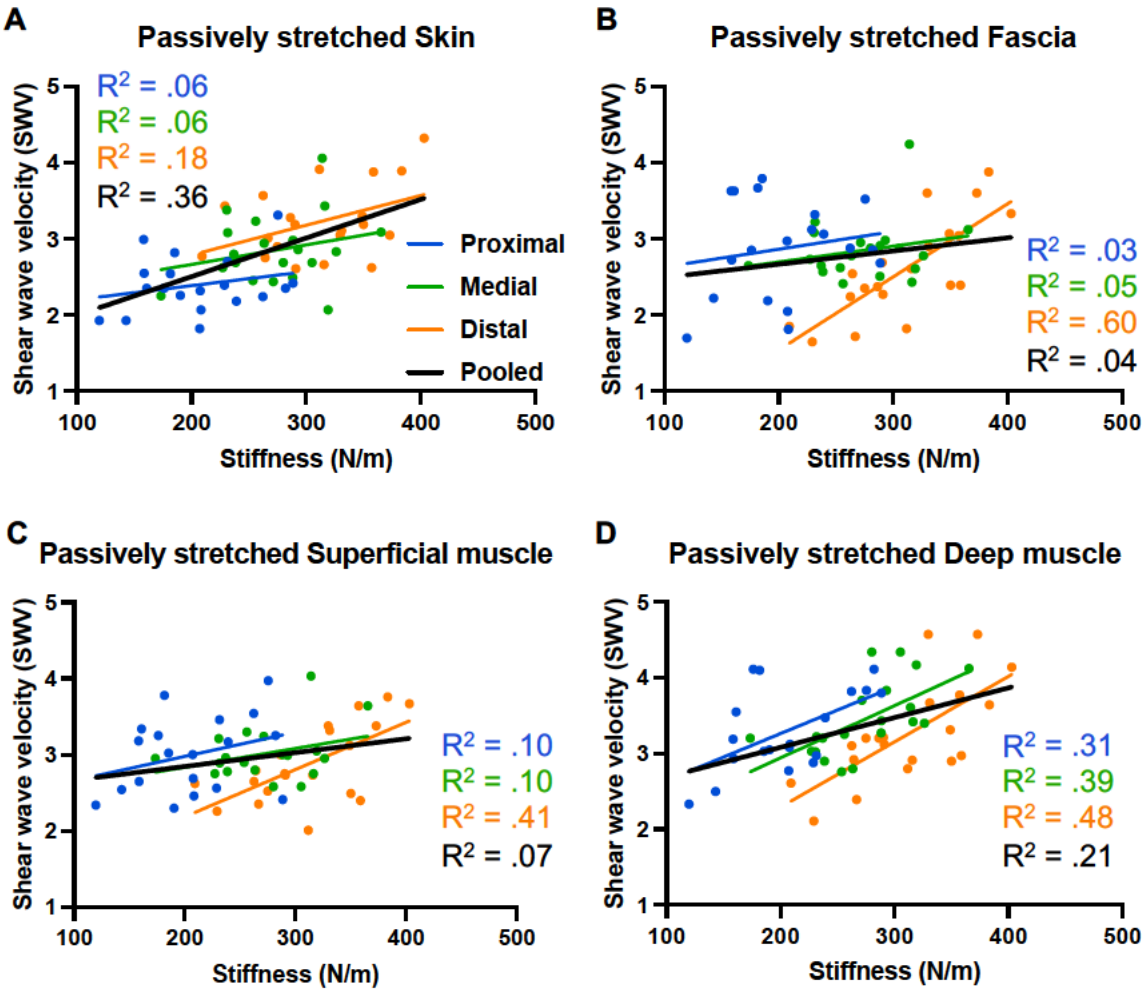

Figure 3.5. The relationship between shear wave velocity, measured by shear wave elastography at the (A) skin, (B) fascia, (C) superficial muscle, and (D) deep muscle and dynamic stiffness, measured the MyotonPRO, including the simple linear regression R^2 values for each region separately and pooled, in the passively stretched condition.

3.4.4. Relationship between the MyotonPRO and subcutaneous adipose tissue thickness

Figure 3.6 plots dynamic stiffness against SAT under all three conditions (REL, NEU, and PAST), and at all three regions (PROX, MED, and DIST). The data shows consistent moderate-strong negative correlations between dynamic stiffness and SAT which, with the exception of the weak relationship at REL MED, are statistically significant in all conditions and locations.

This implies an interference effect of the less stiff SAT on the ability of the MyotonPRO to accurately quantify mechanical properties of underlying muscle.

In REL, Pearson's correlations and simple linear regressions revealed a significant moderate negative correlation between dynamic stiffness and SAT at PROX (r=-.57, P=.006), with 32.34% of variance in stiffness explained by SAT, and a strong correlation at DIST (r=-.82, P<.001), with 66.46% of variance in stiffness explained by SAT, but a weak, non-significant correlation in MED (r=-.38, P=.050), where 14.30% of variance in stiffness is explained by SAT (Figure 3.6A-C).

In NEU, Pearson's correlations and simple linear regressions revealed a strong significant negative correlation between dynamic stiffness and SAT at PROX (r=-.73, P<.001), with 53.51% of variance in stiffness explained by SAT, and moderate correlations at MED (r=-.56, P=.005), with 31.03% of variance in stiffness explained by SAT, and DIST (r=-.69, P<.001), with 47.29% of variance in stiffness explained by SAT (Figure 3.6D-F).

Similarly, in PAST, Pearson's correlations and simple linear regressions revealed strong significant negative correlations between dynamic stiffness and SAT at PROX (r=-.73, P<.001), with 53.01% of variance in stiffness explained by SAT, and DIST (r=-.78, P<.001), with 60.75% of variance in stiffness explained by SAT, and a moderate correlation at MED (r=-.59, P=.003), with 35.00% of variance in stiffness explained by SAT (Figure 3.6G-I).

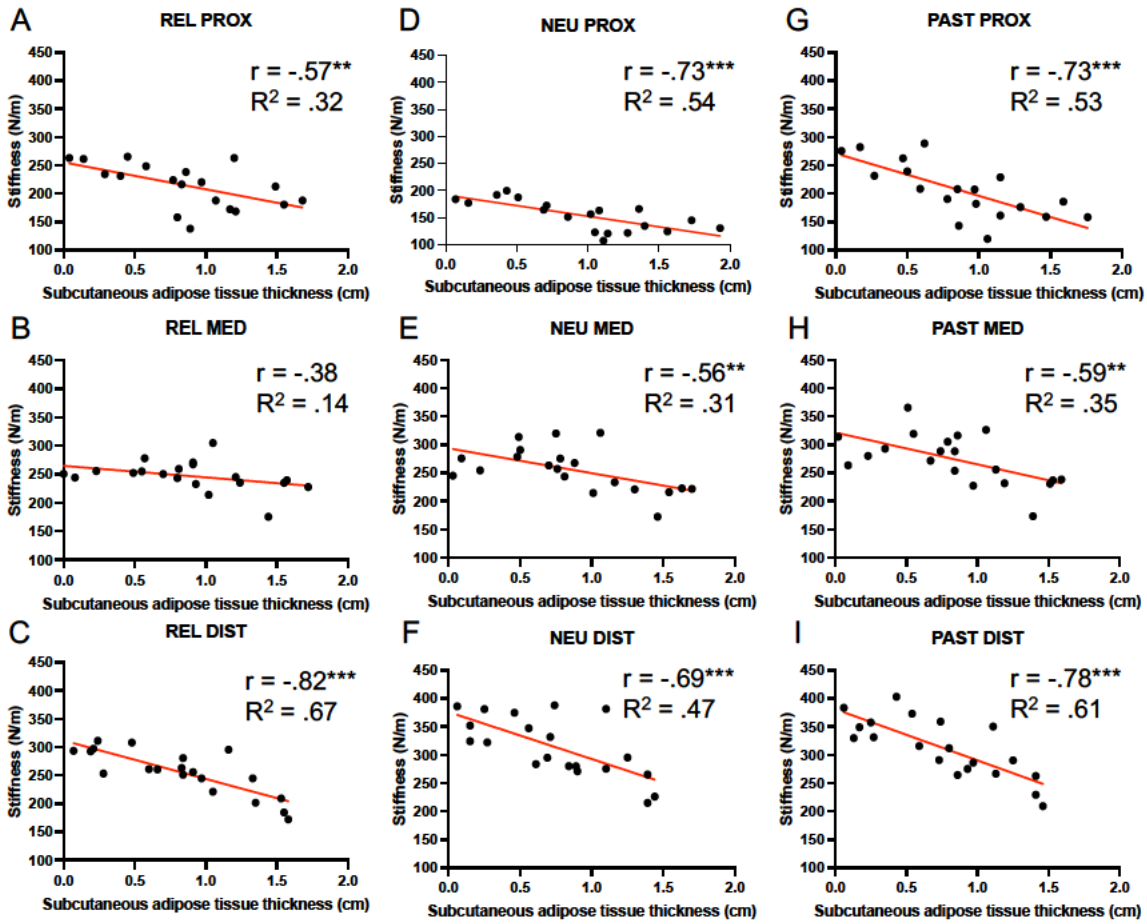

Figure 3.6. The relationship between dynamic stiffness, measured by the MyotonPRO, and subcutaneous adipose tissue thickness, measured using B-mode ultrasound, including Pearson's r value and the simple linear regression R^2 value.

A-C relaxed condition, in order of proximal, medial and distal regions. D-F neutral condition, in order of proximal, medial and distal regions. G-I passively stretched condition, in order of proximal, medial and distal regions. **P<.010, ***P<.001.

3.4.5. Regional differences in subcutaneous adipose tissue thickness

In both REL and PAST, there were no significant differences in SAT between regions (Figure 3.7). In NEU, however, SAT was lower at DIST than PROX (P=.003) and MED (P=.017). When comparing regions across conditions, there are further differences. At PROX, SAT is greater in NEU than in REL or PAST (both P<.001). At MED, SAT is higher in REL than in

PAST (P<.001). At DIST, SAT is larger in REL than in NEU and PAST (both P<.001). These results show that localised flexion causes compression which increases SAT.

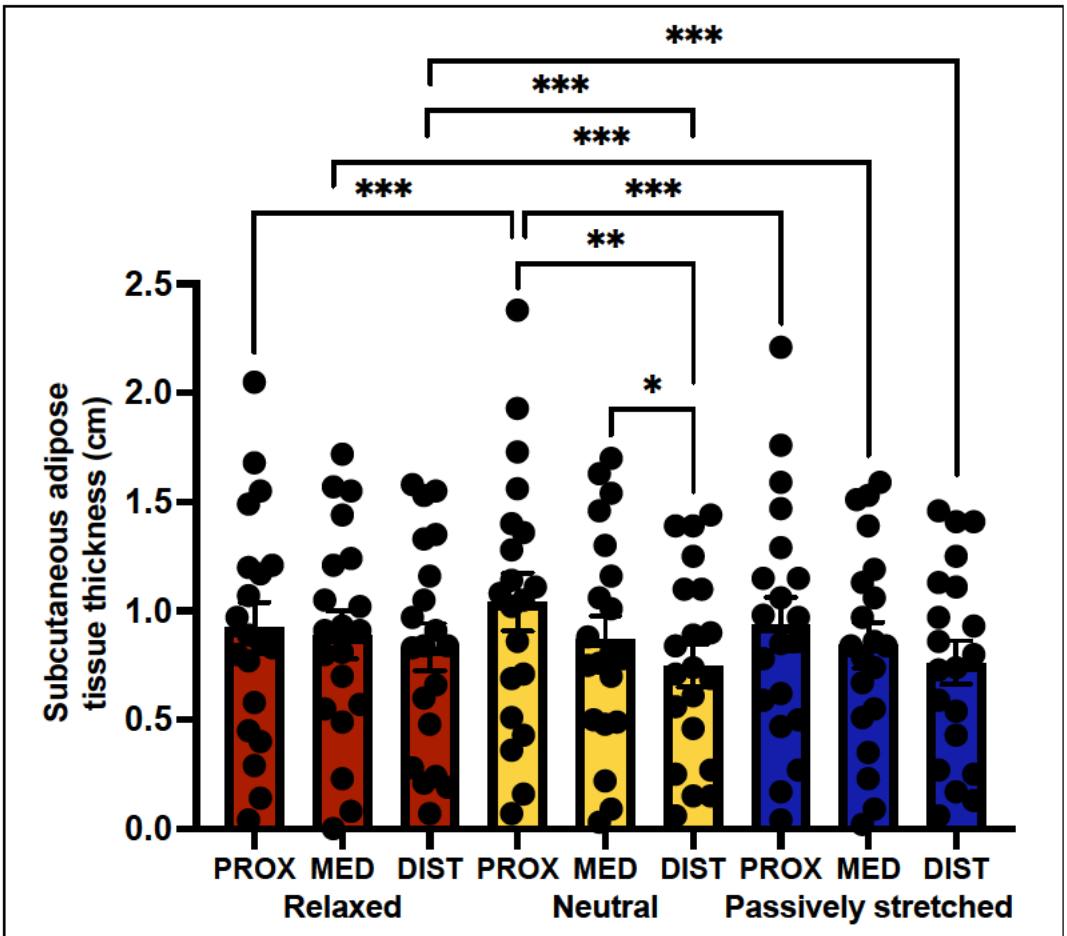

Figure 3.7. The differences between subcutaneous adipose tissue thickness measured at proximal, medial and distal locations in the relaxed, neutral and passively stretched condition by B-mode ultrasound. *P<.050, **P<.010, ***P<.001.

Full mean dynamic stiffness values (in the three locations under each condition) estimated by the MyotonPRO are provided in Appendix 2.

3.5. Discussion

The study aims were to examine the relationship between stiffness of the RF muscle and its supra-adjacent tissue estimated by SWE and the MyotonPRO under different muscle lengths and to assess the impact of SAT on dynamic stiffness values recorded by the MyotonPRO. The present study has three main findings: (1) RF stiffness reported by the MyotonPRO has no agreement with SWV in a supine position, (2) stiffness reported by the MyotonPRO has stronger correlations with SWV of superficial tissue than of deeper tissue, and (3) stiffness reported by the MyotonPRO negatively correlates with SAT regardless of location or condition. Overall, our results show that the ability of the MyotonPRO to estimate RF muscle stiffness is limited by the interference of overlying non-contractile tissue and is not an effective method of assessing mechanical properties in deep structures. Combined, these findings indicate that the MyotonPRO is not an effective alternative method to SWE of assessing RF muscle stiffness. This may be a function of the devices measuring different tissue properties. Since there is a stronger agreement between the two devices at longer muscle lengths, and stiffness reported by the MyotonPRO is negatively related to SAT, use of the MyotonPRO is recommended with the muscle in a stretched state, and in muscles covered by minimal overlying non-contractile tissue if the target is to optimise agreement with SWE.

In REL, there existed no positive correlation between the MyotonPRO and SWV at any location. This is particularly relevant, since measurements of the quadriceps muscles using the MyotonPRO are recommended to be taken with participants in a supine position. These findings support results observed at the VL muscle in a supine condition, where there was no relationship between stiffness assessed by the MyotonPRO and shear modulus estimated by the same model of ultrasound device as in the present study (Bravo-Sánchez et al., 2021). These results would

seem to indicate that the guidance for participant positioning for estimating quadriceps muscle stiffness with the MyotonPRO should not be supine. Alternatively, since there is no agreement between SWE and the MyotonPRO in REL, our results may imply that the devices are measuring two distinct mechanical properties. These two hypotheses based on our results will be discussed below.

We investigated the effect of altering participant positioning (to increase muscle length) on the agreement between SWE and the MyotonPRO. Under these conditions of knee flexion in NEU and PAST, we observed significant weak-moderate positive correlations in the estimation of tissue stiffness between SWE and the MyotonPRO. However, our results reveal that these correlations are stronger at the SKIN than at deeper tissues. This would support a known limitation of the MyotonPRO which is its limited depth of measurement. This may be a result of its mechanism of assessment in which a mechanical compression force is applied by a probe at the skin surface and the measurement of the oscillation of the displaced tissue below. If this mechanism is accurate, it would suggest that a greater preload compression force may enable more effective assessment of deeper structures, including muscle tissue. This is supported by findings presented in Figure 3.6, that shows negative relationships between stiffness measured by the MyotonPRO and SAT regardless of measurement location or position. Furthermore, Figure 3.7 highlights that there is greater SAT in proximal regions and in REL and NEU than in PAST. These findings support the hypothesis that SAT is a limiting factor on estimation of muscle stiffness using the MyotonPRO, since better relationships are observed between the two devices in DIST and PAST, where SAT is lower. This is likely a consequence of the dampening effect on mechanical wave transmission caused by interference of the more compliant adipose tissue overlying the muscle. This is a known limitation of the device, with the manufacturers

advising that the MyotonPRO cannot assess the mechanical properties of tissue covered by >2cm adipose tissue (Myoton®). Our results, however, indicate that this does not seem to be a specific cut-off point whereafter stiffness cannot be assessed but would rather indicate that any amount of adipose tissue can influence the measurement. This relationship may well be linear in nature as the data would suggest that the influence increases linearly with SAT.

These results are similar to those found by Mencel et al. (2021), who observed a significant negative correlation (r = -0.460) between stiffness estimated by the MyotonPRO and skinfold thickness of the RF with participants supine. Our results are further supported by similar findings in the VL muscle, wherein a significant negative correlation (r = -0.459, P = 0.001) was found between MyotonPRO dynamic stiffness and SAT (Bravo-Sánchez et al., 2021). Two studies have found significant positive relationships between muscle stiffness assessed by SWE and the MyotonPRO in the lower leg muscles (medial and lateral gastrocnemius and tibialis anterior) (Feng et al., 2018, Do et al., 2021). Despite these studies not reporting SAT, it is highly likely that SAT overlying the lower leg muscles in these studies was lower than that overlying the RF and VL of the participants in the present study and the research of Mencel et al. and Bravo-Sanchez and colleagues. This assumption is based upon previous extensive research into SAT assessment using ultrasound and skinfold measurements (Müller et al., 2020, Mencel et al., 2021). A stronger correlation between the two outcomes would, therefore, be expected as there is a smaller dampening effect on the MyotonPRO's mechanical impulse caused by SAT. These results therefore suggest that, although increasing muscle length improves the agreement between SWE and the MyotonPRO, it is still greatly limited by the disruption of the measurement caused by non-contractile tissue overlying the targeted muscle. Future research should focus on examining the relationship between SWE, the MyotonPRO and SAT in other

muscles, to examine whether there is a greater agreement between SWE and the MyotonPRO in muscles with less overlying non-contractile tissue. Moreover, mechanistic studies utilising gel phantoms of known stiffness should be used to replicate physiological conditions, similar to those used by Bartsch et al. (2023). These models are made such that less stiff phantoms (representing SAT) of different thicknesses are placed on top of a stiffer phantom (representing muscle) to examine how this affects dynamic stiffness recordings (Kurashina et al., 2023). This may also allow for the calculation of mathematical corrections to account for participant adiposity which would enable more accurate utilisation of the MyotonPRO.

Since our results in REL imply that RF muscle stiffness measurements using the MyotonPRO do not agree with SWE, it should be considered that they may be measuring two distinct mechanical properties. This is likely due to their individual mechanisms of measurement. Due to the measurement approach of the MyotonPRO, it is conceivable that it is providing an estimation of tissue hardness or compressibility as opposed to stiffness. Hardness can be defined as the resistance of a material to localised plastic deformation, and compressibility can be defined as the change in muscle thickness in response to an external compressive force (Parker and Hashmi, 2021). Indeed, the MyotonPRO measurement resembles that of a hardness measurement using a durometer with the difference between the two being the dynamic nature of probe compression by the MyotonPRO (Helili et al., 2021). Both, however, measure tissue displacement parallel to the external compression force. This is different to SWE which induces shear waves perpendicular to the ultrasound transducer orientation, travelling longitudinally along the muscle fascicles. The agreement between SWE and the MyotonPRO in NEU and PAST therefore makes sense, as the two devices are measuring different, but related, mechanical properties which change in the same way as muscle length is increased by knee

flexion. This aligns with findings by Kelly and colleagues of agreement between the MyotonPRO and SWE in the infraspinatus, erector spinae, and MG muscles at rest, 40% and 80% of MVIC (Kelly et al., 2018). These correlations were found when combining data from rest and the two MVIC intensities, wherein both methods reported greater stiffness during contraction and at the higher MVIC. This does not, however, mean that both methods are accurately estimating muscle stiffness. Both muscle stiffness and hardness are known to increase during contraction and with contraction intensity (Yoshitake et al., 2014, Inami et al., 2017). It is therefore possible that, in this study, SWE was estimating the increases in muscle stiffness in response to contraction, and the MyotonPRO was estimating the increases in muscle hardness in response to contraction, resulting in a positive correlation between the two methods, despite measuring two distinct mechanical properties (Kelly et al., 2018). This suggests that in certain conditions SWE and the MyotonPRO may provide similar or different data. This is because they are estimating distinct, but related, mechanical properties. Future research should be conducted which decouples stiffness and hardness, and compares measurements of gel phantoms and human muscle using a durometer and the MyotonPRO to establish exactly what the MyotonPRO is measuring.

There are several limitations to this study. Firstly, although care was taken to ensure there was no compression of the ultrasound transducer on the skin (through use of a probe holder and visualisation of a thick layer of acoustic coupling gel) it is not possible to confirm that this effect was null. This will have had impact on both the measurement of SWV and SAT (Vachutka et al., 2018). Secondly, despite participants being asked to remain relaxed during measurements, it was not possible to ensure participants were completely still and that the degree of muscle relaxation was optimised. Thirdly, although excellent repeatability was

exhibited by the MyotonPRO, the same level of repeatability was not observed for SWV. For intra-day repeatability 5/36 measurement locations exhibited poor repeatability, 16/36 moderate repeatability, and 15/36 good repeatability, with none displaying excellent repeatability. For inter-day repeatability 1/36 measurement locations exhibited poor repeatability, 11/36 moderate repeatability, 19/36 good repeatability, and 5/36 displayed excellent repeatability. Furthermore, due to the minimum sizing of a ROI being 2.2cmx0.5cm on our ultrasound device, some adjacent tissue was included in the SWV assessment of the SKIN and FAS and, as such, the measurements are not entirely representative of solely the target tissue. Finally, in female participants, we did not account for menstrual cycle phase. Whilst evidence for fluctuations in muscle stiffness across the menstrual cycle are weak, there is some evidence to suggest that it may be higher during the follicular phase than during ovulation, measured by both the MyotonPRO and SWE (Saeki et al., 2019, Khowailed et al., 2022). More research is required to understand if and how the menstrual cycle affects muscle stiffness, and the interaction of this with exercise.

3.6. Conclusions

This study demonstrated that to estimate RF stiffness using the MyotonPRO more accurately, the muscle should be lengthened in the PAST condition. Furthermore, the MyotonPRO device underestimates RF muscle stiffness as a consequence of increasing SAT. The findings in this study have relevance in the use of the MyotonPRO in both clinical and athletic populations. Our results indicate that it is better utilised in lean subjects or in muscles covered by minimal overlying non-contractile tissue. It is likely, however, that the MyotonPRO is estimating mechanical hardness and not stiffness. Therefore, mechanistic studies must be conducted, and this study replicated in other muscles to further establish the validity of the MyotonPRO as a

method of estimating muscle stiffness. As a result, subsequent experimental chapters will utilise SWE, but not the MyotonPRO, as a method of researching MSK tissue stiffness.

CHAPTER 4

Shear Wave Elastography Provides Reliable Assessments of Stiffness in the Skin, Fascia, and Both Superficial and Deep Muscle Tissues

A version of this chapter is under review in the Journal of Ultrasound in Medicine & Biology

Rationale

As established by results in Chapter 3, the MyotonPRO is not a valid method of estimating RF muscle tissue stiffness when compared with SWE. The following experimental chapters will, therefore, proceed to estimate muscle stiffness utilising only SWE. Ultrasound is an operator-dependent technique and there is not a comprehensive study assessing the reliability of SWE in assessing the skin and deep fascia overlying the RF muscle, or comparing superficial and deep regions of the muscle at different muscle lengths. This chapter, therefore, aims to address these gaps in the literature, assessing repeatability of SWE in estimating stiffness of the different tissues and depths within muscle. Establishing these facts builds on the existing literature aimed at the development and optimisation of standardised SWE protocols for assessing muscle tissue in research and practice.

4.1. Abstract

SWE has been shown to be a reliable method of estimating stiffness of superficial RF muscle regions at a variety of different muscle lengths. It is important to also understand the ability of SWE to reliably estimate the stiffness of deep muscle tissue and determine if altering muscle length affects this. The ability of SWE to estimate stiffness of the deep fascia is particularly pertinent with interest in the research of its mechanical properties growing due to its roles in perceptions of muscle pain, force transmission and damage during injury. Moreover, understanding the stiffness of skin tissue using SWE is of growing interest due to its implications in skin graft surgeries and dermal ageing. To assess SWE reliability, 20 healthy participants completed 2 visits in which RF SWV was measured twice, separated by 30mins, at four depths (skin - SKIN, fascia - FAS, superficial muscle - SUP, and deep muscle - DEEP) and three muscle lengths (relaxed - REL, neutral - NEU, and passively stretched - PAST).

Relative reliability was determined by calculation of intraclass correlation coefficients and absolute reliability was assessed by calculating the standard error and bias of measurements. SWE reported poor to good relative intra-day reliability (ICC, 0.33-0.82) but good to excellent absolute intra-day reliability (SEM, 0.14-0.25m/s; bias, -0.12-0.07). With regards to inter-day reliability, SWE exhibited moderate to excellent relative reliability (ICC, 0.54-0.91) and good to excellent absolute reliability (SEM, 0.12-0.21m/s; bias, -0.05-0.06). Reliability was highest in the order of PAST, NEU, REL with regards to condition, with SKIN and FAS reliability greater than in the muscle, where SUP measurements reported greater reliability than DEEP. These results suggest that SWE is a reliable method of estimating SKIN, FAS, SUP and DEEP RF tissue, and that to obtain the most reliable results, measurements should be taken from superficial tissue in a stretched state. We also propose a hierarchical categorisation system for the characterisation of absolute reliability of SWE.

4.2. Introduction

SWE is a relatively new technique in the field of MSK biomechanical research (Frulio and Trillaud, 2013). As such, its methodology is still being refined, and standardised protocols need to be developed for its use in both research and practical contexts. This has been reported as a major limitation restricting development in MSK SWE research, with a large-scale scoping review highlighting the heterogeneity in methodology and absence of reporting numerous key methodological components of the measurement protocol in the literature (Cipriano et al., 2022).

The existing literature shows SWE to be a reliable method of estimating RF muscle stiffness at a variety of muscle lengths manipulated by all combinations of lengthening and shortening of

the muscle by hip and knee flexion and extension (Kodesho et al., 2021a, Lee et al., 2021, Coombes et al., 2018, Lacourpaille et al., 2012, Otsuka et al., 2019). However, there is yet to be published a comprehensive analysis of SWE reliability of the RF under these different conditions comparing superficial and deep regions of the muscle. This is important, because excessive levels of deep muscle tissue stiffness have been found in patients with low back pain (Murillo et al., 2019). Understanding deep muscle tissue stiffness is also relevant in researching force sharing and transmission with deep aponeuroses and in assessing rehabilitation from deep muscle injury (Slane et al., 2017, Yagiz et al., 2024). For research into these areas using SWE, it is essential that it is able to repeatably estimate stiffness of deeper tissue. Furthermore, measurement depth is a known limitation of SWE. Greater ROI depths have been shown to yield higher SWV values in muscle and in gel phantom models (Wang et al., 2014, Shin et al., 2016, Wang et al., 2020). This is likely due to a reduced intensity of shear wave generation and propagation in deeper tissue regions. This causes the attenuation effect, wherein SWE reports void areas and regions of excessively high and low SWV within a ROI at a greater acquisition depth. Wang et al. (2020) have previously recommended that a SWV ROI depth of less than 3cm is best for estimating muscle tissue stiffness. It is, therefore, important to determine if this increase in elastogram void saturation and reporting of extreme SWVs impacts the repeatability of SWE measurement.

Overlying and connecting skin and fascial tissue have also not been assessed for SWV reliability, but have been shown to cause heterogeneity in SWE measurements of the RF muscle and are under-researched in the wider literature (Kodesho et al., 2023). There is a growing interest in the fascia within the biomechanics and physiology literature, due to its roles in perceptions of muscle pain, force transmission and concurrent lesion with muscle tissue in strain

injuries, both of which are affected by its biomechanical properties and stiffness (Katayama et al., 2023, Fu et al., 2024, Stecco et al., 2011, Wilke et al., 2019, Kawai et al., 2021a). In addition, there is a need to understand stiffness of the skin, due to its importance in surgical skin replacements and dermal ageing (Graham et al., 2019). For these reasons, the assessment of muscle, fascia and skin stiffness might be valuable in both clinical and athletic contexts. It is, therefore, important to differentially understand the stiffness of the skin and fascia, and whether the mechanical properties of these tissues can be reliably estimated in vivo using SWE.

Another absence in the SWE reliability literature is its quantification by absolute measures of reliability, with most studies just reporting relative reliability using the ICC. However, acceptable values for absolute reliability of SWE need to be determined for SWE to be utilised clinically as a diagnostic tool. This is relevant because muscle stiffness has been shown to change in response to exercise, and in injured and diseased states (Lacourpaille et al., 2017b, Yagiz et al., 2024, Lee et al., 2016). It therefore needs to be established whether the repeatability of SWE falls within the absolute range of these changes in stiffness after exercise, and of the differences between healthy and disordered populations. It is paramount that SWE is able to reliably assess tissue stiffness so that it can detect these meaningful changes and inter-individual differences to aid identification and diagnosis of tissue damage, injury and disease.

To conclude, the literature is currently missing data on the absolute reliability of estimating superficial muscle tissue stiffness with SWE, and both relative and absolute reliability of SWE in evaluating the skin, fascia and deep muscle regions under different levels of strain. The present study therefore aims to assess the influence of muscle length and measurement depth within RF muscle on SWE relative and absolute reliability, as well as assess the device's ability

to reliably assess the fascia and skin tissue overlying the RF. Based on findings in MSK tissue in previous studies, we hypothesise that SWE will exhibit good repeatability in all conditions, tissues and measurement depths, with higher repeatability in superficial tissue and in the PAST condition.

4.3. Materials and methods

4.3.1 Participants

As per Chapter 3 (Section 3.3.1) a total of 20 healthy, active participants (10 males and 10 females; age: 26.3 ± 4.0 years; height: 1.70 ± 0.10 m; weight: 67.7 ± 12.7 kg; BMI: 23.2 ± 2.8kg/m^2) volunteered for this study. Ethical approval for the study was approved by the Science, Technology, Engineering and Mathematics Committee at the University of Birmingham (ERN_0432-Apr2023) and all procedures conformed to the Declaration of Helsinki. Participants provided written informed consent prior to measurements. Sample size was determined by the power analysis described in Chapter 3 (Section 3.3.1).

4.3.2. Experimental design

Experimental design was the same as in Chapter 3 (Section 3.3.2), wherein participants completed two experimental visits conducted on separate days, with two measurements taken per visit, separated by 30min rest.

4.3.3. Experimental procedures

Experimental procedures were as per Chapter 3 (Section 3.3.3). Muscle stiffness was estimated in the same three conditions, with measurements taken in the order of relaxed, neutral, and

passively stretched (Figure 3.1). SWE was only utilised in the medial region (50% of RF muscle length, as identified by B-mode ultrasound).

4.3.4. Shear wave elastography

SWE methodology, and image acquisition and processing were conducted as per Chapter 3, Section 3.3.4, with measurements taken of the skin, fascia, superficial muscle and deep muscle.

4.3.5. Statistical analysis

All statistical analysis was conducted using IBM SPSS software (version 29). In the present study we chose to use SWV as this raw, unprocessed data does not assume the heterogeneity and isotropy of tissue that shear modulus (kPa) does, therefore providing a more accurate estimation of stiffness in the musculoskeletal context (Davis et al., 2019, Lee et al., 2016). Intra- and inter-day repeatability of SWV measurements were calculated separately at all four ROI depths (SKIN, FAS, SUP and DEEP) in all three conditions (REL, NEU and PAST). For intra-day repeatability, data from both visits was used to compare time 1 measurements to time 2 measurements. For inter-day repeatability, the mean values of SWV from the two measurements from each visit were used for analysis. This was done so using ICC as a measure of relative reliability, with standard error of the measurement (SEM) (calculated as the square root of the residual mean square), and bias (calculated via Bland-Altman analysis) representing absolute reliability of the measurements. It is generally accepted that ICC values less than 0.50 indicate poor reliability, values between 0.50 and 0.75 are indicative of moderate reliability, values between 0.75 and 0.90 indicate good reliability and values above 0.90 represent excellent reliability (Koo and Li, 2016). Based on the current lack of evidence for absolute reliability of SWV measurements in the RF deep fascia and muscle, we propose a need to categorise absolute

reliability of SWV as well as relative reliability. Accordingly, we propose that SEM <5% of mean SWV is regarded as excellent reliability, 5-10% as good, 10-15% as moderate and ≥15% as poor (Varol et al., 2024, Valera-Calero et al., 2024). Similarly for bias scores, we suggest that <2% of mean SWV is regarded as excellent, 2-5% as good, 5%-10% as moderate, and ≥10% as poor (Giavarina, 2015). For these calculations we used mean SWV from the 2 time points or visits, for intra- and inter-day reliability, respectively. We have used this ranking system to describe our own results.

4.4. Results

With regards to intra-day reliability, SWV reported moderate to good relative reliability based on ICCs, with good to excellent absolute reliability based on small SEM and bias values relative to mean SWV. This is with the exception of DEEP in REL, where relative reliability was poor. Full intra-day reliability results are reported in Table 4.1, with Bland-Altman plots reported in Figure 4.1.

Table 4.1

Measurement location		Mean SWV		Relative reliability	Absolute reliability			
Condition	Depth	Time 1 (m/s)	Time 2 (m/s)	ICC (lower bound - upper bound)	SEM (m/s)	SEM as a % of mean SWV	Bias (limits of agreement)	Bias as a % of mean SWV
Relaxed	Skin	2.54	2.54	0.69 (0.48-0.82)	0.23	9.06	-0.00 (-0.64-0.63)	0.00
	Fascia	2.00	1.99	0.82 (0.69-0.90)	0.14	7.02	0.02 (-0.36-0.40)	1.00
	Superficial muscle	2.09	2.15	0.60 (0.36-0.76)	0.17	8.02	-0.06 (-0.54-0.42)	-2.83
	Deep muscle	2.25	2.32	0.33 (0.04-0.58)	0.21	9.19	-0.07 (-0.65-0.50)	-3.06
Neutral	Skin	2.67	2.60	0.74 (0.56-0.85)	0.20	7.59	0.07 (-0.48-0.63)	2.66
	Fascia	2.30	2.33	0.69 (0.49-0.82)	0.23	9.94	-0.02 (-0.66-0.61)	-0.86
	Superficial muscle	2.54	2.53	0.64 (0.41-0.79)	0.23	9.07	0.01 (-0.64-0.65)	0.39
	Deep muscle	2.78	2.83	0.75 (0.58-0.86)	0.24	8.56	-0.05 (-0.71-0.61)	-1.78
Passively stretched	Skin	2.84	2.86	0.76 (0.58-0.86)	0.25	8.77	-0.02 (-0.71-0.67)	-0.70
	Fascia	2.81	2.89	0.81 (0.66-0.90)	0.18	6.32	-0.09 (-0.58-0.41)	-3.16
	Superficial muscle	2.98	3.05	0.75 (0.57-0.86)	0.20	6.63	-0.04 (-0.59-0.52)	-1.33
	Deep muscle	3.39	3.51	0.79 (0.63-0.88)	0.24	6.96	-0.12 (-0.77-0.54)	-3.48

Table 4.1. Intra-day reliability statistics for the assessment of skin, fascia and rectus femoris superficial and deep muscle tissue in relaxed, neutral and passively stretched conditions. ICC, intra-class correlation coefficient (single measures); SEM, standard error of the measurement; bias is calculated using the Bland-Altman test.

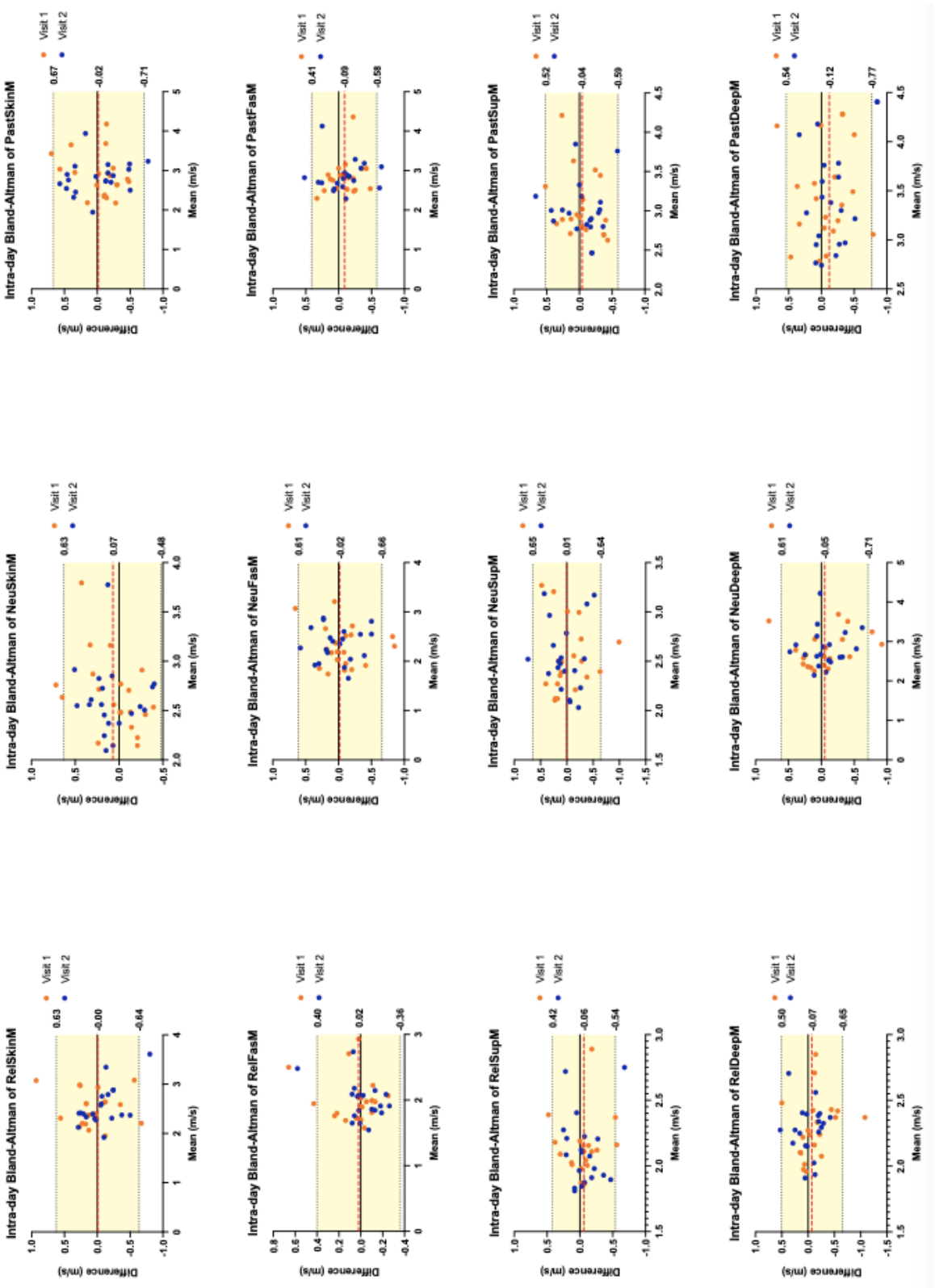

Figure 4.1. Bland-Altman plots representing the absolute intra-day reliability of SWE measurements.

SWE exhibited moderate to excellent inter-day relative reliability and good to excellent absolute reliability with low SEM and bias values relative to mean SWV. Full inter-day reliability results are reported in Table 4.2, with Bland-Altman plots provided in Figure 4.2.

Table 4.2

Measurement location		Mean SWV		Relative reliability	Absolute reliability			
Condition	Depth	Time 1 (m/s)	Time 2 (m/s)	ICC (lower bound - upper bound)	SEM (m/s)	SEM as a % of mean SWV	Bias (limits of agreement)	Bias as a % of mean SWV
Relaxed	Skin	2.52	2.56	0.83 (0.62-0.93)	0.16	6.30	-0.04 (-0.47-0.40)	-1.57
	Fascia	2.01	1.99	0.85 (0.67-0.94)	0.12	6.00	0.02 (-0.31-0.36)	1.00
	Superficial muscle	2.13	2.11	0.71 (0.40-0.88)	0.14	6.60	0.02 (-0.36-0.40)	0.94
	Deep muscle	2.29	2.28	0.54 (0.13-0.79)	0.15	6.56	0.01 (-0.40-0.41)	0.44
Neutral	Skin	2.67	2.60	0.88 (0.72-0.95)	0.12	4.55	0.06 (-0.29-0.41)	2.28
	Fascia	2.29	2.34	0.69 (0.36-0.86)	0.21	9.07	-0.05 (-0.64-0.54)	-2.16
	Superficial muscle	2.53	2.53	0.84 (0.63-0.93)	0.15	5.93	-0.01 (-0.41-0.39)	-0.40
	Deep muscle	2.79	2.83	0.86 (0.69-0.94)	0.17	6.05	-0.04 (-0.51-0.43)	-1.42
Passively stretched	Skin	2.88	2.83	0.84 (0.65-0.94)	0.19	6.65	0.05 (-0.48-0.57)	1.75
	Fascia	2.84	2.86	0.91 (0.79-0.96)	0.12	4.21	0.02 (-0.36-0.32)	0.70
	Superficial muscle	3.04	3.00	0.73 (0.44-0.89)	0.20	6.62	0.03 (-0.52-0.58)	0.99
	Deep muscle	3.46	3.44	0.85 (0.67-0.94)	0.20	5.80	0.03 (-0.53-0.58)	0.87

Table 4.2. Inter-day reliability statistics for the assessment of skin, fascia and rectus femoris superficial and deep muscle tissue in relaxed, neutral and passively stretched conditions. ICC,

intra-class correlation coefficient (single measures); SEM, standard error of the measurement; bias is calculated using the Bland-Altman test.

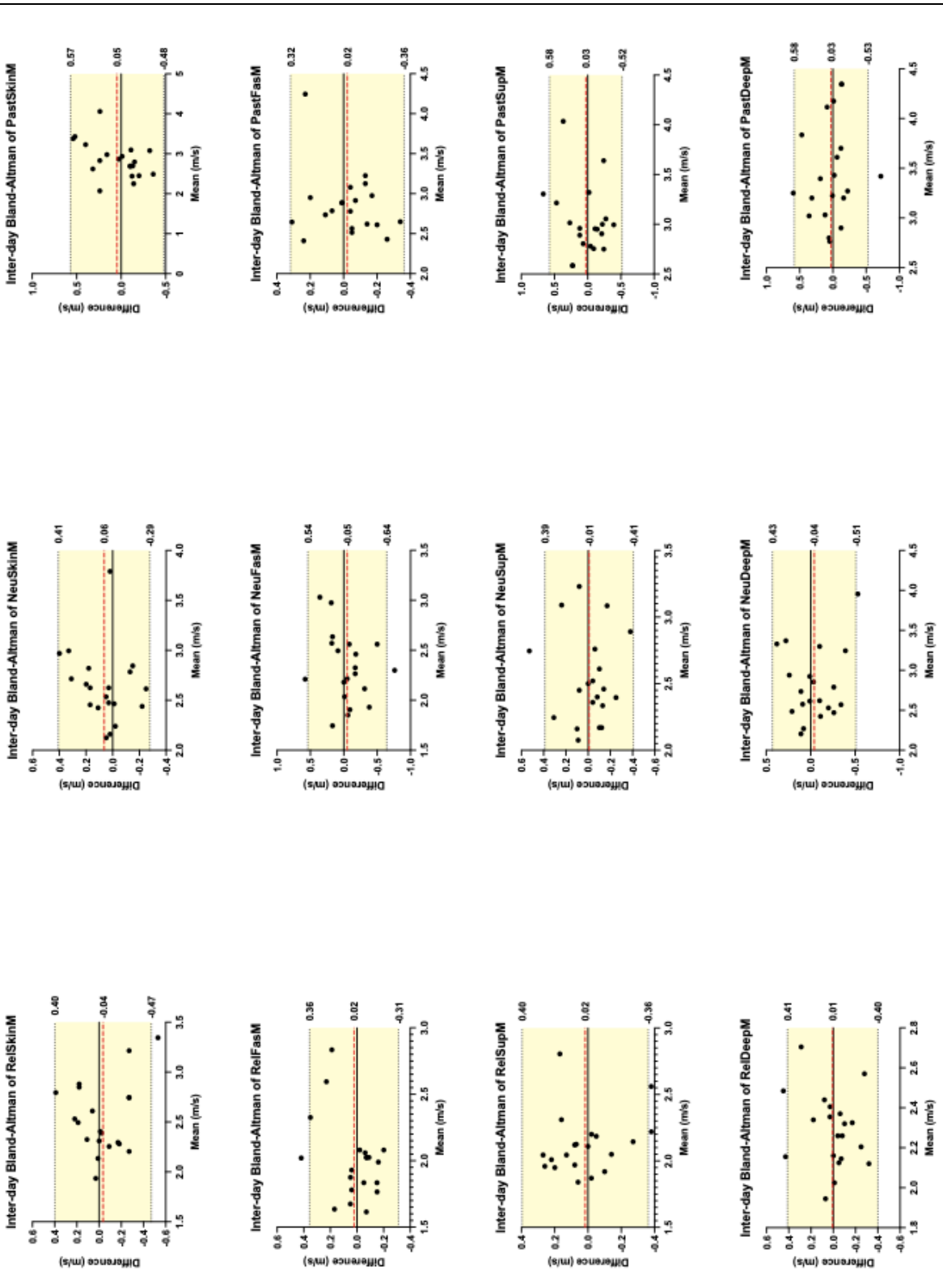

Figure 4.2. Bland-Altman plots representing the absolute inter-day reliability of SWE measurements.

4.5. Discussion

The present study finds estimation of stiffness of RF muscle and its overlying fascia and skin tissue to be reliably assessed by SWE. This is because relative reliability (ICC) in the different muscle conditions was moderate to good both intra- and inter-day. Furthermore, low values for SEM and bias indicate that the absolute reliability of the measurements is good to excellent. SWE can, therefore, be used as a reliable method of estimating skin, fascia and superficial and deep regions of the RF muscle. To the authors' knowledge, this is the first study to show that stiffness of the skin and fascia overlying the RF muscle can be reliably estimated using SWE, and to compare the absolute and relative reliability of assessing superficial and deep regions of the muscle.

Other studies have reported higher ICC values for SWE assessment of the RF than in the present study (Kodesho et al., 2021a, Lee et al., 2021, Coombes et al., 2018, Lacourpaille et al., 2012, Otsuka et al., 2019). These studies utilised AixPlorer and Siemens Healthcare ultrasound devices equipped with higher resolution SWE than the GE Healthcare device used in the present study. This means that they can measure SWV at a higher frequency, and the lower ICC values observed in our study are likely due to temporal limitations of the device. Another study which utilised the GE Healthcare model in estimating vastus lateralis muscle stiffness reported ICCs of 0.80 and 0.62 for intra- and inter-day repeatability, respectively (Bravo-Sánchez et al., 2021). These results more closely align with those found in the present study, highlighting the importance of ultrasound device resolution on SWE repeatability. Furthermore, other studies are flawed in that they do not report the absolute reliability of SWE measurements. In the present study we reported and categorised absolute reliability as good to excellent.

Our findings are particularly relevant in the application of MSK SWE which is commonly used to detect changes in SWV following exercise, and in patient populations compared to healthy controls. The observed changes in stiffness following exercise-induced muscle damage to the knee extensors are large, with Lacourpaille and colleagues reporting a mean increase of 79.4% in knee extensor shear modulus following high-load eccentric resistance training (Lacourpaille et al., 2017b). Furthermore, Heales and colleagues reported a 23.6% increase in RF SWV following eccentric exercise (Heales et al., 2018). Patients with CP suffer with excessive levels of muscle stiffness, causing contractures, limiting joint mobility and inhibiting functional movement. These patients have reported a 52.0% higher LG muscle stiffness, compared to healthy age-matched controls (Brandenburg et al., 2016). Furthermore, a 20% higher SWV of the TA and a 14% higher SWV of the MG was observed in the more affected limb, compared to the contralateral limb of these patients (Lee et al., 2016). The largest SEM we obtained was 0.25m/s, this accounted for only 8.8% of the lower SWV timepoint. This data is from the SKIN in the PAST condition (Table 4.1). It is therefore evident that the observed changes in stiffness as a result of exercise and disease are outside of the device's SEM. This means that SWE is able to detect meaningful changes in SWV due to its low error of measurement and good to excellent absolute reliability.

These results also provide data which aids the optimisation of standardised MSK SWE protocols. When averaging the tissues/measurement depths, both intra- and inter- day ICC ranked in order of PAST, NEU, REL. This indicates that stretching the muscle leads to a greater relative reliability of SWV measurement and that knee flexion is more important than hip extension in this observed muscle lengthening effect on SWV reliability. This aligns with ICCs reported by Kodesho and colleagues throughout passive knee flexion with participants in supine

position, wherein relative reliability (ICC) was higher at greater levels of knee flexion (Kodesho et al., 2021a). When averaging the conditions, the SKIN and FAS had higher intra- and inter-day reliability than muscle tissue, and SUP had higher intra- and inter-day ICCs than DEEP. This is to be expected as measurement depth has previously been reported as a limitation of SWE in both gel phantoms and in vivo in muscle tissue (Wang et al., 2020, Shin et al., 2016). Combined, these results indicate that better reliability of SWE is obtained when scanning more superficial tissue, and with the muscle in a stretched state. These conditions are therefore recommended for SWE measurement in research and practice to obtain the most repeatable measurements. Furthermore, we recommend that future studies report both relative and absolute reliability, with the latter categorised as proposed in the present study.

The present study has several limitations. Participants were asked to remain still and relaxed, and SWV values were extracted from three consecutive visually similar frames, wherein the B-mode images were consistent. However, we did not utilise electromyography to confirm the absence of muscle contraction so it is possible that participants may have, in some instances, not been fully relaxed. The minimum size of a ROI on the GE healthcare ultrasound device is 2.2cmx0.5cm, this meant that some adjacent tissue was included in the SWV estimation of SKIN and FAS tissue and values are not entirely representative of solely the target tissue. Finally, we did not account for menstrual cycle phase in our 10 female participants. This was because there is very little evidence for fluctuations in muscle stiffness across the menstrual cycle or for sex hormone-induced alterations in muscle stiffness (Saeki et al., 2019). This is, however, an under-investigated topic, with some evidence suggesting that muscle stiffness may be higher during the follicular phase than during ovulation (Khowailed et al., 2022).

4.6. Conclusions

Overall, these results are positive findings for researchers and practitioners aiming to utilise SWE in the RF muscle, and in assessment of fascia and skin stiffness. Our results indicate that, despite not always exhibiting excellent relative reliability, the absolute reliability of estimating stiffness with SWV is good to excellent, with small SEMs representing much lower fluctuations in resting passive stiffness than are observed following exercise or in diseased populations. This means that SWE is a reliable method, sensitive to detecting meaningful and clinically relevant alterations in tissue stiffness. Moreover, it is recommended that SWE measurements are taken by experienced ultrasonographers from superficial areas of the muscle and with the muscle beyond its slack length to obtain the most repeatable results. Future research should focus on optimising SWE protocol in different muscles to enable standardisation of SWE measurements between researchers and practitioners. In addition, reliability of SWE should be investigated in assessing other muscles under different levels of tension, as well as investigating the feasibility and repeatability of utilising SWE as a method of estimating tissue stiffness in pathological populations. These findings imply that SWE is a reliable method which can be used to achieve the aims of this thesis; to conduct research developing the mechanistic understanding of MSK tissue mechanics and the effects of exercise on these properties.

CHAPTER 5

Regional Differences in Muscle and Fascial Tissue Stiffness in the Rectus Femoris are Dependent Upon Local Strain

A version of this chapter is under review in the Journal of Biomechanics

Rationale

The previous phase of this thesis contributed to the development of methodological protocols for the estimation of MSK tissue stiffness. Chapter 3 established that the MyotonPRO is not a valid alternative to SWE in the assessment of RF muscle stiffness due to interference on SAT on the measurement. Chapter 4 determined that SWE is a reliable method of assessing both superficial and deep regions of the RF muscle, as well as the fascia and skin overlying it. It also revealed that SWE is most reliable when assessing superficial tissue in a stretched state. The following phase aims to contribute to our mechanistic understanding of MSK tissue mechanical properties by investigating tissue and region-specific differences in muscle stiffness in response to differential localised strain.

5.1. Abstract

Understanding how joint movement regionally affects the stiffness of biarticular muscles contributes to the understanding of muscle biomechanics and strain response, and may have implications in the mechanism of injury occurrence. Mechanical properties of the deep muscle fascia are important in myofascial force transmission and injury, however, its investigation by SWE in the literature is minimal. To determine tissue and region-specific differences in RF stiffness, 20 healthy participants completed two visits in which RF deep fascia and superficial and deep muscle regions were assessed by SWE in three muscle regions (proximal, medial, and distal) and at three muscle lengths (relaxed, neutral, and passively stretched). The deep fascia was consistently less stiff than muscle tissue ($P < 0.05$) in all conditions and regions, except for proximally in the relaxed condition where there were no significant differences. Regional differences in SWV were dependent upon local strain. Hip extension increased proximal SWV of the fascia, superficial and deep muscle above medial and distal regions (all $P < 0.001$), and

conditions of hip flexion ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). Similarly, knee flexion increased distal fascial, superficial and deep muscle tissue SWV above conditions of knee extension (all $P < 0.001$). Stretching the muscle by hip extension and knee flexion removed differences between the regions and increased medial SWV above that in the relaxed and neutral conditions (all $P < 0.001$). These results provide novel insight into regional differences in biarticular muscle and fascial tissue stiffness, implying that local strain increases SWV in the adjacent region. These findings may have implications in force generation and region-specific mechanisms of injury.

5.2. Introduction

As discussed in Section 2.4.4, SWE allows measurement of a specific ROI, allowing both different tissues and different regions within a tissue to be assessed independently. This allows specific assessment of individual muscles in isolation from their surrounding structures, thereby providing insight that performance tests are not able to give. This is particularly relevant due to the anisotropy of muscle tissue, which is therefore not homogenous from origin to insertion, and allows us to differentiate between and gain insight into biomechanical properties of specific tissue regions.

The occurrence of myofascial injury is most common in the proximal third of the RF (McAleer et al., 2022). Therefore, there is a clear need to understand differences in regional RF mechanical properties and how these may change at different hip and knee angles as a result of movement. Kodesho et al. (2021a) quantified regional RF muscle stiffness via SWE throughout passive knee flexion with participants supine, and found stiffness to be consistently higher in proximal regions. Since hip angle was not adjusted, it is possible that these findings may be

accounted for by hip extension the supine position preferentially stretching local proximal muscle regions. Furthermore, this study did not differentiate between superficial and deep muscle regions. There is, therefore, a need to assess regional RF muscle stiffness (both superficial and deep) manipulating muscle length at both the hip and knee joints.

Whilst muscle physiology and mechanics have been extensively researched, the deep fascia has historically been overlooked in the literature. However, interest in it is now gaining traction due to its role in force transmission, its lesion alongside muscle in strain injury, and its contribution to passive muscle mechanical properties (Wilke et al., 2019, Marcucci et al., 2019). Kawai et al. (2021a) found fascial stiffness measured by SWE to be elevated following injury, when compared to the uninjured contralateral limb. This highlights the importance of understanding fascial tissue mechanical properties in developing interventions to both prevent injury, and improve rehabilitation. The literature assessing fascial tissue stiffness with SWE is sparse, and more research is required to understand its mechanical properties at rest in healthy, uninjured subjects. Following this, research can begin to explore the effects of exercise and injury on the mechanical properties of fascial tissue.

The aims of the present study were to determine (1) whether there is a difference between muscle and fascial tissue stiffness, and (2) how changes in muscle length manipulated by changing both hip and knee angles influences regional differences in RF fascial and muscle tissue stiffness. Based on findings in other muscles, we hypothesise that the fascia will have a greater SWV than muscle, and that hip extension will preferentially increase proximal tissue stiffness, whilst knee flexion will preferentially increase distal tissue stiffness.

5.3. Materials and methods

5.3.1. Participants

As per Chapter 3 (Section 3.3.1) a total of 20 healthy, active participants (10 males and 10 females; age: 26.3 ± 4.0 years; height: 1.70 ± 0.10 m; weight: 67.7 ± 12.7 kg; BMI: 23.2 ± 2.8kg/m^2) volunteered for this study. Ethical approval for the study was approved by the Science, Technology, Engineering and Mathematics Committee at the University of Birmingham (ERN_0432-Apr2023) and all procedures conformed to the Declaration of Helsinki. Participants provided written informed consent prior to measurements. Sample size was determined by the power analysis described in Chapter 3 (Section 3.3.1).

5.3.2. Experimental design

Experimental design was the same as in Chapter 3 (Section 3.3.2), wherein participants completed two experimental visits conducted on separate days, with two measurements taken per visit, separated by 30min rest. The mean of these four measurements was used for each participant.

5.3.3. Experimental procedures

Experimental procedures were as per Chapter 3 (Section 3.3.3). Muscle stiffness was estimated in the same three conditions, with measurements taken in the order of relaxed, neutral, and passively stretched (Figure 3.1). SWE was used to estimate fascia, superficial muscle and deep muscle tissue stiffness in the same three regions (proximal, medial and distal).

5.3.4. Shear wave elastography

SWE methodology, and image acquisition and processing were conducted as per Chapter 3, Section 3.3.4, with measurements taken of the fascia, superficial muscle and deep muscle at proximal, medial and distal regions of the muscle (15%, 50% and 85% of muscle length, respectively) (Figure 5.1).

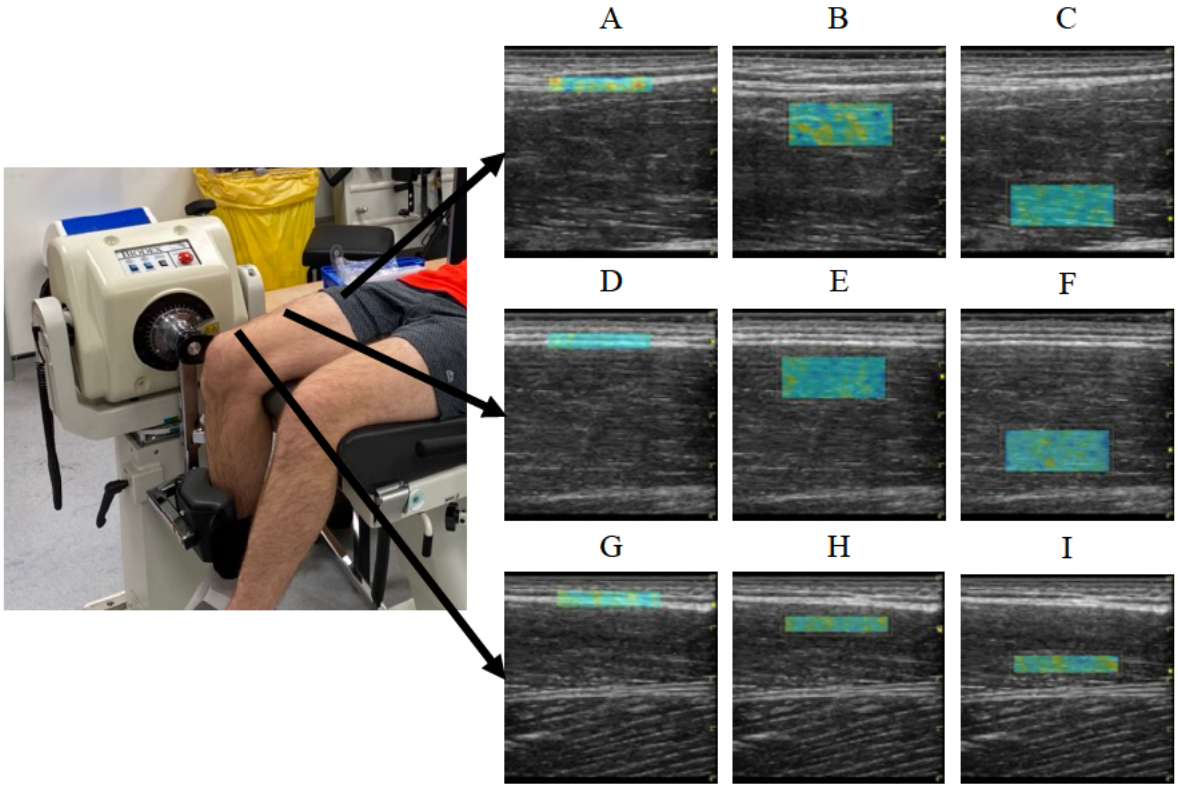

Figure 5.1. Schematic displaying the regions of interest used for shear wave velocity measurements in proximal, medial and distal locations with the participant in the passively stretched condition.

Proximal A) fascia, B) superficial muscle, C) deep muscle; medial D) fascia, E) superficial muscle, F) deep muscle; distal G) fascia, H) superficial muscle, I) deep muscle.

5.3.5. Statistical analysis

All statistical analysis was conducted using IBM SPSS software (version 29). In the present study we chose to use SWV as this raw, unprocessed data does not assume the heterogeneity and isotropy of tissue that shear modulus (kPa) does, therefore providing a more accurate estimation of stiffness in the MSK context (Davis et al., 2019, Lee et al., 2016). All data was normally distributed, determined by the Shapiro-Wilk test. To assess differences in tissue type, two-way ANOVAs were conducted at each location in each condition, with tissue as the within-subjects factor with 3 levels (FAS, SUP, DEEP). To determine the effect of the different conditions on location-specific SWV, at each SWE measurement depth (FAS, SUP and DEEP), a 3 location (PROX, MED, DIST) by 3 condition (REL, NEU, PAST) two-way repeated measures ANOVA was performed. Where appropriate, post hoc pairwise comparisons were conducted using the Bonferroni correction to investigate where the differences were significant. All values reported in text and graphs are mean ± SD unless otherwise specified. For all analyses, statistical significance was set at $\alpha < 0.05$.

5.4. Results

5.4.1. Tissue specific differences in rectus femoris fascia, superficial and deep muscle tissue stiffness

Proximally in the relaxed condition, there were no significant differences between tissue types, or measurement depth within muscle (Figure 5.2A). In every other condition and region, however, fascial tissue was consistently less stiff than muscle tissue. Fascia SWV was significantly lower than both superficial and deep muscle tissue distally in the relaxed condition (both $P < 0.001$). Likewise, in the neutral condition, FAS SWV was lower than both SUP and

DEEP proximally ($P = 0.011$, $P < 0.001$); medially (both $P < 0.001$); and distally (both $P < 0.001$). The same was found distally in the passively stretched condition ($P = 0.002$, $P < 0.001$) (Figure 5.2C-F, I). Additionally, FAS exhibited significantly lower SWV than DEEP in all other conditions (REL MED, $P = 0.002$; PAST PROX, $P = 0.029$; PAST MED, $P < 0.001$) (Figure 5.2B, G, H).

Deep muscle tissue SWV is consistently higher than in superficial muscle tissue (REL MED, $P = 0.007$; REL DIST, $P = 0.002$; NEU PROX, $P = 0.003$; NEU MED, $P < 0.001$; NEU DIST, $P < 0.001$; PAST PROX, $P = 0.018$; PAST MED, $P = 0.006$; PAST DIST, $P < 0.001$) (Figure 5.2B-I).

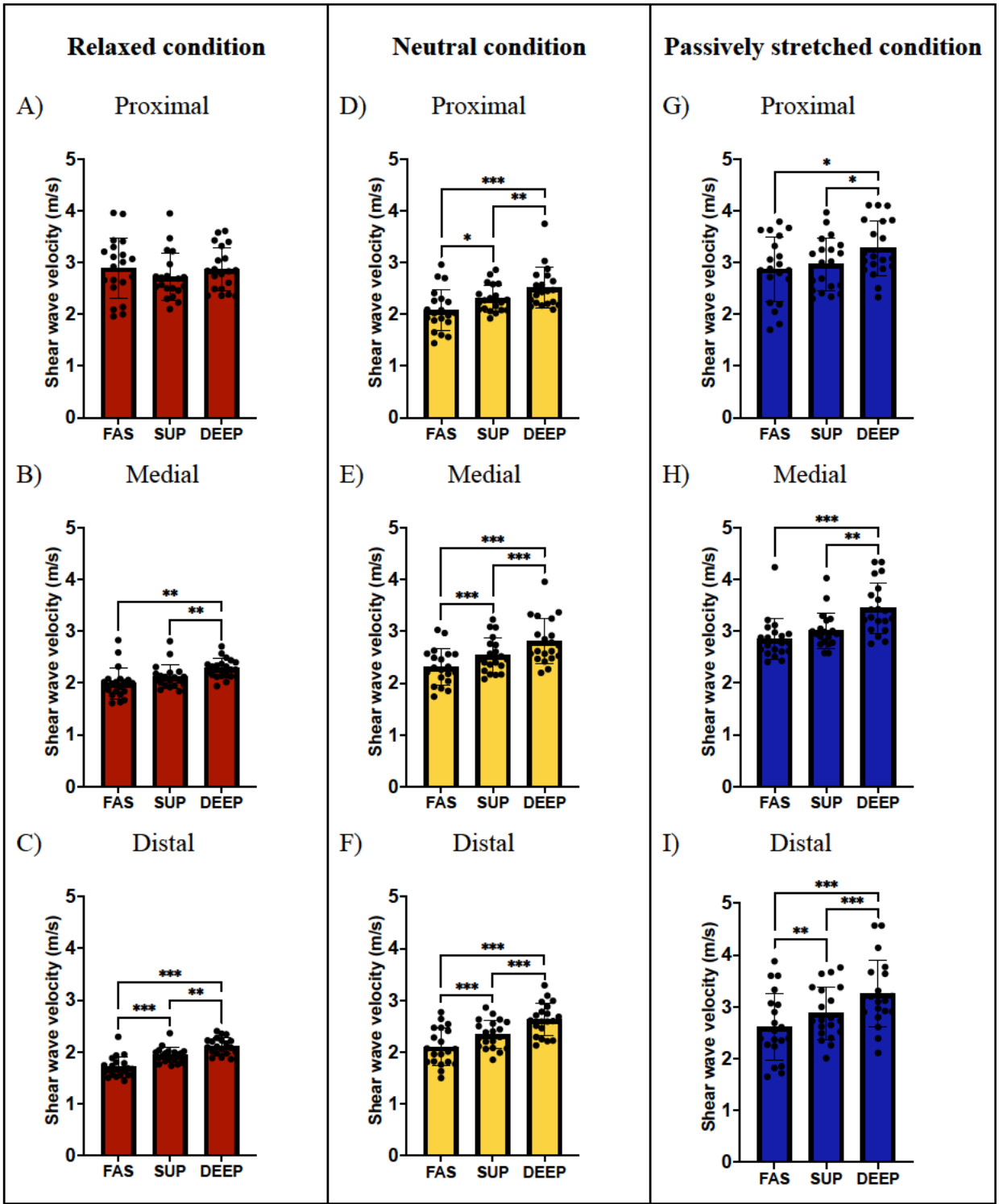

Figure 5.2. Differences in shear wave velocity between fascial, superficial and deep muscle tissue. A-C relaxed condition, in order of proximal, medial and distal regions.
D-F neutral condition, in order of proximal, medial and distal regions. G-I passively stretched condition, in order of proximal, medial and distal regions. *P<0.05 **P<0.01, ***P<0.001.

5.4.2. Regional differences in tissue stiffness

At FAS, SUP and DEEP, there were significant main effects of location ($P < 0.001$, $P < 0.001$, $P = 0.012$, respectively), position (all $P < 0.001$), and a significant location x position interaction (all $P < 0.001$). In all tissues (FAS, SUP and DEEP), SWV is higher proximally when the hip is extended in the relaxed ($P < 0.001$, $P = 0.005$, $P = 0.002$, respectively) and passively stretched conditions (all $P < 0.001$) than when it is flexed in the neutral condition. Similarly, in all of FAS, SUP and DEEP, SWV is higher distally when the knee is flexed in the neutral (all $P < 0.001$) and passively stretched conditions (all $P < 0.001$) than when it is extended in the relaxed condition. This is the same for medial tissue, wherein FAS, SUP and DEEP SWVs are higher with knee flexion in the neutral ($P = 0.001$, $P < 0.001$, $P < 0.001$, respectively) and passively stretched conditions (all $P < 0.001$) than in the relaxed condition. When the hip and knee are extended in the relaxed condition, SWV is higher for all of FAS, SUP and DEEP proximally than medially (all $P < 0.001$) or distally (all $P < 0.001$), and higher medially than distally ($P < 0.001$, $P = 0.001$, $P = 0.003$, respectively). But when the knee is flexed in the passively stretched condition, these regional differences disappear as the fascia and muscle are under tension at both ends. Furthermore, when the hip is flexed in the neutral condition, SWV proximally is no longer higher than it is distally (Figure 5.3). Finally, in the passively stretched condition, when the muscle is under the highest level of tension, SWV of FAS, SUP and DEEP are all higher medially than in the relaxed (all $P < 0.001$) or neutral conditions (all $P < 0.001$).

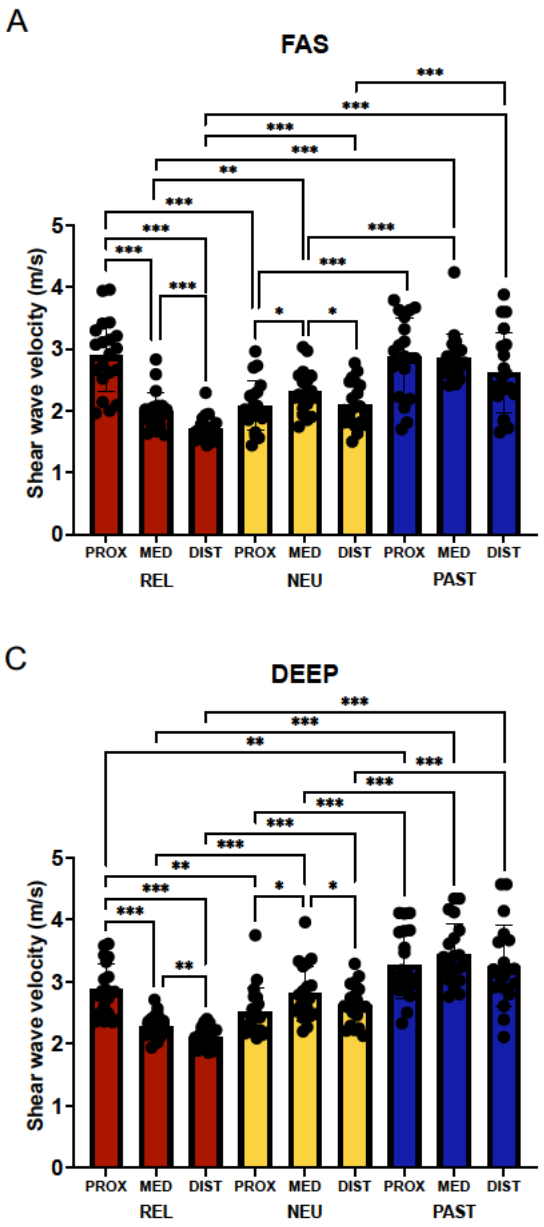

Figure 5.3. Regional differences in shear wave velocity of rectus femoris fascial and muscle tissue between conditions.

REL, relaxed condition; NEU, neutral condition; PAST, passively stretched condition; PROX, proximal region; MED, medial region; DIST, distal region. Regional and conditional differences in SWV at (A) the fascia, (B) superficial muscle and (C) deep muscle. * P<0.05 ** P<0.01, *** P<0.001.

REL NEU PAST

5.5. Discussion

Results from the present study indicate that (1) RF fascial tissue is less stiff than muscle tissue, and that deeper muscle is stiffer than superficial muscle, and (2) fascial and muscle tissue stiffness is higher proximally when the hip is extended, higher distally when the knee is flexed, and at its highest when both the hip is extended, and knee is flexed. In addition, this study highlights the ability of the biarticular RF to be differentially strained along its length based on altering hip and knee angles, which may have implications for force generation, as well as injury risk mechanisms and prevention.

5.5.1. Tissue differences

To the authors' knowledge, this is the first study to compare the stiffness of the deep fascia and muscle tissue in the RF, finding rather comprehensively that the deep fascia is less stiff than muscle in relaxed and stretched states. The exception to this was in the proximal region in the relaxed condition, where there are no significant differences between SWV of the fascia and muscle (Figure 5.2A). We hypothesise that the additional strain on this region caused by hip extension resulted in increased homogeneity of stiffness between the two tissues. Research in other muscle groups, however, has produced conflicting findings. Barzegar et al. (2023) reported higher shear modulus of the GM deep fascia (13.0kPa, 20.40kPa) than of the GM muscle (6.58kPa, 10.83kPa) under both relaxed and stretched conditions, respectively. The opposite was also found in the BF in a relaxed, prone condition with a mean fascial stiffness of 17.9kPa which was nearly double the mean muscle stiffness of 9.0kPa (Wilke et al., 2022). It is important to note, however, that these studies did not report statistical comparisons between the tissues, and that these comments are based purely on observing trends in the data. Our findings highlight intermuscular differences and emphasise the need for more extensive

research throughout muscle groups to establish normative SWV values in muscle and fascial tissue. These intermuscular differences may be a result of adaptations which reflect the functional demand placed on the muscles. Unlike in the GM or BF, SWV may be higher in the RF muscle relative to the deep fascia as a consequence of muscle fibre orientation. Since the RF is a bipennate muscle capable of producing large amounts of contractile force, it has a greater physiological cross-sectional area than the GM and BF muscles, and therefore muscle fibres are more densely packed (Wilson and Lichtwark, 2011). This adaptation to allow high levels of force generation may cause higher SWV in RF muscle tissue relative to fascial tissue as the dense packing of muscle fibres increases stiffness and reduces elasticity, much like ultrasound probe compression (Vachutka et al., 2018). These findings may have implications regarding force transmission and injury risk in the RF deep fascia compared to that overlying other muscles. It is important to identify these relative normative regional values individually in all muscles and their overlying deep fasciae so that practitioners can more confidently interpret SWV data from athletes. This is particularly relevant when attempting to gain insight into injury risk, rehabilitation progress, and readiness to play because stiffness of injured deep fascia has been shown to remain elevated years after injury occurrence (Kawai et al., 2021a).

Furthermore, to our knowledge this is the first study to compare measurement depths of RF muscle stiffness. In all conditions and regions, except for proximally in the relaxed condition, deep muscle exhibited a higher SWV than superficial muscle. These findings are concordant with findings in other muscles that have been examined in the existing literature, including the supraspinatus, multifidus and forearm muscles (Jiang et al., 2023, Murillo et al., 2019, Wang et al., 2020). These findings may be due to deeper muscle fibres attaching to deep aponeuroses. These thick fibrous layers of connective tissue exert an anchoring effect on deeper muscle

fascicles, increasing fascicle strain (Marcucci and Reggiani, 2020). This is supported by findings in the gastrocnemius muscle, where a stiffer aponeurosis was associated with stiffer adjacent muscle tissue (Slane et al., 2017). This would explain our findings, wherein muscle tissue closer to the RF aponeurosis (i.e., deeper tissue) is stiffer than more superficial muscle tissue as a function of tension from the connective tissue to which it is attached. However, it must be considered that these results may be due to a limitation with SWE. Greater acquisition/ROI depths yield higher SWV values both in muscle and in gel phantom models (Wang et al., 2014, Shin et al., 2016, Wang et al., 2020). This is likely due to a decline in the intensity of the acoustic radiation force impulse as it transmits to deeper tissue regions. This phenomenon is also the cause of the attenuation effect, wherein there are greater void areas and areas of excessively high and low SWVs reported within an elastogram of a greater acquisition depth. As a result, it has previously been recommended that a SWV acquisition depth of <3cm is most viable for assessing muscle tissue, although in the previous chapter, we indicate that this may be increased to encompass deeper muscle regions (Wang et al., 2020). Moreover, in the present study the minimum size of a ROI was 2.2cmx0.5cm on our ultrasound device, meaning that some adjacent tissue was included in the SWV assessment of the deep fascia and values are therefore not solely representative of the target tissue. Further methodological research is required to determine the viability of utilising SWE as a method of assessing deep muscle tissue stiffness and determine best practice for assessing SWV of the deep fascia.

5.5.2. Regional differences

To our knowledge, this is the first study to manipulate RF length at both the hip and the knee, and investigate stiffness of fascial and muscle tissue at different depths. Our results show that the observation of higher levels of proximal fascial and muscle tissue stiffness are only a result

of localised tension stress caused by hip extension, since, when the hip is flexed, in the neutral condition, there is no difference between proximal and distal RF regions. This regional behaviour appears characteristic of the biarticular RF muscle, as the same principle is observed in the distal region. Here, fascial and muscle tissue stiffness are greater when the knee is flexed in the neutral and passively stretched conditions than it is in the relaxed condition. There were no regional differences in RF stiffness when the muscle was under tension stress from both tendons in the passively stretched condition, more evenly distributing strain along the length of the muscle. Combined, these findings indicate that regional differences in RF muscle stiffness are dependent upon localised tension stress applied to the tissue. These localised impacts of movement on RF fascial and muscle tissue stiffness may influence risk and mechanism of injury during exercise.

In the present study, we found a higher SWV in medial RF fascial and muscle tissue when the muscle was passively stretched compared to in the relaxed and neutral conditions. This is not a new phenomenon as it is already well-established that increasing muscle length augments muscle stiffness (Le Sant et al., 2015, Itsuda et al., 2024). Specifically in the RF, increasing knee flexion has been shown to be accompanied by increases in RF muscle stiffness with the hip both flexed and extended (Coombes et al., 2018, Xu et al., 2018). Furthermore, another study assessed the biarticular GM muscle and found that both knee extension and dorsiflexion increased shear modulus (Zhou et al., 2020). This study is the first to show that this trend is the same in the deep fascia as it is in muscle tissue.

Our results show that the RF displays a higher SWV proximally when the hip is extended (in PAST and REL) than when it is flexed (in NEU), and higher RF SWV distally when the knee is flexed (in PAST and NEU) than when it is extended (in REL). These findings are supported

by the literature, since when the knee is extended, MG stiffness is higher proximally than it is medially and distally (Zhou et al., 2019, Liu et al., 2020). Similarly, higher levels of strain have been observed in proximal regions of BF cadavers with increasing anterior pelvic tilt (Mendiguchia et al., 2024). These findings therefore appear to be characteristic of biarticular muscles, such that increases in stiffness caused by stretching are not uniformly distributed along the muscle's length, but exacerbated in the region closest to the joint which is stretching the muscle.

With the known heterogeneity of muscle tissue, it is perhaps unsurprising that regional differences in mechanical properties exist within the biarticular RF. Studies utilising electromyography have shown preferential activation of proximal RF regions during hip flexion, highlighting ability of the RF motor units to work independently to fulfil the muscle's dual functions (Watanabe et al., 2021). Furthermore, as described previously, Kodesho et al. (2021a) found that, with the hip extended in a supine position, RF stiffness is consistently higher in proximal regions than in more distal regions regardless of knee joint angle. Our findings in PAST do, however, contrast these results, as we observed no significant differences between regions. This is likely due to heterogeneity in scanning position between our studies. In PAST our participants had a hip angle of 155^{o} to avoid hip overextension, whereas participants were supine with 180^{o} hip angle in the study by Kodesho et al. (2021a), so participants experienced greater hip extension and more tension stress on the muscle from the proximal tendon. Moreover, our study included female participants, who likely had a higher hip ROM and greater flexibility, thus reducing relative tension on the RF from the proximal tendon when compared with the male participants recruited by Kodesho et al. (2021a) (Nagai et al., 2021).

It is noteworthy that these trends were consistent between fascial and muscle tissue, but perhaps unsurprising, since mechanical properties are consistent between materials, wherein application

of a tension stress leads to an increase in material stiffness. Our results show that regional differences in RF muscle stiffness are not consistent, and that regional differences in the SWV of biarticular muscles must be interpreted in the context of participant joint positioning. These findings may have implications in the regional occurrence and mechanism of RF injuries in specific phases of human movement and sporting actions, however, more research is required to understand the link between regional differences in stiffness and injury location to this level of specificity.

5.6. Conclusions

Fascial tissue is consistently less stiff than muscle tissue, regardless of muscle length or muscle region, whilst deep muscle tissue is consistently stiffer than superficial muscle tissue. Regional differences in RF stiffness are not consistent, but are instead altered by manipulating regional muscle strain in response to localised tension stress. Hip extension leads to increases in proximal tissue stiffness, and knee extension leads to increases in distal tissue stiffness. This localised responsiveness to tension may have implications in the region-specific mechanisms of injury, i.e., excessive hip extension is more likely to cause proximal, rather than distal, RF muscle injury. To understand further the relationship between stiffness and injury, research must establish how exercise influences muscle and fascial tissue stiffness in stretched and shortened conditions.

CHAPTER 6

Biceps Femoris Muscle Stiffness Increases After Sprint and Shuttle Run Interventions on Consecutive Days and is Associated with Range of Motion

Rationale

The previous phases of this thesis have established that SWE is a reliable method of estimating MSK tissue stiffness which can be used to detect meaningful changes in stiffness, and that these passive mechanical properties of biarticular MSK tissue are influenced by muscle length and localised stress. The following phase aims to build upon these findings, which have implications in injury mechanism, by determining the effects of maximal running-based exercise interventions on MSK tissue stiffness. This chapter builds upon the existing literature which explores the effects of laboratory-based exercise interventions on muscle stiffness, and explores the relationship between muscle stiffness and ROM.

6.1. Abstract

It is well-established that exercise induces acute alterations in muscle stiffness assessed by SWE but the effects of exercise on stiffness of the deep fascia has yet to be investigated. Previous studies have shown that sprint interventions can lead to an increase in BFlh muscle stiffness, but did not assess lower limb muscles other than the hamstrings. Furthermore, examination of muscle stiffness following exercise on consecutive days is not well understood, with research focusing on the acute impact of one exercise bout. Moreover, the literature has, thus far, not expanded to encompass or compare real-world exercise interventions or the utility of SWE in the applied sports science setting. This study aimed to address these shortcomings and gaps in the literature by evaluating RF and BFlh deep fascia and muscle SWV and ROM in 15 healthy participants pre-, post-, and 24h post- consecutive days of sprint (10 x 40m) and shuttle run (10 x 4x10m) interventions completed in a randomised order. BFlh muscle stiffness significantly increased from baseline immediately after the first intervention ($P < 0.001$) and remained higher than baseline 24h after the second intervention ($P = 0.017$). Interestingly, we also observed a

significant negative correlation between BFlh muscle stiffness and ROM in the active knee extension test (R = -0.535, P = 0.020). Combined, these findings indicate that there is a cumulative effect of exercise on BFlh muscle stiffness, and that high levels of muscle stiffness impede ROM. It is, therefore, recommended that athletes complete regular stretching interventions as part of their recovery programme if the aim is to keep muscle stiffness low and reduce injury risk.

6.2. Introduction

Previous chapters have contributed to the refinement of MSK SWE methodology and the mechanistic understanding of regional deep fascia and muscle stiffness of biarticular muscles, as well as the differences between the two tissues. This experimental chapter aims to build upon this work, and the existing literature, by assessing the utility of SWE in evaluating the mechanical responses of MSK tissue to real-world exercise interventions.

As discussed in the literature review (Chapter 2), different exercise modalities and contraction types have been shown to cause different outcomes on muscle stiffness. Endurance exercise induces a reduction in stiffness, whereas resistance exercise increases stiffness (Andonian et al., 2016, Pournot et al., 2016). This is likely due to the influence of eccentric muscle damage-inducing contractions, which increase muscle stiffness (Lacourpaille et al., 2017b). These findings have been comprehensively reproduced in different muscle groups utilising multiple highly controlled laboratory-based interventions of differing volumes and intensities (Heales et al., 2018, Guilhem et al., 2016, Inami et al., 2022, Goreau et al., 2022). Little is known, however, about the effects of real-world exercise interventions on MSK tissue stiffness, or the utility of SWE in the athletic setting. Some research has investigated the effects of laboratory-

based sprint interventions on hamstring muscle stiffness, finding reduced ST and BFlh passive stiffness but increased BFlh active stiffness (Pimenta et al., 2023, Freitas et al., 2023). Whilst this is interesting, these findings must be investigated further, and in other lower limb muscle groups to determine if they translate to the real-world setting. This research is important, as sprinting is a key performance indicator in team sports, with Faude et al. (2012) finding it to be the most common action in the lead up to goal situations in elite football. In addition, the impact of other training interventions which are commonly used among elite athletes should be investigated and compared. This study will focus on comparing a sprint-based intervention with a shuttle run based intervention, as these are common actions and training modalities in elite football.

Athletes are required to train and compete daily, meaning that recovery from training and competition can be compromised (Carling et al., 2016). Athletic training schedules do not align with the time-points used in research to investigate the effects of exercise on muscle mechanical properties. There is, therefore, a huge gap between the knowledge developed in research settings and practice in the applied setting. One study investigated the effects of two bouts of eccentric exercise, performed on consecutive days, on quadriceps muscle stiffness, finding that VM, VL and RF shear modulus significantly increase after the first bout, but recover to baseline before the second exercise bout. After the second bout, shear modulus then increased similarly to the first bout (Morin et al., 2023). This study did not, however, follow up 24h later to see if there was a sustained or cumulative impact of the consecutive interventions which was not seen 24h after the first intervention. To be more generalisable to the real-world setting, this research needs to be built upon and conducted using field-based exercise interventions, such as sprint and shuttle run interventions completed by athletes, as opposed to highly controlled laboratory-

based protocols. Furthermore, muscle stiffness should be measured 24h after the interventions to determine if there is a cumulative effect of consecutive exercise bouts. It must also be determined if there is an effect of exercise intervention order on alterations in muscle stiffness, as this would influence the structuring of training programmes for elite athletes.

It has been speculated in the literature that elevated levels of muscle stiffness inhibit flexibility and ROM. This is because stretching interventions, both acute and chronic, have been shown to reduce muscle stiffness, with concurrent increases in ROM (Reiner et al., 2021a, Ikeda et al., 2021, Akagi and Takahashi, 2014). Only one study, however, has found a correlation between the two, with Reiner et al. (2024b) finding a significant negative relationship between passive hamstring muscle stiffness and hip flexion ROM in the sit-and-reach test. These findings need validating by utilising other ROM tests, and assessing other muscle groups. Furthermore, since Liu et al. (2024) found local high frequency percussive massage therapy to increase ROM, accompanied by a decrease in deep fascia but not muscle stiffness, it must be considered that mechanical properties of connective tissue may also impact ROM. To our knowledge, however, relationships between deep fascia stiffness and ROM have not been investigated until now.

This study focuses on the effects of sprint and shuttle run interventions to compare the effects on MSK tissue mechanics of maximal velocity running with braking and accelerating forces associated with changes of direction. Since, these training interventions are not completed in isolation in the real-world setting, this study will compare the effects of completing the exercise interventions on consecutive days in both orders.

Based on evidence in the literature and findings in the previous chapters developing MSK SWE methodology and mechanistic understanding of stiffness, knowledge of SWE is sufficient to allow its use in research in applied settings to support athlete health and performance. As such, and based on the existing gaps in the literature, the aims of the present study are to examine how real-world exercise interventions affect stiffness of the RF and BFlh deep fasciae and muscles, ROM and perceived muscle soreness; to determine if there is a cumulative effect of conducting exercise bouts on consecutive days; and to determine if there is a relationship between deep fascia and muscle stiffness and ROM or perceived muscle soreness.

We hypothesise that both interventions will acutely increase both RF and BFlh muscle and fascial tissue stiffness, with the sprint intervention having a greater effect on BFlh tissue and the shuttle run intervention having a greater effect on RF tissue. We also hypothesise that completion of both training interventions will reduce RF and BFlh ROM and increase perceived muscle soreness to the same extent, with a cumulative effect after completing the second exercise intervention, with no effect of exercise order. Furthermore, we hypothesise a negative relationship between muscle and fascia stiffness and flexibility, and a positive relationship between change in muscle and fascia stiffness and perceived muscle soreness.

6.3. Materials and methods

6.3.1. Participants

A total of 15 participants (7 males and 8 females; age: 23.5 ± 3.8 years; height: 1.73 ± 0.10 m; weight: 71.62 ± 14.6 kg; BMI: 23.7 ± 3.1 kg/m^2) volunteered to participate in this study. All participants reported to regularly complete at least 150min moderate or 75min vigorous

physical activity per week, and had no history of MSK injury in the examined muscles. Ethical approval for this study was obtained from the Science, Technology, Engineering and Mathematics Committee at the University of Birmingham (ERN_1431-Sep2023) and all experimental procedures conformed to the Declaration of Helsinki. Participants provided written informed consent at the familiarisation visit, prior to measurements. Sample size was determined via a power analysis (G* Power software) based on previous research with statistical power set at 0.80, an effect size of 0.50 and an alpha level of 0.05 (Pimenta et al., 2023).

6.3.2. Experimental design

Participants completed a familiarisation session and a total of six experimental visits. This study followed a randomised cross-over design in which participants completed two different exercise interventions (sprint and shuttle run protocols) on consecutive days, 24h apart, with a follow up visit 24h later on day three. In a separate week, these interventions were repeated in the opposite order to control for an effect of intervention order on the measured outcome variables (Figure 6.1). The mean time between the first round of follow up measurements and the first exercise session of the second round was 8 ± 7 days (range = 2-26 days). Participants refrained from exercise that involved the legs and consumption of alcohol and recreational drugs for 24h prior to each experimental visit. The dependent variables (SWV, ROM and muscle soreness) were measured pre- and post-exercise on both days, and at the follow up visit (T1, pre-exercise day 1; T2, post-exercise day 1; T3, pre-exercise day 2; T4, post-exercise day 2; T5, 24h follow up). Measurements were repeated twice within the familiarisation session, allowing the calculation of intra-day repeatability of measurements, with comparison to the first pre-exercise measurement (T1) enabling calculation of inter-day repeatability.

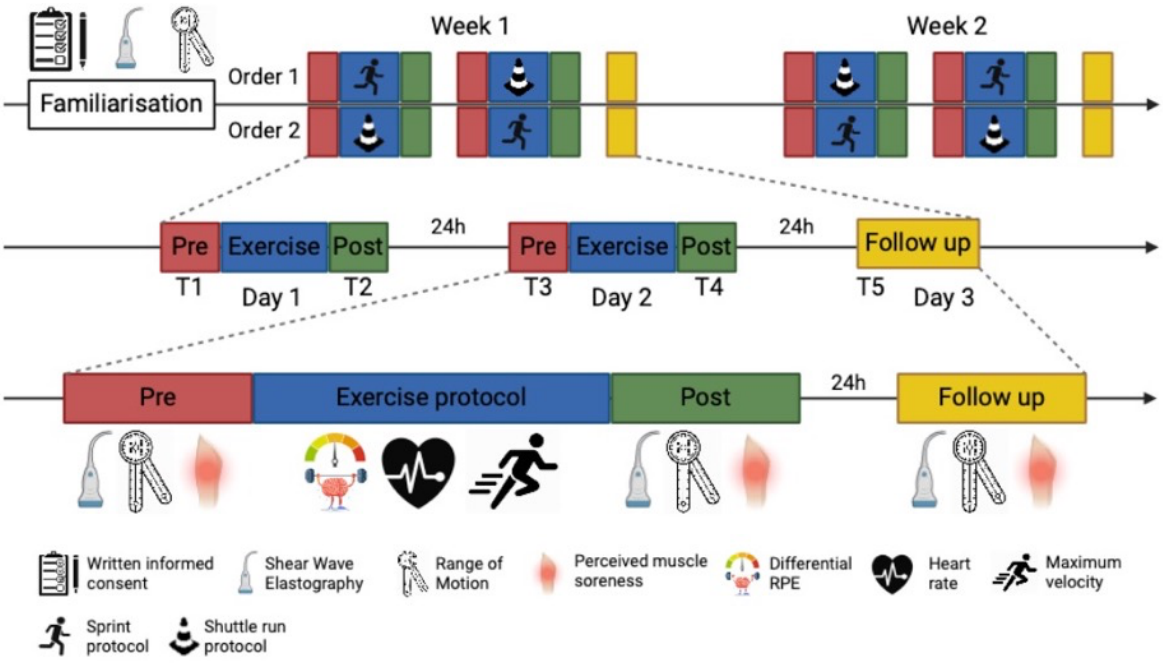

Figure 6.1. Schematic displaying experimental design.
Participants completed either a sprint or shuttle run training intervention on day one, and the other intervention on day two, then return to the laboratory for follow up measurements on day three. This protocol was repeated on a separate week with the order of the exercise interventions reversed. Measurements of muscle stiffness, range of motion and perceived muscle soreness were collected pre- and post- each exercise intervention and at the 24h follow up visit.

6.3.3. Exercise protocols

Sprint protocol – 10 x 40m maximal sprints with a 5m deceleration zone. Participants were given 3min between repetitions to recover, during which differential RPE (dRPE) data was collected, and participants walked the 45m back to the start line, ready for the next repetition.

Shuttle run protocol – 4 x 10m maximal shuttles x10 sets, with 3min recovery between sets. In this recovery time, dRPE was collected.

Before completing either protocol participants completed a self-selected warm up which they deemed sufficient to prepare them for the upcoming exercise. This warm-up was not standardised between participants, but was consistent between trials for each participant. Both exercise interventions were performed outside on a multi-sport tarmac playing surface situated on the university campus, a 5min walk from the laboratory in which measurements were taken. The mean time between finishing the exercise intervention and the commencement of post-exercise SWV measurements was 7min43s ± 45.55s. These exercise interventions were chosen as they reflect real-world training sessions performed by athletes to train and condition maximum velocity sprinting and changes of direction. The interventions were distance and intensity-matched to allow comparison of the interventions to determine the effect of maximum velocity versus changes of direction on mechanical properties of MSK tissue.

6.3.4. Experimental procedures

Participant height (SECA, Hamburg Germany) and weight (Ohaus ChampII, New Jersey USA) were measured in the familiarisation session. The dominant leg, defined as the preferred leg used to kick a football, was selected for SWE and ROM measurements. The laboratory was set at a temperature of 21ºC. SWV was estimated in the RF and BFlh muscles at 50% of femur length. Femur length was determined by measuring the distance from the greater trochanter to the lateral epicondyle, identified by manual palpation with the participant supine on the examination table, and marked onto the skin using a permanent marker. Following this, B-mode ultrasound using a 9-linear array probe (44mm, 2-8MHz) (LOGIQ S8 GE Healthcare, Milwaukee, USA) was used to identify the centre of both muscles with the transducer in the transverse orientation. The transducer was then rotated ~90º to be longitudinally oriented with

the muscle fascicles, and permanent marker was used to mark the four corners of the ultrasound transducer to ensure consistency between measurements. SWV of the deep fascia and superficial muscle tissue was measured at these muscle sites in two different conditions to manipulate muscle length: relaxed (REL), and passively stretched (PAST) in line with procedures in the literature (Lacourpaille et al., 2017b). For measurements of the RF in the REL condition, participants laid supine on the examination table. Unlike in previous chapters, in the PAST condition in this study, participants laid supine on the examination table, with their knees at the end of the table and the lower leg relaxed hanging down off the table to stretch the RF muscle. For measurements of the BFlh in the REL condition, participants laid prone on the examination table. In the PAST condition, participants laid supine on the examination table, with their hip flexed to 90º and knee extended to 120º (180º represents full extension), with the foot fixed in place using an IKD (Biodex System 3 Dynamometer, Biodex Medical Systems), as per Reiner et al. (2024b). During measurements, participants were asked to remain still and fully relaxed, avoiding muscle contractions.

ROM tests were performed to determine RF and BFlh flexibility. The modified Thomas test (TT) was used to assess RF ROM, and the active knee extension (AKE) test was used to determine BFlh ROM, as per standardised protocols (Harvey, 1998, Gajdosik and Lusin, 1983, Cejudo et al., 2020, Cejudo, 2022). To complete the TT, participants stood at the end of the examination table with their gluteal folds on the edge of the table. The participant then laid back on to the table, pulling the non-measured knee in towards their chest and allowing the measured limb to lower down, fully relaxed. To complete the AKE test, participants laid supine on the examination table, with the lumbar spine neutral, the non-measured limb extended relaxed down on the examination table, and the hip of the examined limb flexed at 90º. The examined

limb was stabilised in this position by participants holding onto the posterior thigh, and participants were instructed to actively extend their knee as far as they could without extending their hip. For measurement of knee angle in both tests, a handheld 360 goniometer (Prestige Medical, Northridge, California) was used with the axis placed over the lateral femoral epicondyle, the stationary arm parallel to the femur, pointing towards the greater trochanter, and the movement arm parallel to the fibula, pointing towards the lateral malleolus.

Muscle soreness was assessed using a 10-point VAS, with participants asked, 'How sore do the muscles in your left/right leg feel?'. SWV and ROM were assessed in the familiarisation session and pre-, immediately, and 24h post- every exercise intervention. Muscle soreness was assessed pre-, immediately, and 24h post- every exercise intervention trial (Figure 6.1). During exercise interventions, participants wore GPS devices equipped with integrated heart rate (HR) monitors (Catapult Vector S7 4GHz) to determine key performance indicators (HR and maximum velocity). Each GPS unit was clipped into a specialised Catapult vest positioned between the shoulder blades (Roe et al., 2017). GPS units were switched on before pre-exercise measurements and switched off and docked after post-exercise measurements. Exercise interventions were mapped and pipped by the same researcher (CL) into individual sprint repetitions and shuttle run sets, which were analysed using Catapult OpenField software (Version 3.12.1). Differential RPE was collected after each repetition, with participants shown a 6-20 Borg scale and asked to rate how difficult the exercise felt with regards to 'breathlessness' (B-RPE) and 'leg exertion' (LE-RPE) (McLaren et al., 2016).

6.3.5. Shear wave elastography

As in previous chapters, SWV was estimated via SWE using an ultrasound device equipped with SWE on a 9-linear array probe (44mm, 2-8MHz) (LOGIQ S8 GE Healthcare, Milwaukee, USA) which was positioned in the centre of the muscle (identified with the transducer in the transverse plane), longitudinally oriented to the muscle fascicles at 50% of femur length. All SWV measurements were performed by a PhD student (CL) with 16 months of MSK ultrasound and SWE experience. SWE was measured at both the deep fascia and the superficial muscle, as per image acquisition and ROI positioning protocols reported in Chapter 3 (Section 3.3.4). Similarly, data from SWE videos were processed and analysed as per Chapter 3. Post-exercise measurements were performed consistently in the order of: PAST BFlh, REL BFlh, REL RF, PAST RF for each intervention trial.

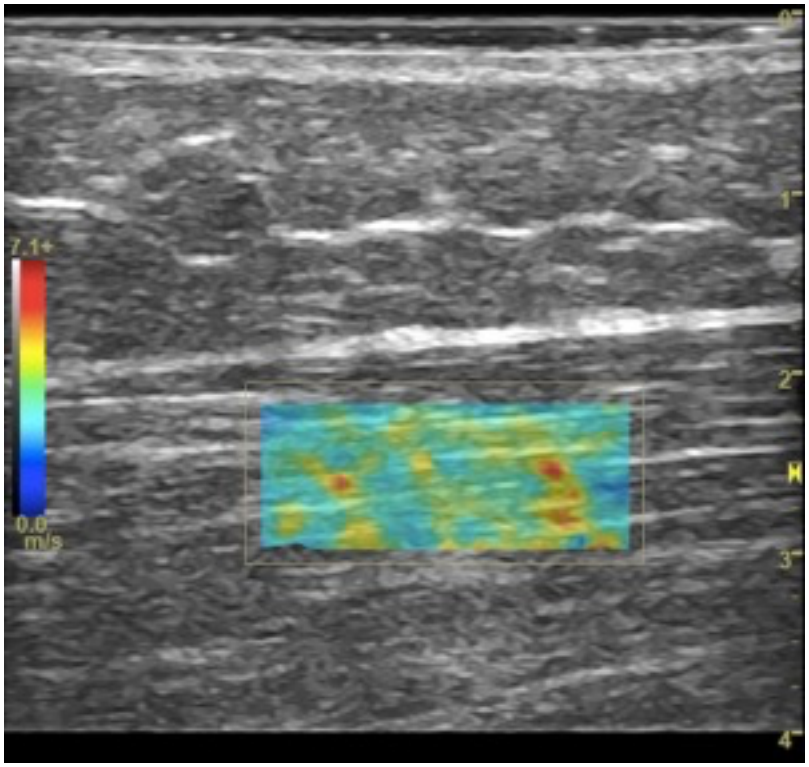

Figure 6.2. Example showing stiffness estimation of the biceps femoris long head muscle using shear wave elastography in the passively stretched condition.

6.3.6. Statistical analysis

All statistical analysis was conducted using IBM SPSS software (version 29). Intra- and inter-day repeatability of SWE measurements were assessed with two measurements taken in the familiarisation session, and comparing the mean of these two values with the measurements taken pre-exercise at each participant's first intervention trial, respectively. ICCs were calculated as a measure of relative reliability, with SEM calculated as a measure of absolute reliability. It is generally accepted that ICC values less than 0.50 indicate poor reliability, values between 0.50 and 0.75 are indicative of moderate reliability, values between 0.75 and 0.90 indicate good reliability and values above 0.90 represent excellent reliability (Koo and Li,

2016). As per Chapter 4, in this study we regard an SEM <5% of mean SWV as excellent reliability, 5-10% as good, 10-15% as moderate and ≥15% as poor. Independent samples T-tests were performed to compare the two exercise interventions in terms of HR (n=14) and RPE responses (n=15), and maximum running velocity (n=15). For this, data was used from the trials in which participants completed each intervention after at least 24h of leg exercise avoidance with data averaged from across the repetitions within the exercise intervention. 3-way (Time*Exercise*Order) repeated measures ANOVAs were performed to determine whether there was an effect of intervention order on each outcome. Since there was no effect of randomisation on any outcomes, 2 (exercise order) by 5 (time) 2-way repeated measures ANOVAs were performed to determine the cumulative effects of the exercise interventions on SWV, ROM and muscle soreness. Simple linear regressions with Pearson's correlations, with 95% confidence intervals (CIs), were performed to determine the relationships between the different measured variables (SWV, ROM, muscle soreness, RPE, HR and maximum running velocity). To determine the relationship between passive tissue stiffness and ROM, we used data from pre-exercise on the first experimental visit. All values reported in text, graphs and tables are mean ± SD unless otherwise specified. For all analyses, statistical significance was set at $\alpha < 0.05$.

6.4. Results

6.4.1. Reliability of the dependent variables

For this study, SWV measurements of the RF and BFlh deep fasciae and muscle reported good to excellent intra-day relative reliability, with ICC values ranging from 0.888-0.985. Absolute reliability was also good to excellent, with SEM values ranging from 2.79-8.38% of mean

SWV. Inter-day relative reliability was moderate to good, except for in the RF muscle which exhibited ICC values <0.500 in both relaxed and passively stretched conditions. Absolute reliability was poor to good with SEM values ranging from 6.66-17.72% of mean SWV. Measurements of RF ROM reported excellent relative and absolute reliability with ICC and SEM values as a percentage of mean SWV of 0.973 and 1.73% intra-day, and 0.938 and 2.66%, respectively. Measurements of BFlh ROM reported good to excellent relative and absolute reliability with ICC and SEM values as a percentage of mean SWV of 0.963 and 1.59% intra-day, and 0.856 and 3.16%, respectively. Full reliability values are reported in Appendix 3.

6.4.2. Exercise variables

Independent samples T-tests revealed no significant differences between the two exercise interventions in terms of mean LE-RPE (P = 0.563), B-RPE (P = 0.270), or HR (P = 0.777) (Table 6.1). Mean maximum velocity was 39.57% higher in sprint trials than in shuttle run trials (P < 0.001).

Exercise intervention	LE-RPE	B-RPE	Maximum velocity (m/s)	HR (bpm)
Sprints	12 ± 2	12 ± 2	7.09 ± 0.91	140 ± 17
Shuttle runs	13 ± 3	13 ± 2	5.08 ± 0.47***	142 ± 21

Table 6.1. Table of exercise variables with independent samples T-tests to compare the sprint and shuttle run interventions. LE-RPE, leg exertion rate of perceived exertion; B-RPE, breathlessness rate of perceived exertion; HR, heart rate; ***, P < .001.

There was no significant main effect of exercise on perceived muscle soreness, but there was a significant main effect of time on perceived muscle soreness (P < 0.001). Post hoc analysis

revealed that T2, T3, T4 and T5 were all significantly higher than T1 (all $P < 0.001$) (Figure 6.3). Perceived soreness is significantly higher at T4, after the second exercise intervention than beforehand at T3 ($P = 0.002$) or at T2 after the first exercise bout ($P = 0.005$). There was no significant interaction effect.

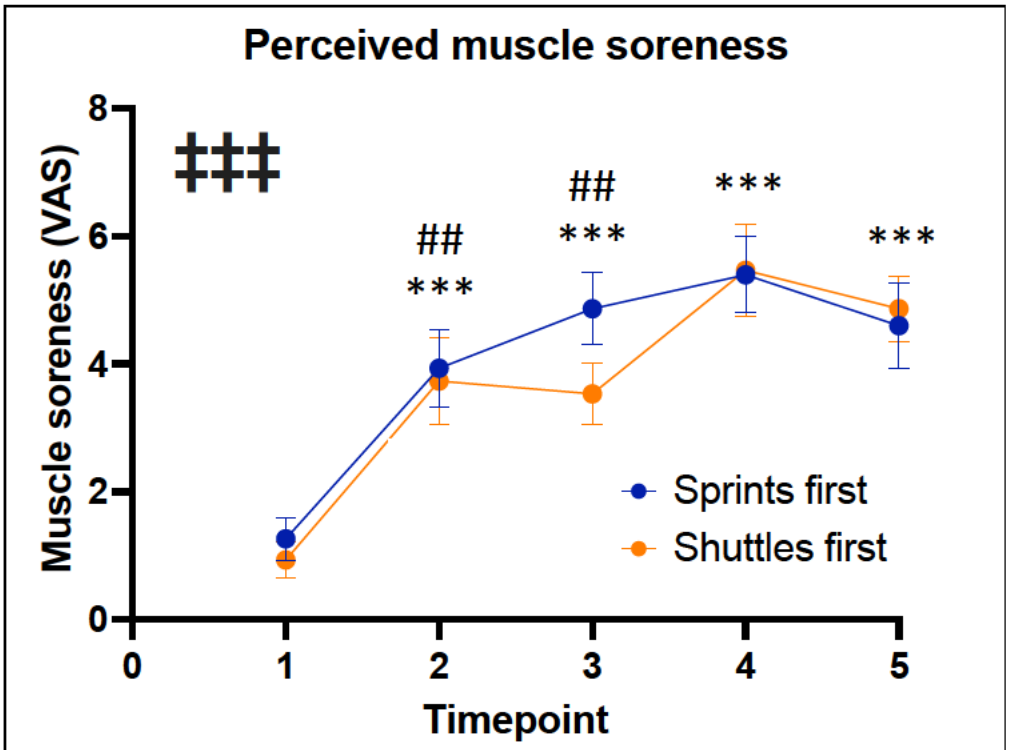

Figure 6.3. Graph displaying the changes in perceived muscle soreness over time. ‡, Significant main effect of time (‡‡‡ $P < 0.001$); *, significantly different to T1 (*** $P < 0.001$); #, significantly different to T4 (## $P < 0.01$); VAS, visual analogue scale.

6.4.3 Shear wave velocity

In PAST RF fascia, there was no significant effect of exercise on relative change in SWV, but there was a significant main effect of time on relative change in SWV ($P = 0.019$), showing SWV increased as a result of the exercise interventions, but post hoc tests show no significant

differences between individual timepoints (Figure 6.4C). There was no significant interaction effect. In PAST RF muscle, there was no significant effect of exercise on relative change in SWV, but there was a significant main effect of time on relative change in SWV ($P = 0.027$), but post hoc tests show no significant differences between individual timepoints (Figure 6.4D). There was no significant interaction effect. In PAST BFlh muscle, there was no significant effect of exercise on relative change in SWV, but there was a significant main effect of time on relative change in SWV ($P < 0.001$), with post hoc analysis showing that SWV was significantly higher at T2 and T5 than at T1 ($P < 0.001$ and $P = 0.017$, respectively). There was no significant interaction effect.

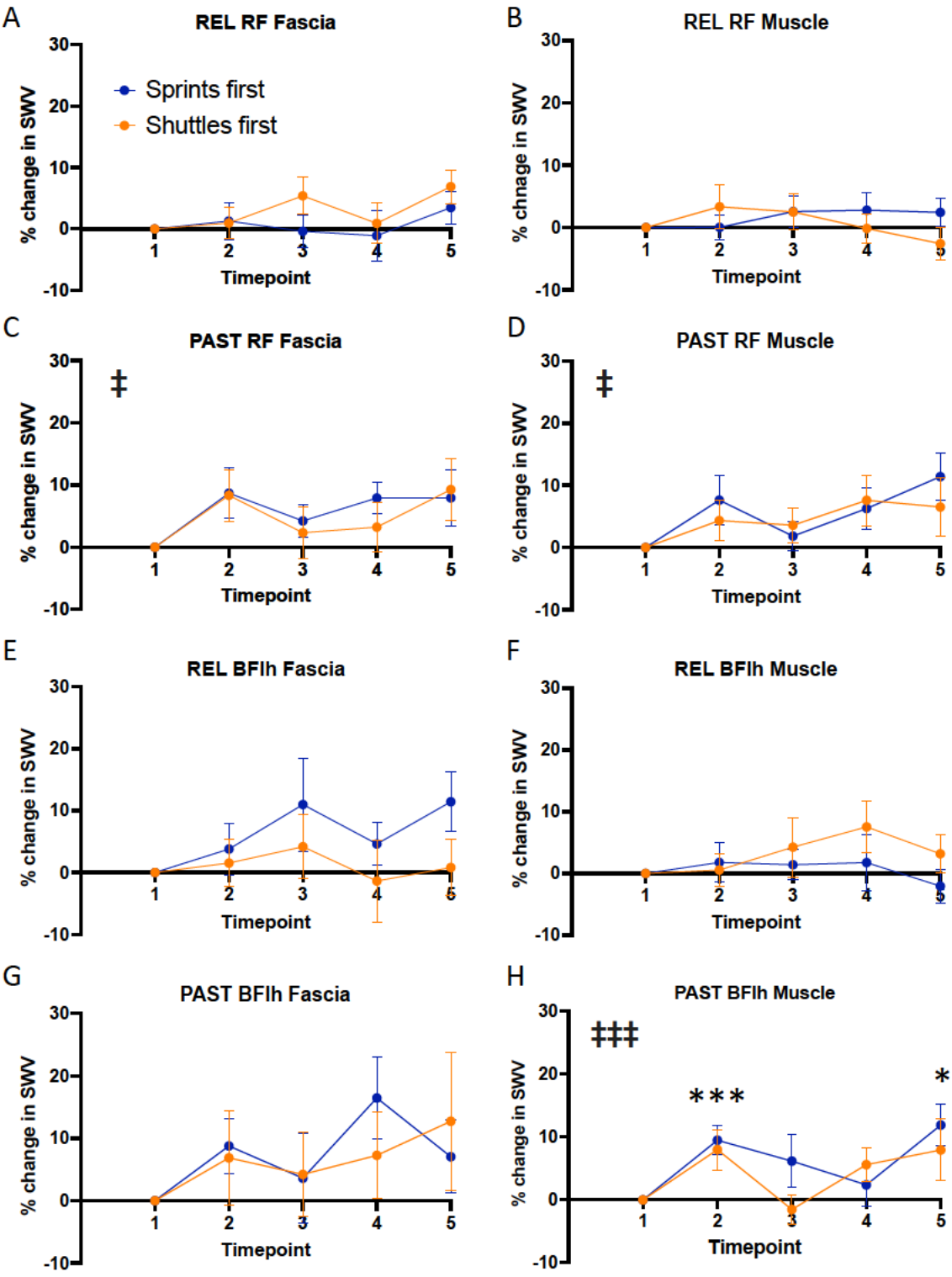

Figure 6.4. Relative changes in shear wave velocity over time from baseline.

‡, Significant main effect of time (‡ $P < 0.05$, ‡‡‡ $P < 0.001$); *, significantly different to T1 (* $P < 0.05$, *** $P < 0.001$); REL, relaxed condition; PAST, passively stretched condition; RF, rectus femoris; BFlh, biceps femoris long head.

There were no significant effects of time on fascia or muscle in REL conditions, or in the fascia in PAST BFlh ($P > 0.05$) (Figure 6.4).

6.4.4. Range of motion and perceived muscle soreness

There was a significant main effect of time on AKE ROM ($P = 0.013$), showing ROM decreased as a result of the exercise interventions, but post hoc tests show no significant differences between individual timepoints (Figure 6.5A). There was no significant main effect of time on TT ROM ($P = 0.070$) (Figure 6.5B).

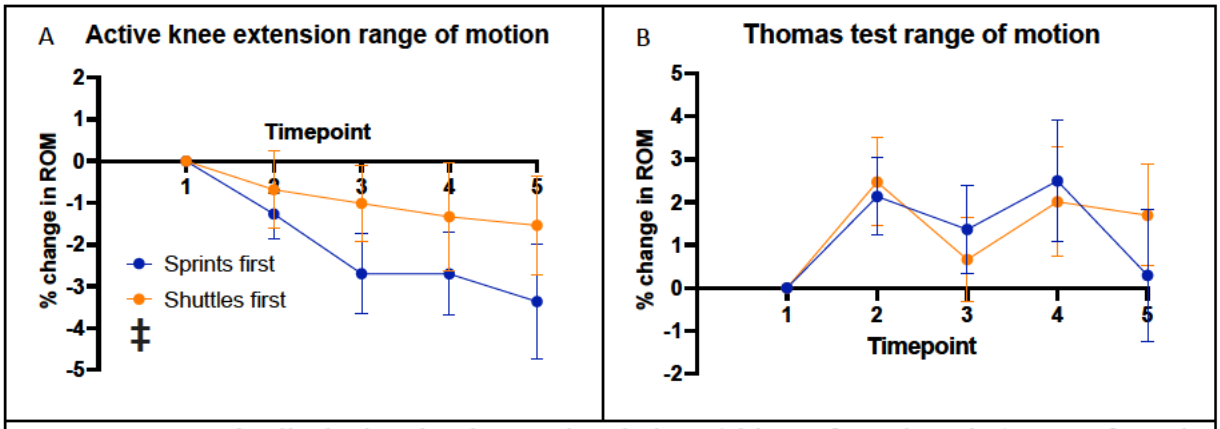

Figure 6.5. Graphs displaying the changes in relative A) biceps femoris and B) rectus femoris range of motion. ‡, Significant main effect of time (‡ $P < 0.05$); ROM, range of motion.

6.4.5. Relationship between stiffness, range of motion and perceived muscle soreness

There was a significant positive correlation between RF muscle SWV in REL and TT ROM ($R = 0.600$ (95% CIs 0.13, 0.85), $P = 0.009$, $R^2 = 0.360$), indicating a higher knee angle (lower flexibility) when SWV of the RF muscle was higher (Figure 6.6A). We observed a significant negative correlation between BFlh muscle SWV in PAST and AKE ROM ($R = -0.535$ (95% CIs -0.82, -0.03), $P = 0.020$, $R^2 = 0.286$), indicating a higher knee angle (greater flexibility) when SWV of the BF muscle was lower (Figure 6.6B). Moreover, a significant negative

correlation existed between the difference in BFlh muscle SWV in REL and PAST conditions and AKE ROM (R = -0.632 (95% CIs -0.87, -0.18), P = 0.006, R^2 = 0.399), indicating a higher knee angle (greater flexibility) when the difference in SWV of the BF muscle was lower between conditions (Figure 6.6C). There were no significant correlations between deep fascia SWV and ROM in either muscle or condition, or in the BFlh muscle in REL or RF muscle in PAST, or difference between REL and PAST in the RF (P > 0.05). We also found no significant relationships between deep fascia thickness and ROM.

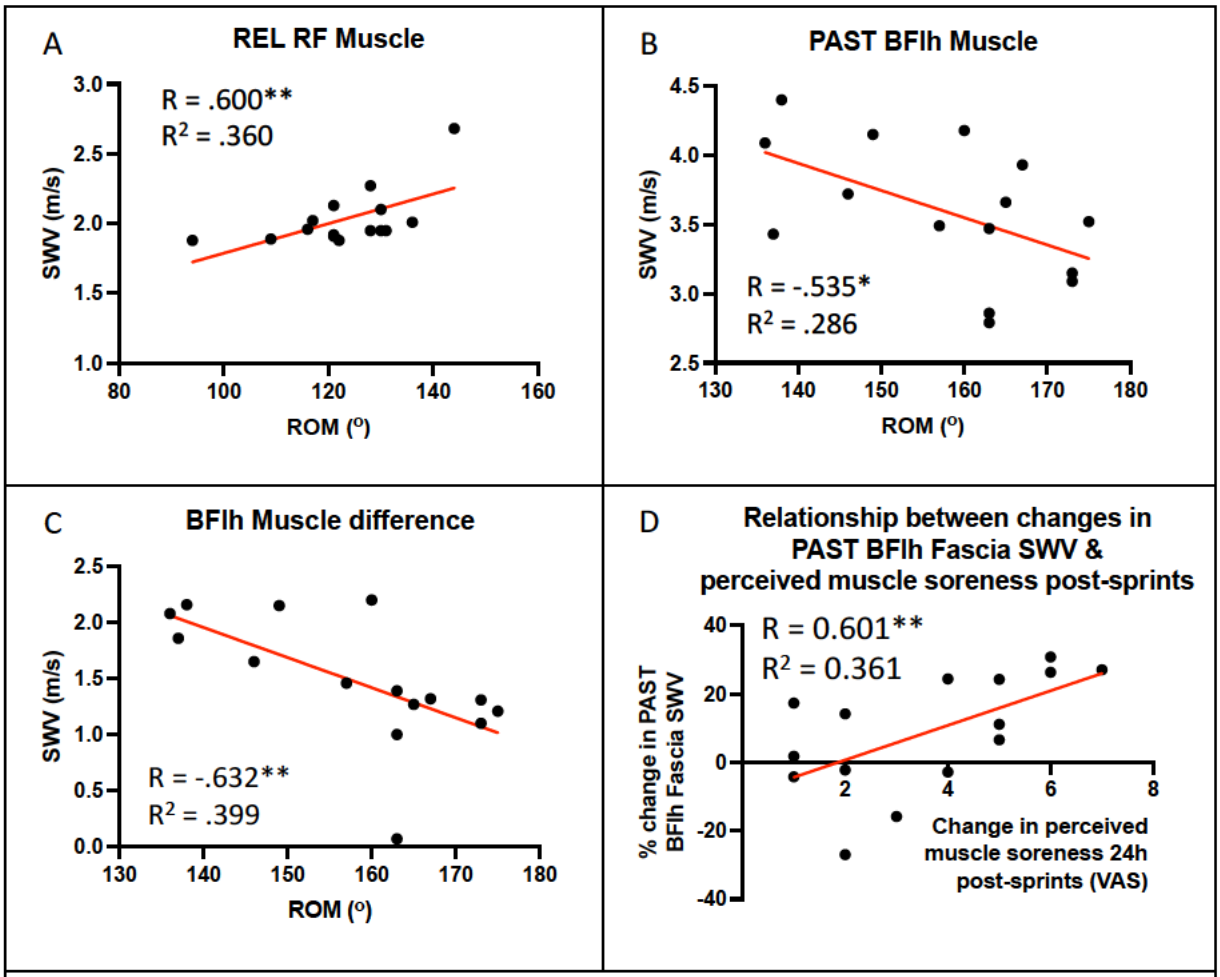

Figure 6.6. Relationships between muscle stiffness and range of motion in A) relaxed rectus femoris muscle, B) passively stretched biceps femoris muscle, and C) the difference between passively stretched and relaxed conditions in the biceps femoris. D) the relationship between % change in passively stretched biceps femoris deep fascia immediately post-sprints and change in perceived muscle soreness 24h post-sprints. *, significant Pearson's correlation (* $P < 0.05$, ** $P < 0.01$). REL, relaxed condition; PAST, passively stretched condition; RF, rectus femoris; BFlh, biceps femoris long head; SWV, shear wave velocity; VAS, visual analogue scale; ROM, range of motion.

We observed a significant positive correlation between the increase in perceived muscle soreness 24h post-sprints and % change in BFlh deep fascia SWV in the PAST condition 0h post-sprints ($R = 0.601$ (95% CIs 0.13, 0.85), $P = 0.009$, $R^2 = 0.361$), indicating that increases

in BFlh deep fascia stiffness are associated with higher levels of muscle soreness 24h post-exercise (Figure 6.6D).

6.5. Discussion

The aims of the study were to examine how real-world exercise interventions affect SWV of the RF and BFlh deep fasciae and muscles, ROM and perceived muscle soreness; to determine if there is a cumulative effect of conducting exercise bouts on consecutive days; to determine if there are differential effects of maximum velocity running and changes of direction on muscle mechanical properties; and to determine if there is a relationship between deep fascia and muscle stiffness and ROM or perceived muscle soreness.

The present study has three main findings: (1) RF and BFlh muscle stiffness increase following sprint and shuttle run interventions on consecutive days, (2) hamstring flexibility reduces after exercise, (3) perceived muscle soreness increases after exercise, is compounded by further exercise and remains above baseline 24h later. We found significant associations which may explain the mechanism behind these findings: (1) higher levels of passive muscle, but not deep fascia, stiffness are associated with reduced flexibility, and (2) sprint-induced increases in BFlh deep fascia stiffness are associated with increases in perceived muscle soreness 24h post-exercise. The implications of each of these findings will be discussed in the context of the existing literature.

6.5.1. Shear wave velocity

In REL conditions, we observed no significant changes in SWV at any timepoint in either the fascia or muscle tissue of the RF and BFlh. This aligns with the existing literature, with

Lacourpaille et al. (2017b) finding eccentric exercise-induced increases in stiffness of the knee extensors and elbow flexors at long, but not short muscle lengths. The authors propose that this is because there is a greater relative formation of actin-myosin cross-bridges at long muscle lengths post-exercise (Chalchat et al., 2023). This is due to the damage-induced Ca^{2+} perturbation and greater sensitivity of muscle fibres to Ca^{2+} when the muscle is beyond its slack length, thus increasing stiffness of elongated muscle tissue above pre-exercise values (Lacourpaille et al., 2017b, MacIntosh, 2010).

With regards to the deep fascia, we found a significant main effect of time on RF fascia stiffness in the PAST condition, but with no differences between specific time points. To our knowledge, this is the first study to explore the effects of exercise on the stiffness of deep fascial tissue, showing that, like muscle tissue, it may be subject to exercise-induced augmentation. Further research is required to recreate and validate these findings using more controlled laboratory-based exercise interventions, such has been performed extensively in muscle (discussed in 2.10).

When SWV of the RF muscle was estimated in the PAST condition, we found the exercise interventions to increase stiffness over time. Here, our results show a similar trend to those found by Morin et al. (2023). In this study, eccentric exercise bouts were completed on consecutive days. RF muscle stiffness increased after the first exercise bout, recovered to baseline 24h later and then increased to a similar extent after the second exercise bout (Morin et al., 2023). Our results show this same trend, but without statistical significance (Figure 6.4D). This is likely because sprint and shuttle run interventions induce less muscle damage than eccentric exercise protocols.

Our results show interesting findings in the BFlh muscle, building on the existing literature. Pimenta et al. (2023) and Freitas et al. (2023) both found significant increases in active BFlh muscle stiffness after 10 x 30m sprints, but these results were not replicated in measurements of passive stiffness. This is likely because both sets of authors estimated passive muscle stiffness at short muscle lengths, under which conditions SWE is not sensitive to exercise-induced increases in muscle stiffness (Lacourpaille et al., 2017b). To the best of our knowledge, this is first study to show that BFlh passive muscle stiffness increases immediately after sprint-based exercise. The presence of these findings in our study, but not those of Pimenta et al. (2023) and Freitas et al. (2023), are because we measured the muscle in an elongated condition (PAST), as we did not find the same at a shorter muscle length (REL). It cannot be ruled out, however, that this discrepancy is due to difference in total volume of our sprint interventions, as the present study involved sprint repetitions over a longer distance (40m). This means that participants were at maximum velocity for a longer amount of time, increasing eccentric load on the BFlh muscle (Bramah et al., 2024). This may explain why smaller post-exercise increases in BFlh muscle stiffness were seen after shuttle runs, as there is less stress placed on the muscle, with a lesser time under tension, but rather with greater loading of tendons due to storage and release of elastic energy upon changes of direction (Roberts and Konow, 2013, Wiesinger et al., 2017). Furthermore, these results support the notion that estimation of muscle stiffness using SWE should be performed with muscles beyond their slack length (Lacourpaille et al., 2017b).

6.5.2. Range of motion

With regards to ROM, we found trends of decreasing flexibility across the course of the intervention period in both muscles, with a significant main effect of time in the AKE for the

BFlh. These results are to be expected as exercise can induce acute reductions in ROM, particularly when accompanied by muscle damage (Peake et al., 2017).

One previous study has found a relationship between muscle stiffness and ROM (Reiner et al., 2024b). Here, Reiner and colleagues observed a significant negative correlation between BFlh muscle stiffness and hip flexion ROM in the sit-and-reach test, but no significant relationship between RF muscle stiffness and hip extension ROM. Our data supports these findings of a relationship between BFlh stiffness and hamstring flexibility. In the PAST condition, we measured SWV of the BFlh under the same conditions (hip and knee angles) as Reiner et al. (2024b) and found a significant negative correlation with AKE ROM. This provides further evidence for the relationship, since we used a different ROM test, focused on knee extension, not hip flexion as in the sit-and-reach test, highlighting the importance of stiffness of the biarticular BFlh muscle in ROM. Moreover, we found a significant negative relationship between AKE ROM and the difference between BFlh muscle SWV in PAST and REL. This is an interesting way to look at the data and aligns with understanding of biomechanical properties, as these two conditions involve absolute hip and knee angles. Therefore, the PAST condition represents a different relative ROM for each participant. For those participants where this angle represents a larger proportion of their maximum ROM, their BFlh muscle was experiencing higher levels of relative strain, and we would expect SWV to be faster. We did not find the same results in the RF muscle; however, this is likely because the PAST condition here was relative to each participant's ROM. Future research should utilise absolute joint angles to create the PAST condition in the RF (as was utilised in the previous experimental chapters) and look to recreate these findings from the BFlh. We also recognise that this novel method of evaluating muscle stiffness as a ratio of PAST:REL may provide greater insights into muscle

mechanical properties and ROM. Unlike Reiner et al. (2024b), the present study found a significant negative relationship between RF muscle stiffness in REL and ROM in the TT. This is likely a consequence of SWE scanning conditions, as Reiner et al. (2024b) had participants seated in an IKD with the knee fixed at 70º and hip fixed at 110º, opposite to our supine REL condition of knee and hip extension. It is plausible that SWV in REL in the present study was more closely associated with ROM as it replicates the hip extension of the TT.

6.5.3. Perceived muscle soreness

We observed expected trends of increased perceptions of muscle soreness over time, with all subsequent timepoints significantly greater than baseline. We also found an interesting significant positive relationship between % change in PAST BF deep fascia SWV immediately post-sprints and perceived muscle soreness 24h post-sprints. Similarly to Xu et al. (2019) we did not, however, find this relationship in muscle tissue. This novel finding may suggest a role for the mechanical properties of the deep fascia in the mechanistic underpinnings of DOMS. The mechanisms behind DOMS are not entirely understood and there is a debate within the literature that perturbed homeostasis within the deep fascia may be a contributing factor (Katayama et al., 2023, Fu et al., 2024). It is possible that receptors in the fascia are sensitive to exercise-induced changes in stiffness that those in the muscle tissue are not (Fede et al., 2021a, Suarez-Rodriguez et al., 2022). Therefore, increases in deep fascia stiffness may drive the sensation of DOMS in the days following exercise. This correlation, however, can of course not imply causation and further research is required to determine the physiological underpinnings of DOMS.

6.5.4. Limitations

As a function of this being an applied study with real-world exercise interventions, it had several limitations. Firstly, exercise interventions were completed outside on a multi-sport tarmac playing surface on the university campus. Consequently, post-exercise measurements were taken after walking back to the laboratory, which took an average of 7min43s ± 45.55s from exercise cessation to beginning post-exercise measurements. This means that both the temporal delay and cool down effect of the walk may have dampened or quashed acute changes in the dependent variables. Furthermore, this means that weather conditions were not controlled for as in the indoor sprint interventions conducted by Pimenta et al. (2023) and Freitas et al. (2023). Secondly, warm up was not standardised between participants, with different warm up protocols prior to sprinting shown to provide different levels of protection against muscle damage (Chen et al., 2018). Thirdly, it must be considered that these findings are specific to our participants and not generalisable to other populations, particularly athletes with higher fitness levels. Finally, due to time constraints, we collected no objective markers of fatigue, such as maximal voluntary isometric contraction torque, or muscle soreness, such as pain pressure threshold using an algometer.

6.6. Conclusions

Our findings suggest that conducting sprint and shuttle run exercise interventions on consecutive days leads to an immediate increase in BFlh muscle stiffness after the first exercise intervention, which is sustained at follow up 24h after the exercise intervention on the following day. This has practical implications for strength and conditioning coaches in planning the weekly training microcycle. Furthermore, our results suggest that stretching interventions aimed at reducing muscle stiffness should be performed regularly as part of recovery from

repetitive exercise bouts to reduce stiffness and increase ROM to reduce risk of injury (Ichihashi et al., 2016, Umegaki et al., 2015). Furthermore, interventions aimed at reducing stiffness of the deep fascia, such as local high frequency percussive massage investigated by Liu et al. (2024), may be important in alleviating DOMS.

CHAPTER 7

General Discussion

7.1. Overview of findings

Since the gold standard measure of tissue stiffness involves invasive tensile testing, other techniques were developed as proxy measures to estimate tissue biomechanical properties, including SWE and the MyotonPRO. The aim of this thesis was to investigate the use of these techniques to non-invasively estimate MSK tissue stiffness, its response to exercise, and to determine the efficacy of doing so in an elite sport setting. The following bullet points summarise the findings of this thesis with specific reference to the thesis objectives.

1. To assess the validity and comparability of the MyotonPRO to SWE as a method of estimating muscle tissue stiffness (Chapter 3).
    a) The MyotonPRO is not a comparable method to SWE in estimating muscle stiffness, because the mechanical transmission involved in its mechanism of measurement is dampened by subcutaneous adipose tissue.
2. To assess the reliability of SWE in assessing skin, deep fascia and muscle tissue stiffness (Chapter 4).
    a) SWE is a reliable method of estimating stiffness of the skin and deep fascia, as well as superficial and deep regions of muscle tissue.
    b) SWE is most reliable at assessing superficial tissue which is under strain.
3. To determine the effects of tissue type, measurement depth, tissue region, and muscle length on stiffness estimated by SWE (Chapter 5).
    a) In the RF, SWV is higher in muscle tissue than in the deep fascia.
    b) Regional differences in stiffness of biarticular RF MSK tissue are altered by local strain preferentially increasing stiffness of the adjacent region.

4. To determine the impact of real-world exercise training interventions on lower limb muscle stiffness, and the associations it has with exercise-related variables (Chapter 6).
    a) Sprint-based exercise interventions increase BFlh muscle stiffness, with a cumulative effect of completing further exercise on the following day, sustaining elevations in muscle stiffness 24h later.
    b) Elevated levels of muscle stiffness impede joint ROM and flexibility.

These findings suggest that SWE, but not the MyotonPRO, is an effective tool for estimating stiffness and monitoring acute and chronic longitudinal changes in MSK tissue stiffness caused by exercise, injury and disease.

7.2. Implications for use of the MyotonPRO to estimate musculoskeletal tissue stiffness

Results from Chapter 3 indicate that the MyotonPRO is not comparable to SWE in the assessment of RF muscle tissue stiffness, particularly in the supine position. This detail is particularly relevant, since the user manual recommends using the MyotonPRO with participants supine to quantify quadriceps muscle mechanical properties (Myoton®). However, when stretching the muscle with knee flexion with the aim of optimising agreement between the two devices, we found only weak positive correlations between estimations of muscle stiffness by SWE and the MyotonPRO, with stronger relationships observed at more superficial non-muscle tissue. Similar to findings by Bravo-Sánchez et al. (2021) in the VL muscle and Mencel et al. (2021) in the RF, we found significant negative correlations between SAT and stiffness estimated by the MyotonPRO. Combined, these results indicate that the MyotonPRO is not a valid alternative to SWE in the assessment of RF muscle tissue stiffness. Accordingly, the subsequent experimental chapters utilised only SWE to estimate MSK tissue stiffness.

Placing our findings in the context of the existing literature, other studies have also found positive correlations between stiffness estimations by SWE and the MyotonPRO, alternatively concluding that these make the MyotonPRO a viable surrogate to SWE (Feng et al., 2018, Kelly et al., 2018, Lee et al., 2021). These studies report Pearson's correlation coefficients culminating in a total of 1 weak positive correlation, 11 moderate positive correlations and just 1 strong positive correlation between stiffness estimated by SWE and the MyotonPRO (Table 7.1). When validating SWE against passive muscle force in tensile testing, however, researchers used the far more conservative regression coefficient of determination (R^2) value to assess the relationship, finding R^2 values above 0.81 (with the vast majority above 0.90) in multiple different regions of human RF, BFlh, ST and SM, and six swine muscles (Kodesho et al., 2021b, Nakao et al., 2023, Liu et al., 2019). The MyotonPRO has therefore not being scrutinised and held to the same rigorous standards as SWE with regards to its comparison to existing techniques before being deemed valid. Strong relationships, not just statistically significant ones, are needed to claim that the MyotonPRO is a valid tool for estimating muscle stiffness. Significant correlations observed by Kelly et al. (2018), including the only strong positive correlation, were found when combining data at rest and during two different contraction intensities. Both SWE and the MyotonPRO reported greater muscle stiffness during contraction and increasing with contraction intensity. As discussed in Chapter 3, however, this does not mean that the MyotonPRO is estimating muscle stiffness as SWE is, but may be measuring muscle hardness instead. Both muscle stiffness and hardness increase during contraction and with contraction intensity (Yoshitake et al., 2014, Inami et al., 2017). Perhaps SWE was estimating the increases in muscle stiffness in response to contraction, and the MyotonPRO was estimating the increases in muscle hardness in response to contraction, resulting in a positive

correlation between the two methods, despite measuring two distinct mechanical properties (Kelly et al., 2018). These correlations should therefore not be considered as representative of the relationship between SWE and the MyotonPRO in estimating muscle tissue stiffness.

Paper	**Muscle**	**Condition**	**Pearson correlation (R value)**
Feng et al. (2018)	Medial gastrocnemius	Passive	0.46*
	Lateral gastrocnemius		0.54*
Kelly et al. (2018)	Infraspinatus	Passive & active combined (0%, 40% & 80% MVIC)	0.35*
	Erector spinae		0.51*
	Medial gastrocnemius		0.71*
Lee et al. (2021)	Rectus femoris	Passive	0.42**
		Active	0.40*
	Tibialis anterior	Passive	0.56**
		Active	0.54**
	Biceps femoris	Passive	0.65**
		Active	0.59**
	Medial gastrocnemius	Passive	0.67**
		Active	0.55*

Table 7.1. Table showing the relationships found between shear wave elastography and the MyotonPRO in a variety of different muscles. * P<0.05, ** P<0.01.

7.2.1. Practical recommendations for use of the MyotonPRO

It must be considered, however, that there is still a real desire to use the MyotonPRO among researchers and practitioners. This is because, if the MyotonPRO is able to accurately estimate muscle tissue mechanical properties, it has the potential to revolutionise the measurement of biomechanical properties in applied practice and research settings due to its ease of use. Moreover, our results draw conflicting conclusions to other studies in the literature which have

observed positive correlations between stiffness estimations by the two devices, deeming the MyotonPRO to be a valid alternative to SWE (Feng et al., 2018, Kelly et al., 2018, Lee et al., 2021). In addition, Kurashina et al. (2023) found a strong significant positive correlation between stiffness of four different gel phantoms measured by SWE and the MyotonPRO. Subsequently, we understand that researchers and practitioners will continue to utilise the MyotonPRO to estimate muscle stiffness. We therefore make the following recommendations based on our findings for best practice to optimise agreement with SWE: measurements should be performed (1) on superficial muscles, (2) in lean populations with minimal overlying SAT, and (3) with the muscle stretched beyond its slack length.

7.2.1. Future directions for research into the efficacy of the MyotonPRO

To more conclusively determine the efficacy of the MyotonPRO as an alternative method to SWE in estimating MSK tissue stiffness, further research must be conducted.

1. Our findings should be scrutinised in other muscle groups to determine if they are representative of solely the RF, or if they apply more broadly throughout the body.
2. Studies comparing SWE and the MyotonPRO should be conducted in different measurement locations and conditions to those currently recommended by the company's user manual to establish if there are protocols which elicit more comparable results between the two devices. This is because we found the strongest relationship between the two devices' estimations of muscle stiffness distally with the muscle locally stretched in NEU and PAST conditions, not medially with participants supine, as recommended. These results indicate that best practice for estimation of muscle stiffness with the MyotonPRO may need further research and modification.

3. Since these findings may also be a consequence of distal RF regions having significantly less overlying SAT than proximal and medial regions (Figure 3.6), research should be performed on lean participants and compared to data from participants with greater SAT to test our hypothesis that the devices show better agreement with reduced overlying SAT.
4. Mechanistic studies should be performed utilising gel phantoms of known stiffness to replicate physiological conditions, similar to those used by Bartsch et al. (2023). These models are made with less stiff phantoms (representing SAT) of different thicknesses placed on top of a stiffer phantom (representing muscle) to examine how this affects stiffness estimation of the target 'muscle' phantom by the MyotonPRO (Kurashina et al., 2023). Conclusions from this research may also allow for calculation of mathematical corrections to account for SAT, enabling more accurate interpretation of data provided by the MyotonPRO.
5. Since we also propose that the MyotonPRO may be estimating muscle hardness, as opposed to muscle stiffness, research should be conducted to determine the relationship between measurements recorded by the MyotonPRO and a durometer in muscle and in gel phantom models.
6. The MyotonPRO also reports measurements of muscle tone and viscoelastic properties (Myoton®). It is important to distinguish between these distinct properties and emphasise that our findings are only representative of the 'dynamic stiffness' measurement provided by the MyotonPRO. Future research should also focus on validating the MyotonPRO in the assessment of these other properties against their own gold standard measures.

7.3. Implications for the use of shear wave elastography to estimate musculoskeletal tissue stiffness

Results in Chapters 4-6 show that SWE is a reliable method of estimating the stiffness of MSK tissue; is sensitive to differences between tissues and regions; and can be utilised effectively in real-world settings to quantify changes in muscle stiffness following exercise. SWE was also able to provide novel insight into how localised strain influences regional differences in biarticular muscle stiffness, and the influence of stiffness on muscle flexibility.

Chapter 4 contributed to the methodological development of SWE measurement protocols by comparing the influence of different conditions and measurement depths on the repeatability of MSK SWV. The novelty of this chapter lies in the reliability assessments of different muscle depths, as well as the skin and fascial tissue overlying the RF muscle, opening new avenues of research in exploring the biomechanical properties of these tissues. This research will have implications in surgical skin grafts and dermal ageing, as well as enhance research into the deep fascia with regards to muscle pain perception, force transmission, and injury mechanism and susceptibility (Katayama et al., 2023, Fu et al., 2024, Stecco et al., 2011, Wilke et al., 2019, Kawai et al., 2021a). This evidence showing repeatability of SWV measurement in deeper muscle tissue is important in allowing advances in research into force transmission from muscle to the deep aponeurosis and monitoring rehabilitation from injury to deep muscle tissue (Slane et al., 2017, Yagiz et al., 2024). The ROI depths used for the estimation of deep muscle tissue stiffness varied between participants based on overlying subcutaneous adipose and fibrous tissue thickness and muscle thickness, but varied from 2.0-4.5cm. Reporting of repeatable SWV measurements up to a depth of 4.5cm increases upon recommendations by Wang et al. (2020), who reported measurement depths of <3cm as the ideal. In addition, this chapter proposes cut-

off points for the categorisation of absolute reliability, with bias and SEM presented as a percentage of mean SWV. Wider adoption of these descriptors of absolute reliability within future MSK SWE literature would enable research to be more easily compared and critiqued, as well as provide a better understanding of the verity of reported differences in SWV between populations and after interventions such as exercise.

Chapter 5 contributed to the understanding of regional biomechanical properties of biarticular muscles, more specifically of the RF muscle and its overlying fascia. The findings are interesting in that they, for the first time, show that RF muscle is stiffer than its overlying fascia, and that both tissues respond locally to strain at either end. This means that hip extension increases SWV of proximal RF muscle and fascia, and knee flexion increases SWV of distal RF muscle and fascia. The results also support the existing literature reporting higher muscle stiffness when the muscle is stretched rather than shortened (Le Sant et al., 2015, Itsuda et al., 2024). The finding of differences between tissues is helpful in contributing to the development of normative values for the deep fascia and superficial and deep muscle tissue regions, however, research is required in larger samples to confirm these findings and truly establish normative values in healthy participants. This is useful, such that in the future it may be possible to quantitatively categorise MSK tissue stiffness. This is important as it may be useful in quantifying muscle damage or risk of injury, similar to the utilisation of SWE to quantitatively diagnose liver cirrhosis severity (Suffredini et al., 2024). These findings also have implications in injury mechanisms and prevention (Mendiguchia et al., 2024). With regards to injury mechanism, these findings may explain that high levels of hip extension are more likely to cause RF injury proximally, because the muscle and fascial tissue is stiffer and therefore closer to its maximum levels of stress and strain before rupture (Kelc et al., 2013, Kalkhoven et al., 2020).

In terms of injury prevention, our results suggest that it may be relevant to prioritise stretching interventions which preferentially target at risk muscle regions based on sport-specific postures and joint positioning (Nakao et al., 2018). For example, sprinters may prioritise stretching the hip flexors and proximal RF, as RF injury is common during the swing phase of gait, where muscle contraction occurs whilst the proximal region is under high levels of strain due to hip extension (Bogwasi et al., 2023). Furthermore, alterations in regional stiffness based on limb positioning may enable the RF muscle to generate force appropriately in line with its dual functions of hip flexion and knee extension. It has already been shown that RF motor units work independently to produce force, with proximal regions more active during hip flexion (Watanabe et al., 2021). Further research needs to be carried out to examine if there is a relationship between or mechanistic advantage of these regional neuromuscular and biomechanical properties of the RF muscle.

Chapter 6 built on the MSK SWE exercise literature by using real-world exercise interventions to compare to the well-established laboratory-based studies. As discussed in detail in Section 2.10, previous literature has shown resistance-based exercise to increase passive muscle stiffness, endurance exercise to reduce passive muscle stiffness, and, albeit with limited research, sprint training to increase active but not passive muscle stiffness (Lacourpaille et al., 2017b, Andonian et al., 2016, Pimenta et al., 2023). In addition, one study examined the effects of a laboratory-based eccentric exercise intervention completed on consecutive days, finding no cumulative effect of exercise, but instead sufficient recovery to baseline after 24h, followed by an increase in stiffness after the second bout comparable to that of the first, with no 24h follow up (Morin et al., 2023).

The findings from our study suggest that sprint-based exercise interventions completed on consecutive days, specifically sprints and shuttle runs, do induce cumulative effects on muscle mechanical properties. Elevated levels of BFlh muscle SWV were observed 24h after the second exercise bout, which were not seen 24h after the first bout. This is the first study to show that exercise on consecutive days culminates in a delayed increase in muscle stiffness. These findings have implications in recovery processes performed as part of the weekly microcycle in elite sport, indicating that strategies aimed at reducing muscle stiffness (discussed in Section 2.13) should be utilised regularly.

Findings in Chapter 6 also indicate that higher levels of muscle stiffness inhibit flexibility. We observed a significant positive correlation between RF muscle stiffness and TT ROM, and a significant negative correlation between BFlh muscle stiffness and AKE ROM, i.e., higher levels of muscle stiffness are associated with reduced flexibility in both muscles. These findings align with our understanding of muscle mechanics, since stiffer tissue is more resistant to deformation and elongation in response to a stretching tension stress. These results support the limited existing literature, with the use of the AKE test in the assessment of the BFlh and the findings in the RF adding novel insight (Reiner et al., 2024b). This implies that practitioners in the elite sport setting can consider utilising SWV measurements as a proxy for ROM tests in some instances. This applies if they are already prioritising time with athletes to take SWE measurements and do not also have time for ROM assessments. This is important in this setting due limited access time to athletes and the overwhelm caused by taking excessive assessments (Sands et al., 2019). Furthermore, these findings imply that reduced muscle stiffness following a stretching intervention is the likely mechanism behind the observed increases in ROM (Nakao et al., 2018, Akagi and Takahashi, 2014). This supports the previous recommendation of recovery strategies, such as stretching interventions, to reduce muscle stiffness and injury risk,

and enhance recovery, particularly during periods of high training volume and fixture congestion which cause muscle stiffness to accumulate.

7.3.1. Practical recommendations for using shear wave elastography

It has been established by our findings in Chapter 4 that SWE can be utilised in research settings to advance the knowledge of skin and deep fascia mechanical properties, and in deep as well as superficial muscle regions. Although, superficial regions are still preferable when assessing muscle stiffness with SWE unless specifically investigating deep muscle properties, as they are more reliable. We recommend that researchers and practitioners calculate and report the absolute reliability of their measurements to understand if their results are within the device's minimal detectable change and establish if findings are of clinical significance.

Results in Chapter 5 indicate that researchers and practitioners should be aware that SWV values will differ regionally based on participant limb positioning. This is important to consider when designing methodological protocols and when comparing findings from studies which use different SWE measurement protocols. This heterogeneity in study design and methodological reporting has impeded advances in the field of MSK SWE (Cipriano et al., 2022).

7.3.2. Future directions and applications for shear wave elastography

Based on the current state of the literature and following on from our findings, several gaps remain within the literature. These research questions and proposed directions for future research and application of SWE are as follows:

1. For MSK SWE to be successfully utilised within clinical and athletic populations, normative values for all populations must be established in different muscle groups and in both sexes. This may enable quantitative categorisation of MSK tissue stiffness for diagnosis of disease and identification of meaningful fluctuations in muscle stiffness in response to exercise, as has been implemented in the liver (Suffredini et al., 2024). This would facilitate the use of SWE in longitudinal monitoring of disease progression and symptom severity in clinical populations and of injury risk and recovery status in athletes.
2. Future research should address our results in Chapter 5 (regional differences in RF stiffness), and investigate if these findings are consistent among other biarticular muscles, or are solely characteristic of the RF muscle and fascia.
3. With regards to MSK SWE research in the field of sport and exercise science, more applied, real-world research is needed to build upon the well-established impacts of laboratory-based interventions and determine and compare how real-world training interventions impact muscle stiffness.
4. Building on this, more research should be conducted to determine the efficacy of different recovery strategies in reducing exercise-induced increases in muscle stiffness.
5. There is a need to better understand the relationship between exercise-induced increases in muscle stiffness and muscle damage (Lacourpaille et al., 2017b). To do this, studies should utilise eccentric exercise protocols and determine the association between increases in muscle stiffness and Z-band streaming from muscle biopsies.
6. Further research should also be performed in clinical populations to test the effectiveness of treatments on reducing elevated levels of symptom-derived MSK tissue

stiffness, as has been conducted in patients with Parkinson's disease utilising deep brain stimulation and dopamine precursor medication.

7.4. Summary

This thesis demonstrates that the MyotonPRO is not a comparable alternative to SWE in the measurement of muscle stiffness; SWE is a reliable technique in MSK tissue; the RF is regionally affected by local strain; and real-world maximal running-based exercise interventions induce cumulative augmentation of muscle stiffness in the RF and BFlh, with sprint interventions acutely increasing passive BFlh muscle stiffness. In conclusion, SWE is a reliable method of estimating muscle stiffness which can be utilised in research settings to gain a deeper understanding of MSK tissue biomechanics; clinically to diagnose, monitor progression of, and assess treatment effectiveness in diseased populations such as cerebral palsy; and in athletic and rehabilitative settings to evaluate injury risk and recovery status from exercise.

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Appendix 1

Measurement location			Pearson correlation		Simple linear regression		
Condition	Region	Depth	R value	P value	R^2 value	Lower bound	Upper bound
Relaxed	Proximal	Skin	0.183	0.226	0.034	-24.244	52.046
		Fascia	0.347	0.073	0.120	-8.714	54.243
		Superficial muscle	0.474	**0.020**	0.225	1.980	78.065
		Deep muscle	0.169	0.245	0.029	-30.471	61.152
	Medial	Skin	-0.050	0.418	0.002	-38.633	31.573
		Fascia	0.091	0.351	0.008	-34.584	50.323
		Superficial muscle	0.061	0.399	0.004	-48.755	62.550
		Deep muscle	0.097	0.342	0.009	-55.032	81.988
	Distal	Skin	0.009	0.485	0.000	-86.183	89.403
		Fascia	0.290	0.107	0.084	-37.859	157.635
		Superficial muscle	-0.022	0.463	0.000	-144.615	132.365
		Deep muscle	0.104	0.332	0.011	-97.821	149.984
	Pooled	Skin	-0.023	0.430	0.001	-29.044	24.351
		Fascia	-0.214	0.052	0.046	-29.314	2.776
		Superficial muscle	-0.182	0.084	0.033	-38.450	6.888
		Deep muscle	-0.267	**0.020**	0.071	-48.049	-1.055
Neutral	Proximal	Skin	-0.321	0.090	0.103	-105.955	21.585
		Fascia	0.060	0.403	0.004	-30.700	38.919
		Superficial muscle	0.064	0.397	0.004	-47.321	60.928
		Deep muscle	0.317	0.093	0.101	-11.694	55.952
	Medial	Skin	0.300	0.099	0.090	-18.502	82.853

		Fascia	0.301	0.099	0.090	-19.194	86.449
		Superficial muscle	0.240	0.154	0.058	-27.927	83.838
		Deep muscle	0.364	0.057	0.133	-8.588	73.013
	Distal	Skin	0.139	0.280	0.019	-34.032	60.937
		Fascia	0.713	**<.001**	0.508	54.048	156.799
		Superficial muscle	0.564	**0.005**	0.318	30.145	189.003
		Deep muscle	0.510	**0.011**	0.260	14.118	157.116
	Pooled	Skin	0.662	**<.001**	0.438	62.955	117.016
		Fascia	0.262	**0.023**	0.069	1.170	105.190
		Superficial muscle	0.236	**0.036**	0.055	-5.669	125.857
		Deep muscle	0.315	**0.008**	0.099	12.333	110.156
Passively stretched	Proximal	Skin	0.243	0.158	0.059	-33.309	97.352
		Fascia	0.176	0.235	0.031	-24.814	51.511
		Superficial muscle	0.309	0.099	0.095	-17.295	77.353
		Deep muscle	0.555	**0.007**	0.308	11.626	87.839
	Medial	Skin	0.252	0.142	0.063	-22.334	71.721
		Fascia	0.232	0.162	0.054	-27.962	79.986
		Superficial muscle	0.318	0.086	0.101	-19.545	101.961
		Deep muscle	0.623	**0.002**	0.388	21.327	91.507
	Distal	Skin	0.423	**0.032**	0.179	-2.796	94.373
		Fascia	0.774	**<.001**	0.600	37.227	87.825
		Superficial muscle	0.640	**0.001**	0.410	26.882	105.493
		Deep muscle	0.695	**<.001**	0.482	27.270	84.696
	Pooled	Skin	0.600	**<.001**	0.359	45.804	96.023

		Fascia	0.196	0.069	0.038	-7.271	51.419
		Superficial muscle	0.261	**0.023**	0.068	0.702	74.280
		Deep muscle	0.454	**<.001**	0.206	25.096	79.722

Results from Pearson's correlations, and simple linear regressions between shear wave velocity and dynamic stiffness, measured by the MyotonPRO, in relaxed, neutral and passively stretched conditions; proximal, medial and distal, and pooled, regions; and skin, fascia, superficial muscle and deep muscle (Chapter 3).

Appendix 2

	Relaxed	Neutral	Passively stretched
Proximal	213.65 ± 38.43	153.33 ± 27.66	205.22 ± 50.78
Medial	246.46 ± 26.51	255.01 ± 38.81	273.97 ± 44.39
Distal	253.70 ± 39.87	313.60 ± 52.23	312.40 ± 52.76

Mean ± SD of dynamic stiffness (N/m) estimated by the MyotonPRO in the three measurement locations (proximal, medial, distal) under the three conditions to manipulate muscle length (relaxed, neutral, passively stretched), showing the sensitivity of the MyotonPRO to changes in muscle length.

Appendix 3

Intra-day reliability:

Measurement		Mean values		Relative reliability	Absolute reliability	
Shear wave elastography		Shear wave velocity (m/s)				
Muscle Condition	Tissue	Time 1	Time 2	ICC (lower bound - upper bound)	SEM (m/s)	SEM as a % of mean SWV
Rectus femoris Relaxed	Fascia	1.92	1.90	0.941 (0.838-0.980)	0.063	3.29
	Muscle	2.08	2.09	0.919 (0.777-0.972)	0.063	3.03
Rectus femoris Passively stretched	Fascia	3.35	3.39	0.985 (0.957-0.995)	0.105	3.12
	Muscle	3.45	3.52	0.954 (0.870-0.984)	0.122	3.50
Biceps femoris Relaxed	Fascia	2.23	2.13	0.963 (0.876-0.988)	0.130	5.96
	Muscle	2.19	2.20	0.918 (0.775-0.972)	0.071	3.23
Biceps femoris Passively stretched	Fascia	2.71	2.73	0.888 (0.699-0.961)	0.228	8.38
	Muscle	3.61	3.55	0.973 (0.921-0.991)	0.100	2.79
Range of motion		Range of motion (º)		Relative reliability	Absolute reliability	
Test		Time 1	Time 2	ICC (lower bound - upper bound)	SEM (º)	SEM as a % of mean ROM
Modified Thomas test		123	124	0.973 (0.918-0.991)	2.133	1.73
Active knee extension		156	156	0.963 (0.896-0.987)	2.481	1.59

Intra-day reliability statistics for the assessment of rectus femoris and biceps femoris long head fascia and superficial muscle tissue in relaxed and passively stretched conditions (Chapter 6). ICC, intra-class correlation coefficient (single measures); SEM, standard error of the measurement; bias is calculated using the Bland-Altman test.

Inter-day reliability:

Measurement		Mean values		Relative reliability	Absolute reliability	
Shear wave elastography		Shear wave velocity (m/s)				
Muscle Condition	Tissue	Day 1	Day 2	ICC (lower bound - upper bound)	SEM (m/s)	SEM as a % of mean SWV
Rectus femoris Relaxed	Fascia	1.91	1.86	0.733 (0.384-0.901)	0.145	7.69
	Muscle	2.08	2.03	0.327 (-0.208-0.710)	0.176	8.55
Rectus femoris Passively stretched	Fascia	3.37	3.31	0.556 (0.065-0.827)	0.526	15.76
	Muscle	3.48	3.38	0.385 (-0.146-0.742)	0.397	11.56
Biceps femoris Relaxed	Fascia	2.18	2.07	0.713 (0.341-0.893)	0.377	17.72
	Muscle	2.20	2.11	0.668 (0.276-0.873)	0.158	7.33
Biceps femoris Passively stretched	Fascia	2.72	2.83	0.675 (0.270-0.878)	0.437	15.75
	Muscle	3.58	3.60	0.829 (0.561-0.939)	0.239	6.66
Range of motion		Range of motion (º)		Relative reliability	Absolute reliability	
Test		Day 1	Day 2	ICC (lower bound - upper bound)	SEM (º)	SEM as a % of mean ROM
Modified Thomas test		123	123	0.938 (0.827-0.979)	3.282	2.66
Active knee extension		156	158	0.856 (0.630-0.949)	4.956	3.16

Inter-day reliability statistics for the assessment of rectus femoris and biceps femoris long head fascia and superficial muscle tissue in relaxed and passively stretched conditions (Chapter 6). ICC, intra-class correlation coefficient (single measures); SEM, standard error of the measurement; bias is calculated using the Bland-Altman test.

Appendix 4 - Study 1 participant information sheet

UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: Investigating the intra- and inter- day reliability of shear wave elastography and myotonometry for quantifying muscle tissue mechanical properties

Investigators:
Cameron Ley; *Tel*: [redacted], *Email*: [redacted]
Dr Barry Drust; *Email:* [redacted]
Dr Eduardo Martinez Valdes; *Email:* [redacted]

An invitation to take part:
Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

Technical terminology:

Shear Wave Elastography (SWE) – an ultrasound-based method of estimating muscle tissue stiffness

MyotonPRO – a handheld device used to estimate muscle stiffness via non-invasive palpation of the muscle

Isokinetic Dynamometer (IKD) – a chair-like machine used to set joint angles

Electromyography (EMG) – a non-invasive method used to quantify muscle electrical activity

Total Time commitment:
The total time commitment for this study is 4 hours.
The first visit will take up to 2 hours and the following 2 visits will last up to an hour.

What is the purpose of the study?
Shear wave elastography and MyotonPRO measurements provide an insight into muscle mechanical properties, more specifically, muscle stiffness. The purpose of this study is to understand the intra- and inter-day reliability of these measurements; regional differences in stiffness along the rectus femoris muscle; how muscle length affects these measurements; and the influence of subcutaneous, non-contractile tissue on their reliability.

Why have I been chosen?

You have been chosen because you are:

- Aged between 18 and 40
- Healthy, active 2+ times per week
- Have no history of musculoskeletal disorders

1. Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason for up to two weeks after your last laboratory visit. You should feel under no pressure to participate. Please also bear in mind that all information collected will be kept strictly confidential in compliance with university regulations on research data management.

2. What will happen to me if I agree to take part?

You will be invited to the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham for 3 visits. An investigator will explain the nature of the procedures to you in detail. You are encouraged to ask questions prior to and throughout the study protocol if there is anything you do not understand or feel comfortable with. You will then be asked to sign a consent form. You will also have your height and weight measured. Following provision of informed consent, you will be familiarised with experimental methods.

What do I have to do for the measurements made during the experimental visit?

Before the experimental visits

You will be asked to refrain from exercising, alcohol consumption and use of recreational drugs for 24h prior to the visit. All 3 visits will be done at the same time of the day.

During Experimental Visits

In visit 1, 3 locations will be identified on your thigh, using ultrasound imaging to visualise the appropriate anatomical structures. Muscle stiffness will be measured at these 3 locations using ultrasound shear wave elastography and a MyotonPRO device whilst you are supine (lying on your back) on a massage table, lying on the isokinetic dynamometer with your knee flexed at 90^{o}, and sat up in the dynamometer with your hip flexed at 110^{o} and knee flexed at 90^{o}. EMG will be used to confirm the muscle is in a relaxed state. Following 30min rest in a seated position, these measurements will be repeated. This protocol is identical at all 3 visits.

3. What are the possible disadvantages and risks of taking part?

There are no adverse effects to expect from this protocol.

Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms.

4. What are the possible benefits of taking part?

SportExR undergraduates who participate in this study will receive credit towards their research hour requirement for each data collection session they attend.

At your request, you will find out about your muscle mechanical properties.
In the event of any incidental findings, we will advise you to visit your GP and will contact your GP directly to provide any necessary information with your consent.

5. Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. In accordance with the Data Protection Act 2018 (DPA 2018/GDPR) all physical data (questionnaires, consent forms, manual entry forms) will be stored in a locked cabinet in a locked room that only researchers will have access to. Electronic data will be stored on password protected computers, both of which will only be accessible by researchers named on this information sheet. In accordance with the University of Birmingham's data protection guidelines all data will be stored securely for a minimum of 10 years after which it will be permanently destroyed.

6. What will happen to the results of the research study?

The results of this study are expected to be published anonymously in a scientific journal and within a PhD thesis; however, names of participants will never be published.

7. Who is organising and funding the research?

Liverpool Football Club are funding the research.

8. What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection, by notifying us via email or telephone. If you withdraw, the data collected to date cannot be erased but it will not be used in any data analysis or publications. Finally, if you took part in this study for research hours, you will receive the amount of hours you completed.

9. Can I obtain feedback from the study?

Yes, if you wish to know the results of the study a summary of the results can be provided once the study has concluded. On the Consent Form, there is a space to indicate if you would like to receive a study summary.

10. Do you have any further questions or concerns?

If you have any further questions about the study please feel free to contact:
Cameron Ley; *Tel*: [redacted], *Email*: [redacted]
Dr Barry Drust; *Email:* [redacted]

This study has been reviewed by the University of Birmingham Science, Technology, Engineering and Mathematics Ethics Review Committee.

Appendix 5 - Study 2 participant information sheet

UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: Investigating the cumulative effects of consecutive exercise bouts on muscle mechanical properties and range of motion

Investigators:
Cameron Ley; *Tel*: [redacted], *Email*: [redacted]
Dr Barry Drust; *Email:* [redacted]
Dr Eduardo Martinez Valdes; *Email:* [redacted]

An invitation to take part:
Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

Technical terminology:

Shear Wave Elastography (SWE) – an ultrasound-based method of estimating muscle tissue stiffness

MyotonPRO – a handheld device used to estimate muscle stiffness via non-invasive palpation of the muscle

Total Time commitment:
The total time commitment for this study is 10 hours.
This is broken down into a familiarisation session lasting 1h, 4 intervention trials lasting 2h, and 2 follow-up sessions lasting 30min each. The trials will take place on 2 separate weeks (2 intervention trials a week). These weeks do not need to be consecutive, but trials will all be at the same time of day.

What is the purpose of the study?
Shear wave elastography and MyotonPRO measurements provide an insight into muscle mechanical properties, more specifically, muscle stiffness. The purpose of this study is to understand how exercise affects muscle stiffness, and the relationships these changes have with range of motion.

Why have I been chosen?

You have been chosen because you are:

- Aged between 18 and 40
- Healthy, active 2+ times per week
- Have no history of musculoskeletal disorders

11. Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason for up to two weeks after your last laboratory visit. You should feel under no pressure to participate. Please also bear in mind that all information collected will be kept strictly confidential in compliance with university regulations on research data management.

12. What will happen to me if I agree to take part?

You will be invited to the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham for 7 visits. An investigator will explain the nature of the procedures to you in detail. You are encouraged to ask questions prior to and throughout the study protocol if there is anything you do not understand or feel comfortable with. You will then be asked to sign a consent form. You will also have your height and weight measured. Following provision of informed consent, you will be familiarised with experimental methods.

What do I have to do for the measurements made during the experimental visit?

Before the experimental visits

You will be asked to refrain from exercising, alcohol consumption and use of recreational drugs for 24h prior to the visits. All experimental visits will be done at the same time of the day.

During Experimental Visits

The first week comprises a session familiarising you with the measures, and 2 experimental sessions 24h apart. One will involve pre-exercise measures, a straight-line sprinting exercise protocol (10x40m, 3min rest between repetitions), and post-exercise measures. The other will involve pre-exercise measures, a shuttle run sprinting exercise protocol (10 repetitions of 4x10m shuttle runs, 3min rest between repetitions), and post-exercise measures. With a follow-up visit 24h later. Another week comprises the same structure, but without the familiarisation session. The order of the 2 exercise interventions is switched in the second week.

13. What are the possible disadvantages and risks of taking part?

Performing vigorous exercise carries the following risks that we feel you should be made aware of, as well as some of the things we are doing to minimise these risks:

• Sensations of fatigue and physical exhaustion – this will be short-lived and will subside in a few minutes upon exercise cessation

• Fainting – often related to physical exhaustion and then suddenly stopping, this will be mitigated by the provision of water and a short cool-down period prior to post-exercise measurements, furthermore the exercise interventions include sufficient rest periods, designed to ensure the exercise is not exhaustive

• Cardiovascular event (e.g., myocardial infarction or 'heart attack') – this is a small risk, particularly for healthy individuals who are unaccustomed to physical activity. We will also ensure that you are warmed up appropriately prior to the exercise interventions and will be monitoring your heart rate and general disposition when exercising to minimise the risk of a cardiovascular event

Trained investigators will supervise the exercise tests, and there is an AED situated less than 50m from the Bournbrook sports pitches, where exercise will take place. If at any time during the test you want to stop, you can signal as instructed and the test will be stopped. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those described above.

14. What are the possible benefits of taking part?

SportExR undergraduates who participate in this study will receive credit towards their research hour requirement for each data collection session they attend.

At your request, you will find out about your muscle mechanical properties.
In the event of any incidental findings, we will advise you to visit your GP and will contact your GP directly to provide any necessary information with your consent.

15. Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. In accordance with the Data Protection Act 2018 (DPA 2018/GDPR) all physical data (questionnaires, consent forms, manual entry forms) will be stored in a locked cabinet in a locked room that only researchers will have access to. Electronic data will be stored on password protected computers, both of which will only be accessible by researchers named on this information sheet. In accordance with the University of Birmingham's data protection guidelines all data will be stored securely for a minimum of 10 years after which it will be permanently destroyed.

16. What will happen to the results of the research study?

The results of this study are expected to be published anonymously in a scientific journal and within a PhD thesis and undergraduate dissertations; however, names of participants will never be published.

17. Who is organising and funding the research?

Liverpool Football Club are funding the research.

18. What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection, by notifying us via email or telephone. If you withdraw, the data collected to date cannot be erased but it will not be used in any data analysis or publications. Finally, if you took part in this study for research hours, you will receive the amount of hours you completed.

19. Can I obtain feedback from the study?

Yes, if you wish to know the results of the study a summary of the results can be provided once the study has concluded. On the Consent Form, there is a space to indicate if you would like to receive a study summary.

20. Do you have any further questions or concerns?

If you have any further questions about the study, please feel free to contact:

Cameron Ley; *Tel*: [redacted], *Email*: [redacted]
Dr Barry Drust; *Email:* [redacted]

This study has been reviewed by the University of Birmingham Science, Technology, Engineering and Mathematics Ethics Review Committee.