THE APPLICATION OF ULTRASOUND SHEAR WAVE ELASTOGRAPHY TO MEASURE MUSCLE STIFFNESS IN PREMIER LEAGUE ACADEMY FOOTBALLERS

By

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ABSTRACT

Muscle stiffness has received major interest in recent years as it is believed that lower extremity stiffness is an important factor in musculoskeletal performance and injury prevention (Pruyn et al. 2012, Butler et al. 2003). An advancing technique to measure stiffness, ultrasound shear-wave elastography, has been designed to quantify mechanical and elastic tissue properties to provide an estimate of muscle stiffness (Brandenburg et al. 2014, Talijanovic et al. 2017). Thus, the aim of this thesis is to assess the application of ultrasound shear wave elastography (USWE) to measure muscle stiffness in premier league academy football.

Study 1

The purpose of the systematic review is to assess the variation in methods used to measure the shear modulus of the rectus femoris using USWE in order to measure muscle stiffness, as the current literature shows variation between studies. From this information, we aim to establish an evidence-based protocol for measuring shear wave elastography, and to develop a methodology that is suitable for research within an elite practical setting by identifying which aspects of the procedures and protocols are essential.

Study 2

The aim of this study was to quantify muscle stiffness using USWE across different age groups and to determine the relationship between muscle stiffness and measures of physical performance in premier league academy footballers. Alongside USWE measurements, the athletes performed a vertical hop test, to assess a holistic measure of lower-limb stiffness, to compare against muscle stiffness measured from the USWE. Rectus femoris stiffness

measured from USWE was related to increased CMJ eccentric and concentric peak force, whilst increased vertical stiffness levels appear to be beneficial across a range of performance tasks including acceleration, maximal strength and jump performance.

Study 3

The purpose of this research is to provide information on the change in muscle stiffness using shear wave elastography (USWE) following a 90-minute premier league academy football match. No significant difference was found between the pre-scan and post-scan for muscle stiffness ($p = 0.118$) though a mean decrease of -0.17m/s decrease was observed for the players who played 90-mins with a moderate effect size of 0.45. A moderate negative correlation between the difference in muscle stiffness and Total Distance ($r = -0.6291$), and a moderate positive correlation with number of sprints performed ($r = 0.5745$) though these were not significant ($p = > 0.05$).

Study 4

The aim of the case series approach was to (1) assess how consecutive soccer-specific stimulus influences the response of muscle stiffness measured by USWE over a 5-day longitudinal analysis, and (2) to establish how a consecutive training stimulus of different themed training sessions may influence the acute change of muscle stiffness over a 4-day longitudinal analysis with the addition of a performance marker (CMJ) to help interpret the impact of the stiffness. The case series establishes that the type of stimulus, and the series in which soccer-specific stimulus is performed can affect the individual muscle stiffness response.

This research has helped to provide an interesting insight into how USWE may be used within a professional sports team and provided a platform for future research to help integrate USWE into monitoring and readiness processes within premier league academy football.

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LIST OF ABBREVIATIONS

ACC	**Accelerations**
ACL	**Anterior Cruciate Ligament**
AIIS	**Anterior Inferior Iliac Spine**
Ca^{2+}	**Calcium Ions**
CK	**Creatine Kinase**
CMJ	**Countermovement Jump**
COD	**Change of Direction**
CRP	**C-Reactive Protein**
CUR	**Countermovement Utilisation Ratio**
CV	**Coefficient of Variation**
DEC	**Decelerations**
ECM	**Extracellular Matrix**
GPS	**Global Positioning Systems**
HR	**Heart Rate**
HRR	**Heart Rate Recovery**
HRV	**Heart Rate Variability**
HSR	**High-Speed Running**
IKD	**Isokinetic Dynamometer**
ICC	**Intra Class Correlation**
MEMS	**Micro-electrical mechanical systems**
MDC	**Minimal Detectable Change**
MTU	**Musculotendinous Unit**
MVC	**Maximal Voluntary Contraction**

M+2	**Matchday Plus Two**
PAP	**Post-Activation Potentiation**
RF	**Rectus Femoris**
ROI	**Region of Interest**
RSI	**Reactive Strength Index**
ROI	**Region of Interest**
SD	**Sprint Distance**
SEM	**Standard Error of Measure**
SSC	**Stretch-Shortening Cycle**
SWC	**Smallest Worthwhile Change**
SWS	**Shear Wave Speed**
USWE	**Ultrasound Shear Wave Elastography**
VL	**Vastus Lateralis**
VM	**Vastus Medialis**

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CHAPTER 1

GENERAL INTRODUCTION

Muscle stiffness, which is a fundamental mechanical property of muscle function, has received major interest in recent years as it is believed that lower extremity stiffness is an important factor in musculoskeletal performance and injury prevention (Pruyn et al. 2012, Butler et al. 2003). Stiffness is defined as the resistance of an elastic body to resist deformation in response to an applied force (Latash & Zatsirosky, 1993). This can be characterized using Hooke's Law, which states the force required to deform the body is equal to the spring constant multiplied by the distance of deformation (Pruyn et al. 2016). While stiffness may be necessary for performance, the optimal level of stiffness is less certain as both high or low amounts may lead to changes in performance and/or injury potential (Butler et al. 2003, McMahon & Cheng, 1990, Arampatzis et al. 1999).

Studies have demonstrated that lower extremity stiffness is related to performance as a consequence of the storage and return of elastic energy in the stretch-shortening cycle in running and jumping activities (Spurrs et al. 2003, Kerdock et al. 2002). The literature has revealed that optimizing lower-extremity stiffness can enhance an athletes' sporting performance through an increase in running speeds (Bret et al., 2002), change of direction speed (Serpell et al. 2014), jump performance (Arampatzis et al. 2001) and force production (Turner and Jeffreys, 2010). Yet, relatively high levels of stiffness are also related to repetitive stress and soft-tissue injuries such as hamstring strains (Watsford et al. 2010, Kawai et al. 2021b). The majority of the research within this area has assessed a measure of lower-limb stiffness using methods such as the vertical hopping test, therefore, relatively few studies have characterised in detail the relationship between individual muscle stiffness and physical performance. It is yet to be established if performance is improved due to stiffness of individual units working in isolation or whether stiffness of the combined lower body structure working together increases performance. Measuring the stiffness of an individual unit, therefore, might give insight into the contribution and importance of each specific component of stiffness against the whole unit, which may be able to provide meaningful information on the relationship between the optimal levels of stiffness and performance. This may be especially true for populations such as Premier League football players who are required to complete training programmes that maximise performance while minimising the potential for injury.

In the English Premier League, footballers can complete up to 11,429 ± 701m of Total Distance during match-play but are also expected to tolerate around 600 changes of directions (COD), perform up to 36 sprints, and even perform up to an average of 16 vertical jumps during the course of a game (Allen et al., 2023, Harper et al, 2018, Rampini et al. 2007, Reily & Thomas, 1976, Di Salvo et al, 2010, & Rienzi et al. 2000). The physical demands and expectations of a Premier League professional football player are to train and compete on a weekly basis over a 9-month season. In some circumstances during fixture congestion, this can result in playing up to 3 games within 7 days (Thomas, 2017). In an attempt to maintain a competitive advantage for the team, a number of objective and subjective markers are collected in order to measure a player's readiness and recovery status (Mohr et al. 2016, Carling et al. 2015). Examples of the data collected for such purposes include; Examples of the data collected for such purposes include; counter-movement jump (CMJ) profiling and strength diagnostic assessments (Adductor Squeeze and 90/90 Isometric Hamstring etc.) pre- and post- match play to assess readiness and fatigue, player subjective wellness questionnaires (muscle soreness, sleep quality, fatigue, stress) to evaluate player status, and the utilisation of micro-electro mechanical systems (MEMS) in linear running to assess metrics such as step balance and fatigue index (Thorpe et al. 2016, Buchheit, 2014, Romagnoli et al. 2016, Saw et al. 2016). While such markers may give an indication of a player's acute response to a game or assess their wellness and physical preparedness in the lead up to a game, they do not reflect an objective internal indicator of the structural properties of muscular tissue, such as muscle stiffness, that may be a more direct reflection of a player's physiological readiness. As football related activities are frequently associated with muscle damage and severe inflammatory responses (Nedelec et al. 2012, Thorpe & Sunderland, 2012), the ability to accurately quantify these properties could significantly impact the monitoring process used within the sport by providing a better reflection of the actual consequences of the total physical load experienced by the players.

An advancing technique to measure stiffness, ultrasound shear-wave elastography, has been designed to quantify mechanical and elastic tissue properties to provide an estimate of muscle stiffness (Brandenburg et al. 2014, Talijanovic et al. 2017). This technology provides a quantification of shear elastic modulus which provides an estimation of muscle stiffness in a selected region of interest in the muscle resulting in the provision of detailed information from a localised area (Sarabon et al, 2019). The muscle stiffness value is represented by a metric called shear modulus which is a ratio of shear stress (the force required to cause deformation of an object) to shear strain (the actual deformation of an object under shear stress) in the muscle. While the exact physiological mechanisms that cause muscle stiffness are still debated within the literature, current hypotheses suggest the following factors may be important (1) disruption in calcium ion (Ca^{2+}) homeostasis (Lacourpaille et al., 2017), and an increase in the amount of cross-bridge attachments (Tanner et al. 2011) (2) changes in titin and sarcomere structure (Brynnell et al. 2018, van der Pijl et al. 2020, Wang et al. 2001), and (3) increases in collagen cross-linking that elevate fascial stiffness (Smith et al, 2021 & Kawai et al, 2021b).

The ability of ultrasound shear-wave elastography to provide an internal marker of muscle stiffness in a localised region of the body with a non-invasive methodology and with real-time visual feedback make it a potentially viable monitoring tool for soccer. This research will examine two potential use cases for USWE to provide useful information in a "real-world" football monitoring program. (1) An investigation into the relationship of muscle stiffness and physical performance tasks will validate whether USWE could potentially be used as a predictor of physical performance and a recruitment tool in Premier League academy football, and (2) by measuring the adaptive response of the shear modulus following different soccer-specific stimuli (e.g. training/matches), will paint a picture of the impact of these different exercise patterns on the demands placed on the neuromuscular system may be able to be obtained. This data may provide a more detailed understanding of the load placed on these tissues than can be obtained using more traditional external physical outputs provided by MEMS or other typically used wearable sensors.

AIMS AND OBJECTIVES

Thus, the aim of this thesis is to investigate the application of ultrasound shear wave elastography to support performance in Premier League academy football. The aims of this thesis are as follows:

- To develop an applied methodology for how USWE can be integrated into an Premier League academy football club.

- To assess the relationship between muscle stiffness and physical performance tasks in Premier League academy footballers.

- To measure the adaptive response of muscle stiffness following a competitive 90-min Premier League academy match-play stimulus.

- To measure the adaptive response of muscle stiffness following a series of soccer-specific training in individual Premier League academy footballers.

The aims and objectives of the research thesis are going to be fulfilled by completion of the following 4 studies:

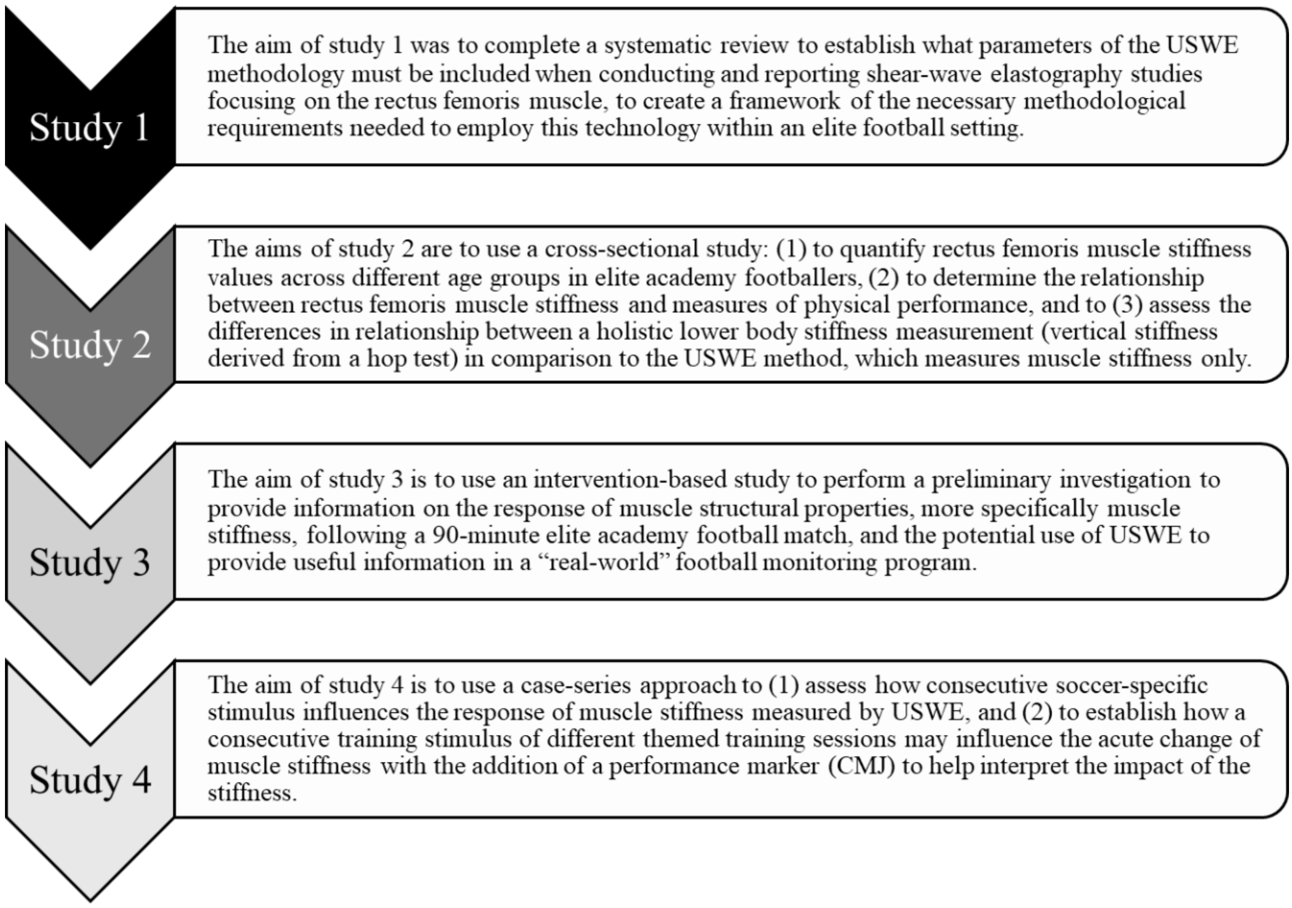


Figure 1.1: How the thesis is going to fulfil the aims and objectives for the research project.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

This aim of the literature review is to critically investigate the current state of the literature on (1) the current monitoring processes and challenges in elite football, (2) the relationship of muscle stiffness to physical performance and (3) the muscle stiffness response to different exercise modalities. In addition, a definition of stiffness will be scrutinised and potential mechanisms of how muscle stiffness is caused will be discussed, and how this may potentially have an impact on injury susceptibility and age. Therefore, the aim of this review is to evaluate the role of muscle stiffness measured via USWE in its relationship to physical performance and the response to exercise, and how this can be implemented within an elite football setting.

PHYSICAL DEMANDS OF ELITE FOOTBALL

Defining the physical outcomes of elite football

To perform at the very highest level of elite soccer, in leagues such as the English Premier League, a player must be physically capable, whilst exhibiting exceptional technical and tactical skills, in an attempt to positively influence the outcome of a match (Wrigley et al, 2012). Soccer can be described as an invasive field game that is characterised by an intermittent activity profile where high-intensity anaerobic efforts are superimposed on a background of aerobic activity (Bangsbo et al, 1991 & Drust et al., 2000). Modern day elite footballers are expected to have the endurance and aerobic capacity to play at a high level for 90 + minutes, whilst being able to tolerate maximal eccentric contractions in decelerating and sprinting mechanisms during match-play. In the English Premier League, elite footballers can complete up to 11,429 ± 701m of Total Distance during match-play but are also expected to tolerate around 600 changes of directions (COD), perform up to 36 sprints, and even perform up to an average of 16 vertical jumps during the course of a game (Allen et al., 2023, Harper et al, 2018, Rampini et al. 2007, Reily & Thomas, 1976, Di Salvo et al, 2010, & Rienzi et al. 2000). Peak ground reaction forces during the task of a horizontal deceleration alone can generate up to six times body mass (Harper et al. 2019), which inevitably provides an extremely large eccentric stimulus, without considering the aerobic capacity necessary to repeat all of the other running, sprinting, COD and jumping activities for the duration of a 90-min football match. All of these physical actions, as well as unorthodox, powerful movements such as tackling, twisting, and attempting to maintain or gain possession of the ball while exerting physical force against an opponent (Mohr et al., 2003) can help to influence the outcome of the soccer match. Approximately 80-90% of the total distance in an elite soccer match is comprised of low to moderate intensity actions (speeds 0 – 19.8 km/h), and the remaining 10-20% is comprised of high intensity activities (speeds >19.8 km/h) (Carling et al., 2008; Rampinini et al., 2007; Reilly and Thomas, 1976; Rienzi et al., 2000). The 10-20% of total distance covered performing high intensity activities performed by both elite and youth players (speeds >19.8 km/h), are commonly regarded the most important to the match outcome. This is due to the perception that these actions are directly linked to the match situations leading to either scoring or preventing goals/goal scoring opportunities (Faude et al., 2012).

THE IMPORTANCE OF MUSCLE STIFFNESS

Definition of stiffness

Stiffness is defined as the resistance of an elastic body to resist deformation in response to an applied force (Latash & Zatsirosky, 1993). This can be characterized using Hooke's Law, which states the force required to deform the body is equal to the spring constant multiplied by the distance of deformation (Pruyn et al. 2016). Thus, the relationship between stress and strain, as characterized by Hooke's Law, establishes the mechanical property of a material. As muscle stiffness is the focus of this research project, the mechanical property we are interested in is the shear modulus, which is a measure of the ability of a material to resist deformation under stress to provide a measurement of muscle stiffness.

The causes of muscle stiffness

While the exact physiological and biomechanical mechanisms that cause muscle stiffness are still debated within the literature, current hypotheses suggest the following factors may be important: (1) disruption in calcium ion (Ca^{2+}) homeostasis (Lacourpaille et al., 2017), and an increase in the amount of cross-bridge attachments (Tanner et al. 2011) (2) changes in titin and sarcomere structure (Brynnell et al. 2018, van der Pijl et al. 2020, Wang et al. 2001), and (3) increases in collagen cross-linking that elevate fascial stiffness (Smith et al, 2021 & Kawai et al, 2021b). A mechanism, proposed by Lacourpaille and colleagues, hypothesises that changes in muscle stiffness post-exercise are facilitated by disruptions in calcium ion (Ca^{2+}) homeostasis (Lacourpaille et al., 2017). The disruption in Ca^{2+} homeostasis is caused by myofibrillar disruptions as a consequence of damaging eccentric exercise which increases stable actin-myosin cross-bridge formation (Whitehead et al., 2001, Herzog, 2014). Titin, which modifies the force generating region of muscle sarcomeres, has also been proposed to link to muscle stiffness. The observation is that titin increases in active muscle and therefore increases the binding of actin and titin to cause an increase in muscle stiffness (Herzog, 2018). Brynnell and colleagues (2018) tried to establish titin's contribution to skeletal passive stiffness through RNA sequencing in mice and stated that titin is the dominant determinant of physiological passive stiffness. Furthermore, research by Kawai and colleagues has highlighted that the fascia and extracellular matrix (ECM) may influence muscle stiffness. Their research examined hamstring injuries, and discovered that the stiffness of the fascia increased post-injury for years after the initial incident (Kawai et al., 2021a). It is hypothesized that the hamstring injury could have caused an increase in collagen cross-linking, specifically at the site of the injury, which would have led to greater shear modulus values in that region because of the build-up of scar tissue (Forrest and Jackson, 1971). Research from Zhou et al. (2022) has demonstrated that USWE displays good reliability in measuring fascial tissue stiffness, thus, more research needs to be performed to understand more comprehensively how the fascia and ECM influences muscle stiffness.

METHODOLOGIES USED TO MEASURE MUSCLE STIFFNESS

Methodologies used to measure muscle stiffness.

Currently in the literature, lower extremity stiffness is predominantly measured using vertical stiffness, leg stiffness and joint stiffness. These methods do not provide a measure of individual muscle stiffness, and whilst relationships have been found between stiffness and performance, it is yet to be established if performance is improved due to stiffness of individual muscles working in isolation or whether stiffness of the combined lower body musculature working together increases performance. An example of methods which have been previously used to calculate vertical or leg stiffness can be illustrated in research from Pruyn and colleagues (2016) with approaches such as the vertical hop test and free-oscillation techniques. The vertical hop test, also known as the 10-5 hop test, involves 15 consecutive hops on a force plate system. During the test, participants are encouraged to jump as high as possible on their 1st rep and jump as quickly as possible for the remaining jumps. Five acceptable consecutive hops are generally used for analysis (typically 6th to 10th jump) (Hobara et al., 2013), with the average of contact and flight time data from these hops then used to calculate stiffness based on Dalleau's et al. (2004) equation, a method that has been shown to be a reliable and valid way of measuring leg stiffness (Dalleau et al., 2004).

$$K_N = \frac{M \times \pi(T_f + T_c)}{T_c{}^2\left(\frac{T_f + T_c}{\pi} - \frac{T_c}{4}\right)}$$

Where M was total body mass, Tc the ground contact time and Tf the flight time

The oscillation tests used to measure whole leg stiffness can also be performed on a force plate system or by utilising a load cell. However, the free-oscillation technique comprises of a controlled displacement to the leg, which causes perturbation. Pruyn et al. (2012) explains that the oscillation of the leg following the perturbation can be used to calculate whole leg stiffness. Although the research from Pruyn and colleagues (2016) has helped to provide significant findings in relation to whole lower limb stiffness, these techniques cannot be used to examine individual muscle tissue, or to comprehensively understand which specific structures in the lower limb exhibit stiffness.

With new technologies, such as USWE and Myotonometry, showing excellent within-day reliability, and high between-day reliability (Pruyn, Watsford and Murphy, 2016) at measuring individual muscle stiffness, we can begin to look at the effect individual muscle stiffness has on performance and how this information can be used to help inform practitioners. Similarly to the free-oscillation technique aforementioned, Myotonometry, performed with a handheld MyotonPRO device, can administer a brief, gentle tap to cause a natural oscillation within an individual muscle tissue of choice to receive information on mechanical properties (Pruyn, Watsford and Murphy, 2016). As the MyotonPRO is a wireless device that can produce instantaneous feedback on mechanical properties of individual muscle sites, it makes the device both practical and attractive for applied sport scientists to collect data in an applied setting. Although the MyotonPRO has shown excellent reliability in estimating muscle stiffness and seems to provide a practical solution to collecting information on mechanical properties in the applied field, there are limitations to consider. Similar to the USWE, as explained in Chapter 4, there is inconsistency in the methodology to measure muscle stiffness with the MyotonPRO (Kalkhoven and Watsford, 2018) meaning that the results obtained from different research studies are difficult to compare. Another limitation of the Myoton-Pro is that the device cannot measure muscle stiffness when there is greater than 2cm of subcutaneous adipose tissue, meaning the measurements of participants with greater body fat percentages can be affected by overlying fascial, adipose, and skin tissue. Despite the correlation in measures of muscle stiffness from the MyotonPro and USWE stated by Feng et al. (2018), this thesis will concentrate on the muscle stiffness measurements derived from the USWE, which is considered a more gold-standard way of collecting muscle stiffness within the current literature.

Introduction to Ultrasound Shear Wave Elastography (USWE)

An advancing technique to measure stiffness, ultrasound shear-wave elastography, has been designed to quantify mechanical and elastic tissue properties to provide an estimate of muscle stiffness (Brandenburg et al. 2014, Talijanovic et al. 2017). The use of USWE in the clinical medical field is already widely established, as the technology has been recognised to successfully diagnose liver cirrhosis (Fruilo and Trillaud, 2013) and breast masses (Cosgrove et al., 2012) as the USWE can successfully detect the difference in muscle hardness. This technology provides a quantification of shear elastic modulus which provides an estimation of muscle stiffness in a selected region of interest (ROI) in the muscle resulting in the provision of detailed information from a localised area (Sarabon et al, 2019). The muscle stiffness value is represented by a metric called shear modulus, which is a ratio of shear stress (the force required to cause deformation of an object) to shear strain (the actual deformation of an object under shear stress) in the muscle. USWE works by administering shear waves into a specific ROI in a muscle tissue via an ultrasound probe. The estimated muscle stiffness is based upon the speed in which the induced shear waves propagate in the tissue of interest, whereby faster propagation is used to infer stiffness muscle. The shear wave velocity is measured by the ultrasound probe using B-mode ultrasonic imaging, which is described as the shear modulus measurement. Previous research from Lacourpaille and colleagues (2012), and Dubois and colleagues (2015) has shown that USWE provides a reliable measurement in measuring muscle stiffness at rest and during passive stretching in the lower limb muscles and can therefore be beneficial in applied and clinical research projects which require an accurate assessment of muscle mechanical properties. The ability of USWE to provide an internal marker of muscle stiffness in a localised region of the body with a non-invasive methodology and with real-time visual feedback make it a potentially viable monitoring tool for muscle tissue properties of elite footballers.

STIFFNESS IN RELATION TO PHYSICAL PERFORMANCE

Based on the definition and causes of muscle stiffness, it would be deemed necessary to develop methods of measuring muscle stiffness in athletes to enhance understanding of maximising muscular performance and minimise injury risk. The literature has revealed that optimizing lower-extremity stiffness can enhance an athletes' sporting performance through an increase in running speeds (Bret et al., 2002), change of direction speed (Serpell, Ball, Scarvell, Buttfield and Smith, 2014), jump performance (Arampatzis, Schade, Walsh and Brüggemann,

2001) and force production (Turner and Jeffreys, 2010). Turner and Jeffreys (2010) theorized that by optimising muscle stiffness, athletes can improve performance by directing energy storage and release to tendons, where the elastic properties of the tendons can enhance physical performance. However, it has been suggested that stiffness only improves performance in fast stretch shortening cycle activities, with research into the effects of muscle stiffness and slow stretch shortening finding contrasting evidence (Laffaye, Bardy, & Durey, 2005; Pruyn, Watsford, & Murphy, 2014; Wu et al. 2010; Kubo et al., 2007). Cavagna (1970) proposed that a more compliant system will absorb and transfer greater amounts of elastic energy at a given force, increasing force outputs and slow stretch shortening performance outcomes. However, numerous research since has found relationships between slow SSC and stiffness (Wu et al. 2010; Kubo et al., 2007).

The relationships discussed below between stiffness and physical performance traits such as maximal strength, running capability, and jumping performance, are all caused by an adaptative chronic physiological response to a regular training stimulus. When a regular training stimulus is performed with a desired physical outcome, the muscle tissue will progressively adapt over time. These chronic adaptations are different from acute physiological responses, which occur immediately before, during, or after exercise. These acute physiological changes can be indicative of an interference to the muscle that are only temporary in nature, yet chronic adaptations occur gradually and generally last as long as an exercise program is maintained. Therefore, there is potential scope that measuring stiffness could be a viable option to predicting physical performance outcomes. If a was an evidential relationship between that and the desired physical trait, which could be extremely useful for elite sports team in talent identification and recruitment.

Relationship of Muscle Stiffness and Strength

Conflicting findings have been noted in studies looking at the effect of muscle stiffness on measurements of strength. He et al. (2021) found that males who have undergone an ACL repair, had a positive, moderate correlation with vastus medialis muscle stiffness and quadricep peak torque and average power during an isokinetic muscle strength test. This may be due to the importance of stiffness in the transition of tension generated by the contractile components of the muscle, with the actomyosin cross bridges being important in the production of maximal force and consequently sporting performance. This has also been supported in healthy adults, with Alfuraih et al. (2019) showing a moderate correlation between vastus medialis stiffness and isokinetic knee flexion torque and power (r= 0.603; 0.613) and knee extension torque and power (r= 0.636; 0.600). However, not all research has found that muscle stiffness has an effect on strength. Dumke and colleagues (2010) found that muscle stiffness had no effect on strength and power performance when measured using an isometric squat. However, the validity of the free oscillation technique utilised to measure the muscle stiffness has been questioned, as it only provides an estimation of passive muscle stiffness, which may not be relevant during strength and power movements where muscle stiffness could be altered by increased muscle activation. Kalkhoven and Watsford (2017) also found individual measures of rectus femoris, biceps femoris and gastrocnemius stiffness did not correlate with any strength measures, whilst Ando and Suzuki (2019) also found no correlations in the medial gastrocnemius with isometric strength tests.

Relationship of Muscle Stiffness and Running

Similar to the relationship to muscle stiffness and strength, the research into the effect of muscle stiffness and running is also varied, mainly due to the vast physiological demands of different running events. When considering sprint performance, Miyamoto, and colleagues (2019) found that higher passive vastus lateralis stiffness was negatively correlated to 100m times ($r = -0.470$) meaning that increased stiffness would help to increase 100m sprint performance. It has also been proposed that this increased stiffness supports the repositioning of the limb during the swing phase of sprinting which allows for increased ground contact time and superior performance. Takahashi et al (2018) also found that a higher passive stiffness of the plantar flexor muscle group was related to improved sprint performance ($r= -0.334$), which they proposed allowed for an increase in ground reaction forces and reduction in ground contact times. These findings have been further supported by Yamazaki and colleagues (2021) who found passive medial gastrocnemius stiffness was negatively correlated to 100m times during 4 different contraction velocities, and Kalkhoven and Watsford (2017) who stated that rectus femoris stiffness was directly related to sprint speeds, with significant negative relationships in 10m, 20m, 30m, 40m, 50 and 60m sprint times. These studies emphasize the importance that individual muscle groups have on influencing sprint performance, however, these studies all take measurements of passive muscle stiffness. Miyamoto and colleagues (2019) analysed the relationship with sprinting and active muscle stiffness (during contraction) , and shown a much weaker relationship, with 100m times being positively correlated to the active vastus lateralis stiffness ($r = 0.468$) meaning reduced sprint performance.

However, whist the aforementioned literature takes into consideration sprint performance, the physiological make up of long distances runners is extremely different, and consequently so is the effect of muscle stiffness on running performance. Miyamoto and colleagues (2019) discovered that higher passive vastus lateralis stiffness correlated with inferior 5000m running speed ($r = 0.423$). This may be due to an increase in stiffness resulting in an increase in metabolic energy cost during antagonist co-contraction, but more research is needed in this area. However, this relationship may be specific to individual muscles, as Ueno et al (2018) found that increased passive plantar flexor stiffness was correlated with personal best times in 5000m races ($r = -0.401$), with plantar flexor stiffness being higher in the trained individual vs. untrained individuals. Research from Kubo and colleagues (2017) supports this, and states that the difference in passive plantar flexion stiffness is due to muscle stiffness, not any contributions from the tendons, which indicates enhancing plantar flexion stiffness can potentially enhance running performance. Further research from Dumke and colleagues (2010) also found that increasing gastrocnemius and soleus stiffness is significantly related to improved running economy at competition level speeds which is believed to be due to increased stiffness reducing muscle activation and decreasing energy expenditure. It is worth noting however, in the majority of these studies, including the research from Miyamoto and colleagues (2019), muscle stiffness was measured in a sitting or lying down position in a passive condition, which is not directly related to the joint angles or muscular condition that is relevant during running which may have an effect on the stiffness outcomes.

Relationship of Muscle Stiffness and Jump Performance

Another key determinant of sport is jumping, with the quadriceps being identified as a key muscle in jump performance (Peng, 2011). This is more specifically in jumps that utilise the fast stretch shortening cycle, as efficient storage and release of elastic energy is vital in these types of jumps for optimal performance. Significant correlations have been measured between improved drop jump height ($p = 0.03$) and drop jump reactive strength index (RSI) ($p = 0.01$) when comparing a group of stiff athletes vs. compliant athletes, and when ranked by rectus femoris stiffness (Kalkhoven and Watsford, 2017)). This may be due to stiffer rectus femoris increasing concentric force production by working in a more optimal force velocity range. However, similar results have been supported in the lower limb, with significant correlations between drop jump performance and stiffness of the lateral gastrocnemius (r=0.66) , medial gastrocnemius (r=0.47) and soleus (r=0.67) (Pruyn, Watsford and Murphy, 2014). The medial gastrocnemius stiffness has not only been shown to correlate with fast SSC actions, but also concentric strength, as measured by a squat jump. In the study by Pruyn and colleagues (2014), they reported significant differences between the squat jump scores between the stiffer group and compliant group as measured by medial gastrocnemius (r=0.20). This highlights the importance of the medial gastrocnemius in braking phase of jumping actions when the eccentric load is high, and storage of elastic strain energy takes place (Kyroloinen et al. 2003). All these studies represent the importance of muscle stiffness in fast SSC actions and concentric force, both of which are important for sporting success.

However, whilst muscle stiffness may provide an insight into fast SSC performance and concentric strength, it may be less beneficial in measuring slow SSC performance and reactive strength. Kalkhoven and Watsford (2017) found no differences in CMJ between groups with high quadricep stiffness compared to groups with lower quadricep stiffness when ranked on stiffness of the rectus femoris, biceps femoris and medial gastrocnemius. Similar results have been reported by Pruyn, Watsford and Murphy (2014) who found no differences in CMJ scores between a group with stiff muscles and a group with compliant muscles in the lateral gastrocnemius, medial gastrocnemius, and soleus. That being said, in a recent study, Djuric and colleagues (2023) explored the relationship between the passive muscle stiffness of four lower limb muscles (GL, GM, VL, VM), with vertical jumping performance and countermovement utilisation ratio (CUR) to understand in more detail the association between performance and shear modulus. The CUR, which is the ratio of CMJ to squat jump performance, has been suggested as a useful indicator of power performance in athletes in an evaluation of their SSC. The results of the study revealed although muscle stiffness was not related to jump performance, there was significant positive relationship between VL and VM shear modulus and CUR, which may indicate that higher muscle stiffness in these specific quadricep muscles are advantageous for fast SSC activities (Djurić et al., 2023).

Relationship of Muscle Stiffness to Specific Sports Performance

Whilst the aforementioned research primarily focuses on physical attributes which are performed in isolation, such as jumping, running and strength, some studies have looked at individual muscle stiffness in sporting actions. This may provide scope that the use of USWE within an elite football setting could be meaningful. Sheehan, Watsford and Pickering Rodriguez (2018) have looked at individual muscle stiffness in golfing performance, with their results finding bicep femoris significantly correlated with handicap (r = 0.45). They believe this increased stiffness allowed for an increased rate of force development, as the stiffer bicep femoris reduces the negative effects of the electromechanical delay by moving out of the toe region, and consequently enhancing golfing performance. However, they also found, linked to this, that reduced stiffness of the upper body may be beneficial to increased club head speed. This reduced stiffness in the upper body is also supported by Colomar, Baiget and Corbi (2020) who seeked to look at the influence of upper body stiffness on tennis performance, specifically in the serve and forehand velocity. Their results shown that decreased stiffness, predominantly in the pectoralis major, may be correlated to increased serve speed (r = -0.29) and forehand velocity (r= -0.45). This may be related to their slow SSC nature of upper body movements, where compliant muscles may be beneficial to be able to absorb and reuse elastic energy. More specifically to this thesis, Kalkhoven and Watsford (2018) assessed the mechanical stiffness of several lower-body muscles and compared this against athletic performance variables in 22 sub-elite footballers. When the authors ranked the footballers on their rectus femoris muscle stiffness, the relatively stiff group exhibited superior sprint, agility and drop jump performance ($p < 0.05$), whilst gastrocnemius medialis and biceps femoris were not related to performance.

Relationship of Muscle Stiffness, Performance and Age

In addition, to the authors knowledge, no researcher has investigated the effect of muscle stiffness and performance between different age groups. However, previous research looking at both vertical and leg stiffness have found increases in stiffness with age in pre- and post-pubescent boys (Di Giminiani and Visca, 2017; Laffaye et al., 2016) with the increase in age being associated with an increase in body mass, which therefore increases leg stiffness to maintain spring mass model behaviour on ground contact time (Granata et al., 2002). Simiarly, Korff and colleagues (2009) found age-related increases in stiffness and superior performance in the stiffer individuals, and these results were replicated even when body mass was accounted for, implying that age-related differences in performance during cannot solely be explained by differences in body size. However, in contrary to this, a longitudinal study in team sport by Lehnert et al. (2020) found no changes in muscle stiffness in boys from the ages of 14 to 16. More research is needed to establish if there are age-related changes to stiffness, especially muscle stiffness, and the effect that this may have on performance. These age-related changes may also be a function of training exposure, and the potential musculoskeletal adaption which may take place when athletes increase their training volume and intensity as they get older. This may be even more relevant in a study population of Premier League academy footballers where football-specific stimuli and strength and conditioning exposure are carefully and strategically periodised to increase as they progress through the age groups. Establishing these stiffness characteristics for Premier League academy footballers may help in areas such as talent identification, injury prevention and exercise programme prescription.

MUSCLE STIFFNESS AND INJURY RISK

Despite the literature showing that there is evidence supporting the relationship of muscle stiffness and improved performance capability, there is limited research which infers that too much stiffness can also increase the risk of injury, hence why this muscular property may be important to monitor. If the principle of material stiffness is applied to muscle stiffness, when a material becomes too "stiff," and can no longer cope with the increase in stress and strain, it escalates the likelihood of the material breaking down or a muscle rupturing or tearing for example, despite the performance benefits which are associated with improved muscle stiffness levels (Kelc et al. 2013). Therefore, understanding the optimal level of muscle stiffness to balance both performance optimization and injury risk minimization seems important.

Using a similar methodology to the research conducted by Pruyn et al. (2016), Watsford and colleagues utilised a free oscillation technique and unilateral hopping test to calculate vertical and whole leg stiffness in professional male Australian rules footballers in the 2006 pre-season to assess the relationship between stiffness and hamstring injury risk (2010). The results of this research exhibited that athletes who reported a hamstring injury during the season displayed higher bilateral hamstring and leg stiffness in the preseason of the season in which the injury was sustained in comparison to the other respective players. Therefore, it appears that a high bilateral stiffness and leg stiffness may be a determinant in the risk of sustaining a hamstring injury. That being said, when considering the players who did get injured during the season, the hamstring stiffness was significantly higher on the non-involved limb ($p = 0.01$), which may associate the increased injury risk with lack of strength.

On the contrary, Kawai and colleagues also discovered a relationship between muscle stiffness and hamstring injury in the years following the injury incident, when examining soccer players with prior hamstring injuries (2021a, 2021b). The results of this research documented that muscle and fascial stiffness in the hamstring muscle and fascia at sites of previous injury were higher in the injured leg, regardless of age and BMI. These findings could provide useful insight to sport science and medical practitioners in the monitoring of injury history and show the significance of implementing injury prevention programmes to prevent re-injury, especially if these changes to the injury site can last for years post-injury.

MONITORING AND SCREENING IN ELITE FOOTBALL

The effectiveness of current practices used to monitor fatigue and recovery within elite football.

Carling and colleagues (2018) performed a review on the monitoring of post-match fatigue in professional soccer and stated that uncertainty exists around the real-world impact of research regarding post-match fatigue monitoring and its usefulness in informing readiness to play in professional soccer players. In professional football, different levels of monitoring are performed, ranging from more basic neuromuscular assessments such as a CMJ or adductor squeeze, to more advanced assessments such as blood biomarkers and saliva testing, all in aid of trying to examine the physical state of their respective athletes. Thorpe et al. (2016) performed a body of research examining the monitoring of fatigue in elite soccer players and investigated some of the current practices. Subjective wellness questionnaires are extremely common in both elite football and endurance sports, as a quick and inexpensive method to understand fatigue and wellness status, without having to ask the athlete to exert any physical energy (Buchheit 2014 & Coutts et al, 2007). As efficient and cost-effective as a subjective wellness questionnaire can be, the influence of the questionnaire is heavily reliant on the athlete being honest and not manipulating their response. An assessment of neuromuscular function following match-play is also common practice within elite football, using jump protocols such as the countermovement jump (CMJ) to provide a reliable method to measure neuromuscular fatigue (Gathercole et al. 2015 & Watkins et al. 2017). The high intensity and intermittent nature of elite football justifies the use of a CMJ to provide information on the neuromuscular fatigue in elite football players. That being said, practitioners in elite football can be discouraged of using a CMJ, which encourages the player to push maximally in a plyometric movement, when all other efforts following match-play are to promote recovery and adaptation. Similarly to the subjective wellness questionnaire, the limitation of the CMJ for monitoring fatigue is being heavily reliant on player application, and ensuring they provide maximal effort to make the test worthwhile. Previous research has shown that heart rate (HR) measures such as submaximal heart rate, heart rate recovery (HRR) and heart rate variability (HRV) can help to monitor fatigue, and aerobic adaptation (Buccheit, 2014). HRV is among the monitoring tools that are increasing in popularity in sports such as elite football, due to its measurement ease and validity to provide an internal load measure without the athlete being able to influence their score (Sanchez-Sanchez et al. 2021). Buccheit and colleagues (2016) identified the root mean square of successive differences (RMSSD) from HRV as an effective variable to assess parasympathetic tone, which has been shown to be associated with readiness to perform, and to be a good monitoring tool to optimize the training process. Creatine kinase (CK) is also widely used as a monitoring tool to make inferences on fatigue and readiness in elite football (Szigeti et al. 2023, Yapal et al. 2022, Scott et al. 2016, Lazarim et al. 2009 &

Russel et al. 2016). Although CK provides a predictor of muscle damage, as the measurement comes from a blood assessment, it is impossible to isolate where the muscle damage is localised to help assist in recovery interventions. Research from Thorborg and colleagues (2017, 2023) has shown that the Copenhagen five-second squeeze is a valid indicator of specific hip and groin function in elite football players. Unlike the other tests aforementioned, the adductor squeeze helps to isolate a specific area of the body, to determine the probability of suffering an overuse groin injury, which is important to measure based upon the changes of direction and repetitive ball strikes completed in elite football (Moreno-Perez et al. 2019). However, the challenge of requiring the player to maximally exert force in an isometric contraction following a muscle damage-inducing bout of match-play is a persisting subject. Based upon some of the challenges listed above with monitoring and screening in elite football, USWE may provide a solution which allows a practitioner to assess a localised muscle tissue, without the need for physical exertion following match-play, and where the player cannot manipulate or influence the outcome of their measurement.

APPLICATION OF USWE TO MEASURING A RESPONSE TO EXERCISE

As aforementioned, measuring the acute physiological adaptation to an exercise bout is different to measuring the chronic adaptation to a series of exercise. In the section below, the application of USWE to measure a response to a single bout of exercise is assessed in order to analyse how different types of exercise may influence the muscle stiffness response.

Relationship of Muscle Stiffness derived from USWE and Muscle Damage

The ability of ultrasound shear-wave elastography to provide an internal marker of muscle stiffness in a localised region of the body with a non-invasive methodology and with real-time visual feedback make it a potentially viable monitoring tool for soccer. A developing body of research has observed that changes in muscle stiffness may be sensitive to changes in muscle damage (Lacourpaille et al. 2017, Green et al. 2011, Chalchatt et al. 2020). Lacourpaille and colleagues (2014) performed a study which analysed the time-course effect of exercise-induced muscle damage (EIMD) on localised muscle mechanical properties using USWE, which raised interest in the possibility of a relationship between muscle stiffness measured by USWE and muscle damage, and the potential implications this could have in the world of professional sport. The study performed by Lacourpaille demonstrated a relationship between an increase in shear modulus during a passive muscle stiffness assessment following eccentric exercise of the knee extensors and elbow flexors with subsequent force loss in a muscle function assessment after 48 hours. Bouillard et al. (2012) also provides support for such relationship by finding that the shear modulus follows changes in torque during a fatiguing isometric contraction. Guilhelm et al. (2016) and Lacourpaille et al. (2014) further emphasise the potential relationship to muscle damage, as they also display a rise in shear modulus within 1 hour of eccentric exercise in the elbow flexors and knee extensor muscles respectively, which re-iterates that shear-wave elastography could potentially be used as a proxy marker for muscle damage, as Lacourpaille et al. (2017) stated. Further research is required to support this theory, but if USWE is able to provide a proxy marker of muscle damage immediately following a bout of exercise, without the need for a muscle biopsy or an assessment of muscle function 48hr- post-activity, it would exponentially improve the ability of an applied sport scientist or medical professional to monitor recovery status and fatigue in elite football players following match-play.

Increase in Muscle Stiffness Response Following Exercise

There seems to be a trend in the literature that eccentric-based resistance exercises cause an increase in muscle stiffness, in agreement with the research from Lacourpaille and colleagues above (Guilhem et al., 2016 & Chalchatt et al., 2022). Guilhem and colleagues (2016) displayed an increase in gastrocnemius medialis muscle stiffness 5mins- post 10 x 30 repetitions of eccentric maximal plantarflexions, whereas Chalchatt and colleagues (2022) experienced an increase in rectus femoris and vastus lateralis muscle stiffness across a 7-day period following a 45min downhill walk. Although the majority of the current literature supports that eccentric exercise causes an increase in muscle stiffness, the research conducted from Xu and colleagues (2019) does show some conflicting findings. The study protocol consisted of of 75 maximal eccentric knee extensions and despite a significant increase in rectus femoris stiffness, there was a significant decrease in vastus lateralis stiffness, and no change observed in vastus medialis stiffness. Even though the mechanism of a knee extension may require different levels of activation from each quadricep muscle, this does highlight that there may be intermuscular variability may be a factor to consider when measuring the response to exercise.

Decrease in Muscle Stiffness Response Following Exercise

On the contrary, the bulk of the literature that displays a decrease in muscle stiffness following exercise, is comprised of endurance or aerobic-based exercise bouts (Andonian et al., 2016, Morales-Artacho et al., 2017, Sadeghi et al., 2018, Fouré et al., 2022). The current hypotheses by these authors suggest the immediate decrease in muscle stiffness following endurance exercise may be a result of cross-bridge attachment release induced by aerobic-based exercise. Morales-Artacho and colleagues (2017) displayed a decrease in biceps femoris, semitendinosus, and semimembranosus muscle stiffness following 15mins of cycling, and Sadeghi et al. (2018) discovered a decrease in soleus, rectus femoris and semitendinosus muscle stiffness following 4-mile, and 13.1mile runs respectively. Moreover, there were also results obtained following various extreme mountainous ultra-marathon events in research from Andonian et al. (2016) and Foure and et al. (2022), showing decreases in the rectus femoris, vastus medialis and vastus lateralis, as well as the gastrocnemius medialis, gastrocnemius lateralis and soleus muscle stiffness respectively. The extreme nature of the ultra-marathons, however, make it difficult to draw detailed and confident conclusions as the extreme distance and unique elevation cause supra-physiological stress which may not be common with more traditional bouts of endurance exercise. Foure et al. (2022) proposed that a consequence of the ultra-marathon may be the development of inflammation and muscle swelling as a protective mechanism to combat the extreme load.

SUMMARY

From the literature review above, it does seem as though the current monitoring and screening processes in elite football are missing a technology such as USWE, which can measure the muscular properties of individual muscle sites, to provide an objective and internal indicator of an elite footballers' physiological readiness. In the literature, it does seem to display an optimal level of muscle stiffness, where superior stiffness can enhance performance, but growing research shows that increased muscle stiffness also heightens injury susceptibility (Kawai, 2021b). Maloney (2024) discusses that the relationship between stiffness and injury is likely "U-shaped," and where this "U" sits relative to stiffness is likely different depending on type of injury and physical profile of the athlete, with factors such as fatigue also likely influencing this relationship further. Therefore, it seems important to perform further research with the USWE in order to develop a standardised methodology of deploying USWE to assist in the implementation of monitoring muscle stiffness in elite football. The research examining the causes of the underpinning physiological mechanisms behind muscle stiffness is poorly understood within the literature, and requires further research to understand the phenomena. Muscle stiffness has been shown to have conflicting findings when assessing the relationships between strength, running, and jumping activities, re-iterating that further research is required to confirm the relationships between stiffness and physical performance to assess whether USWE can be utilised as a potential tool to evaluate athleticism. When investigating how muscle stiffness responds to exercise, on the whole, it seems that endurance-based exercise leads to a decrease in muscle stiffness, whilst eccentric-resistance exercise has conveyed the alternate response, with an increase in muscle stiffness. The research from Lacourpaille et al. (2017) shows there may be a potential relationship between muscle stiffness and muscle damage which would be extremely useful in an elite football setting, but more research is required to better understand it.

CHAPTER 3

APPROACH TO THE PROBLEM

The outcome of the literature review has identified three distinct phases of research to investigate the effectiveness of USWE to measure muscle stiffness in a Premier League academy football setting (Figure 1). The first phase of research will focus on the methodological processes of USWE, and how this can be implemented within an Premier League academy football setting. The use of USWE to assess the musculoskeletal system in sports medicine and athlete monitoring is very novel, and there is not yet an evidence-based, gold-standard methodology in practice to use USWE within an Premier League academy sports setting. The current literature emphasises an inconsistency in the methods used to estimate muscle stiffness USWE, with irregularities in key variables. By performing a systematic review, we aim to highlight the key variables which are consistent throughout the literature, and underline which aspects of the methodology have more variation. From this information, we aim to establish an optimal protocol, based on current knowledge, to estimate muscle stiffness with shear-wave elastography, and to develop a methodology that is suitable for research within a practical setting.

The following two phases of research, physical performance, and readiness/response to exercise, have both been characterized as potential use cases for USWE in an Premier League academy football setting, with the literature indicating that USWE could be important during both of these distinct phases of research. The second phase of research will focus on the relationship between muscle stiffness and physical performance. The literature has emphasized that stiffness may be necessary for performance and has been shown to enhance an athletes' sporting performance through an increase in running speeds, change of direction speed, jump performance, and force production. Therefore, this phase of research will investigate further the relationship between muscle stiffness and physical performance in Premier League academy footballers to determine the effectiveness of utilising USWE as a predictor of physical performance. Preliminary research has been completed on sub-elite footballers using the USWE, but there has been no investigation into the superior physical capability and athleticism of Premier League academy footballers.

The third phase of research will investigate the effectiveness of USWE to be a monitoring tool in assessing readiness and response to exercise in Premier League academy football. Although the literature has reported that different exercise modalities may cause distinct muscle stiffness responses, to the authors knowledge, there is no research which examines the muscular response to a soccer-specific stimulus, whether that be a match or training stimulus. This phase of research will therefore explore the muscle stiffness response to an (1) Premier League academy football match, and a (2) series of training to show the effectiveness of USWE to provide insightful information in a “real-world” football monitoring programme.

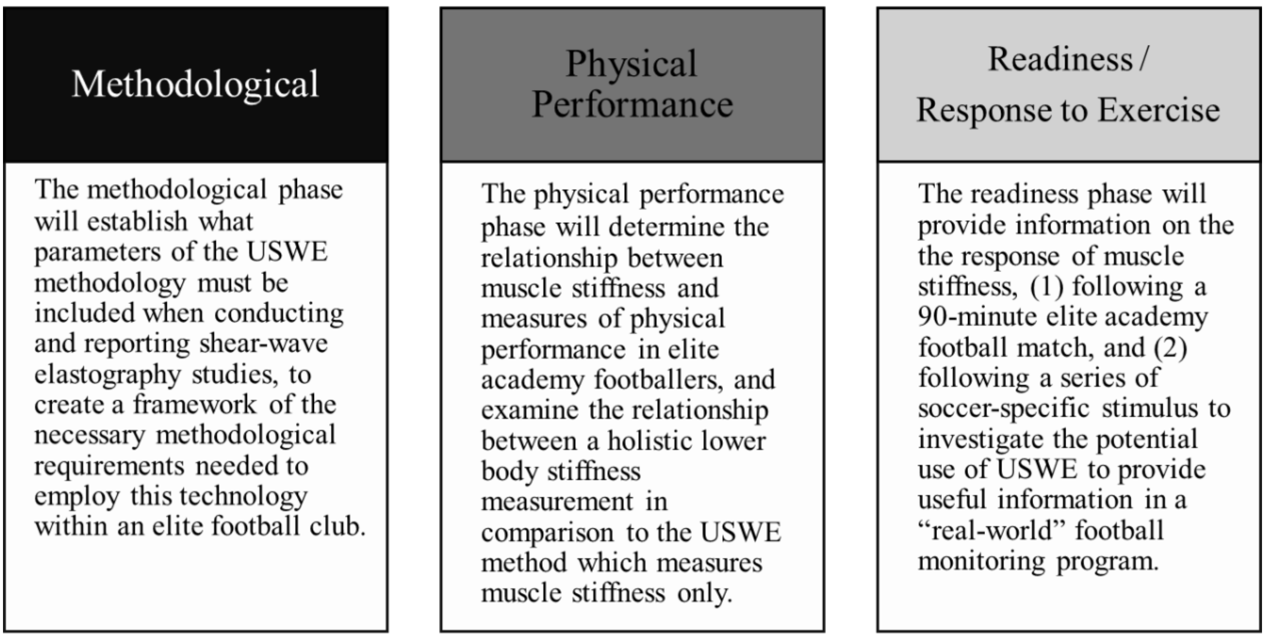


Figure 3.1: The three distinct phases of research during this thesis: (1) Methodological, (2) Physical Performance, and (3) Readiness / Response to Exercise

It is also worth noting, that the delivery of this thesis was affected by COVID-19. Although best efforts were preserved during this period, it did cause disruption to the timeframe of collecting data because of government lockdowns, and premier league restrictions on being in contact with Premier League academy footballers. The commencement of the PhD registration period was December 2019, and COVID-19 lockdowns and preventative measures began in March 2020 and lasted until December 2021. Therefore, this condensed the period of data collection to between January 2022 and May 2022 (the end of the 2021/2022 season), based upon the minimum period of registration ending in December 2022, to allow for adequate time to perform the statistical analysis on the results of the studies. The phase of research which was most heavily affected was the readiness and response to exercise phase, which was originally planned to be broken into two sub-categories: (1) efficacy and (2) effectiveness. Please find attached the summary of planned research prior to the disruption of COVID for the readiness and response to exercise phase of the thesis in the Appendix (Appendix 9.1)

CHAPTER 4

A SYSTEMATIC REVIEW OF THE METHODOLOGY TO ESTIMATE MUSCLE STIFFNESS OF THE RECTUS FEMORIS USING USWE

INTRODUCTION

The purpose of the systematic review is to assess the variation in methods used to measure the shear modulus of the lower-limb using shear-wave elastography in order to estimate muscle stiffness. The systematic review by Cipriano et al. (2022) highlights the lack of data collection and reporting standards. The absence of standardized procedures for collecting and reporting USWE data make it particularly challenging to compare data across article, and to establish normative data (Cirpiano et al. 2022). Costantino and colleagues (2021) have recently published guidelines and recommendations for conventional ultrasound in rheumatic and musculoskeletal diseases, and it seems necessary to create USWE-specific guidelines for authors to abide by, to ensure standardisation across USWE studies. The review from Cipriano and colleagues (2022) emphasises an inconsistency in the reporting of methods used to estimate muscle stiffness with shear-wave elastography, with irregularities in key variables such as scanning procedure, number of repetitions, scans per repetition, condition of muscle during the scan, joint angle, participant position, region of interest and the image processing. By performing this review, we aim to highlight the key variables which are consistent throughout the literature, and underline which aspects of the methodology have more variation. From this information, we aim to establish an optimal protocol, and provide practical recommendations, based on current knowledge, to estimate muscle stiffness with shear-wave elastography on the rectus femoris muscle, and to develop a practical methodology to be used within an Premier League Academy football club.

The rectus femoris was selected as the measurement site for USWE throughout this research thesis for several reasons. The rectus femoris is a muscle with substantial demands from a soccer-specific stimulus, considering its bi-articular anatomy and ability to perform knee extension and hip flexion. in the kinematics and kinetics of movements such as sprinting, jumping and kicking a football, which is specific within this scope of research (Valera-Calero et al. 2024, Brophy et al. 2007, Cerrah et al. 2011 & Mendiguchia et al. 2013). The research from Kalkhoven and Watsford (2017) re-iterates the rectus femoris’ importance in physical performance and elite football, showing a significant relationship between rectus femoris muscle stiffness and sprint speeds, drop jumps and change of direction (Peng et al. 2011). Ekstrand et al. (2011, 2013) illustrated that thigh injuries are the most common muscle injury in football, with 79% of all muscle injuries occurring in the thigh, of which 19% are related to the quadriceps and predominantly the rectus femoris (Cross et al. 2004) which emphasises the need to monitor and track this muscle group to avert injury risk, as well as maximise performance. In the systematic review from Cirpiano and colleagues (2022), the rectus femoris was the 2nd most studied lower-limb muscle group, only behind the gastrocnemius medialis. Casey et al. (2022) stated that the size and accessibility of the rectus femoris make it a practical choice when assessing an area of muscle with ultrasound, which may explain why it is so prevalent in the literature. Studies by Tas et al. (2017) have also demonstrated the high reliability of USWE to assess muscle stiffness of the rectus femoris muscle.

Therefore, the aim of this systematic review is to establish what parameters of the shear-wave elastography methodology must be included when conducting and reporting shear-wave elastography studies, specifically on the rectus femoris muscle. By having a greater understanding of the methodological processes used within the shear wave elastography literature, we will be able to create a framework of the necessary methodological requirements needed to employ this technology within a Premier League football club.

METHODS

Search Strategy

The following electronic bibliographic databases were searched: PubMed, MEDLINE (OVID interface), Web of Science, Scopus, SportDiscus. In addition to electronic bibliographic database searching, as the Methodological Expectations of Cochrane Intervention Reviews (MECIR) standards suggest, the reference lists of the included studies were hand-searched for additional relevant studies that have been missed with the search. Any relevant in-progress work that has not been published yet, was also identified by contacting relevant authors in the field. To minimise the risk of publication bias, grey literature was included too, and it was accessed via the British national bibliography for report literature (BNBRL), OpenGrey database, ProQuest Dissertations & Theses Global and EThOs. If deemed necessary, the authors of potentially eligible studies were contacted to check/confirm whether they had published their study or not. Previous systematic reviews on the same topic were searched too if applicable. However, to our knowledge no previous systematic review exists on this topic. The keywords which were specifically selected and used within the search strategy in the aforementioned electronic bibliographic databases were as follows:

((Shear wave Elastography OR Elastography OR Ultrasound OR Shear Wave Ultrasound Elastography) AND (Shear wave Velocity OR Shear Modulus OR Youngs Modulus OR Muscle Stiffness OR Muscle Elasticity OR Leg Stiffness OR Passive Muscle Stiffness OR Viscoelastic Properties OR Elastic Modulus OR Muscle Damage OR Elastic Properties OR Mechanical Properties OR Active Muscle Stiffness OR Lower Extremity Stiffness) AND (Lower limb OR Leg OR Quadriceps OR Rectus Femoris)).

Inclusion and Exclusion Criteria

Only human participants were included in the review (animal studies and studies with cadaveric specimens were excluded). Studies assessing participants with an illness/injury (for example: Cerebral Palsy/Inflammatory Myopathesis/Duchenne Muscular Dystrophy) were excluded from the review as the aim of the study was to limit the range of participants to normal, healthy individuals and athletes, as the aim was to develop a methodology that was suitable for research within a Premier League football club. Studies which used other types of elastography (such as Magnetic Resonance Elastography, Supersonic Shear Imaging, Transient Ultrasound Shearwave Elastography, Acoustic Radiation Force Impulse Imaging, Contrast Enhanced Ultrasound) or other types of muscle stiffness measuring devices (such as MyotonPro) were also excluded from the review. Studies assessing tendon stiffness, nerve properties, blood flow or stiffness of arteries were excluded from the study unless there were also measurements on muscle. All types of observational studies using quantitative methods were eligible for inclusion (including cohort, case-control, cross-sectional studies, case series and case reports). There was no restriction in terms of the setting or department that the research has been conducted. Based on the scoping searches, it was most likely that the setting of most studies were in a laboratory within a University department. As the purpose of the systematic review was to analyse the methodology performed when utilising USWE, studies were also excluded from the review if no reliability measure on the methodology was present.

Inclusion Criteria	Exclusion Criteria
Human participants	Animal and Cadaveric Studies
Studies including Rectus Femoris measurements	Studies with only participants with identified illness or injury
All observational studies	Studies with participants below 16 years old, or older than 65 years old.
Full text. scientific papers written in English	Studies not including Rectus Femoris measurements (for example, Upper Body, Torso or other lower-limb muscles)
	Studies using any other type of elastography
	Studies only assessing tendon stiffness
	Studies only assessing nerve stiffness
	Studies using any other technological devices to measure muscle stiffness.
	Studies which did not display a reliability measure for the methodology performed

Table 4.1 – Inclusion and Exclusion Criteria of the studies eligible for selection

Data extraction

The titles and/or abstracts of studies retrieved using the search strategy and those from additional sources were screened independently by two review authors (Jordan Slack (JS) and Ignacio Contreras (IC)) to identify studies that potentially met the inclusion criteria outlined below. This included citations and abstracts of potentially eligible studies identified and it allowed the identification and removal of any duplicates before the start of the screening process. The full texts of these potentially eligible studies were then retrieved and independently assessed for eligibility by JS and IC. Any disagreements between them over the eligibility of particular studies was resolved through discussion with a third independent reviewer (Eduardo Martinez-Valdes (EMV)).

A standardised, pre-piloted form was used to extract data from the included studies for the assessment of study quality and for evidence synthesis. The screening forms were pilot tested first by both reviewers on a small number of articles, to ensure their effectiveness. The screening process began with the screening of the titles/abstracts of the identified studies against the inclusion/exclusion criteria by the two reviewers (JS and IC) independently. If further information was required to support the screening process, the authors of the study were contacted. Extracted information is summarised in Table 1.

Two review authors extracted the data independently, and discrepancies were identified and resolved through discussion (with a third author where necessary). If a study consisted of ambiguous or inconclusive results (e.g. unpublished data), the authors were contacted. If the corresponding authors did not respond and further clarification was necessary to classify a study as eligible, this study was excluded. If multiple versions of the same study existed, they were collated, and the primary authors were contacted for further clarification. Similarly, if two or more potentially eligible studies appeared to use the same sample, the authors were contacted to clarify whether the results were duplicated or not. On both occasions, duplicates were removed, and one version was selected and justified.

Table 4.2. Characteristics of included studies	
Date of data extraction:	
Information about data	**Data extracted**
General study information	Title Authors Year of publication
Study Methodology	Study design

	Study setting (including country) Sample size Individuals' characteristics (age, gender, height, weight) Examiner Experience Anatomical Location Scanned Participant Position Joint Angle (associated with the muscle being scanned) Scanning Procedure Scanning Condition (Passive Condition or Contraction) Number of Scans per repetition Ultrasound System Used Probe Size Probe Orientation Region of Interest Depth of Region Image Processing Data Analysis Sampling Frequency Reliability Measure
Outcome	To establish the variability in the reporting of methodological choices
Funding, declaration of conflict of interest	Funding information Conflicts of interest of authors

Table 2 – Data extracted from each eligible study for the purpose of the Systematic Review.

Strategy for data synthesis and Analysis of Sub-Groups

A narrative synthesis was used for the systematic review, as a meta-analysis was not feasible due to the heterogeneity of the data. The narrative synthesis approach adopted a textual approach to tell a story based on the findings of the studies included, which included a degree of statistical data manipulation. The included studies were organised into smaller groups, to make the process of narrative synthesis more manageable, increase the homogeneity and answer specific questions. The analysis was grouped into (1) methodological details, (2) ultrasound-specific details and (3) reliability outcomes. The methodological details focused on variables such as identification of where to scan, participant position, joint angle, equipment used to maintain position, scanning condition and sets/repetitions used. The ultrasound specific details observed the ultrasound machine used, probe size and orientation, and region of interest (ROI). The reliability outcomes analysed the reliability measure, focusing on the statistical test used such as intra-class coefficient (ICC), coefficient of variation (CV) or standard error of measure (SEM). As an aim of this systematic review was to establish an optimal methodology for shear-wave elastography within a Premier League sports team, there will also be a sub-group analysis on any studies which perform rectus femoris measurements on athletes. As the scoping searches only identified limited research on athletes, the athlete analysis was not broken up into further sub-groups, and any research including an athlete population was included.

RESULTS

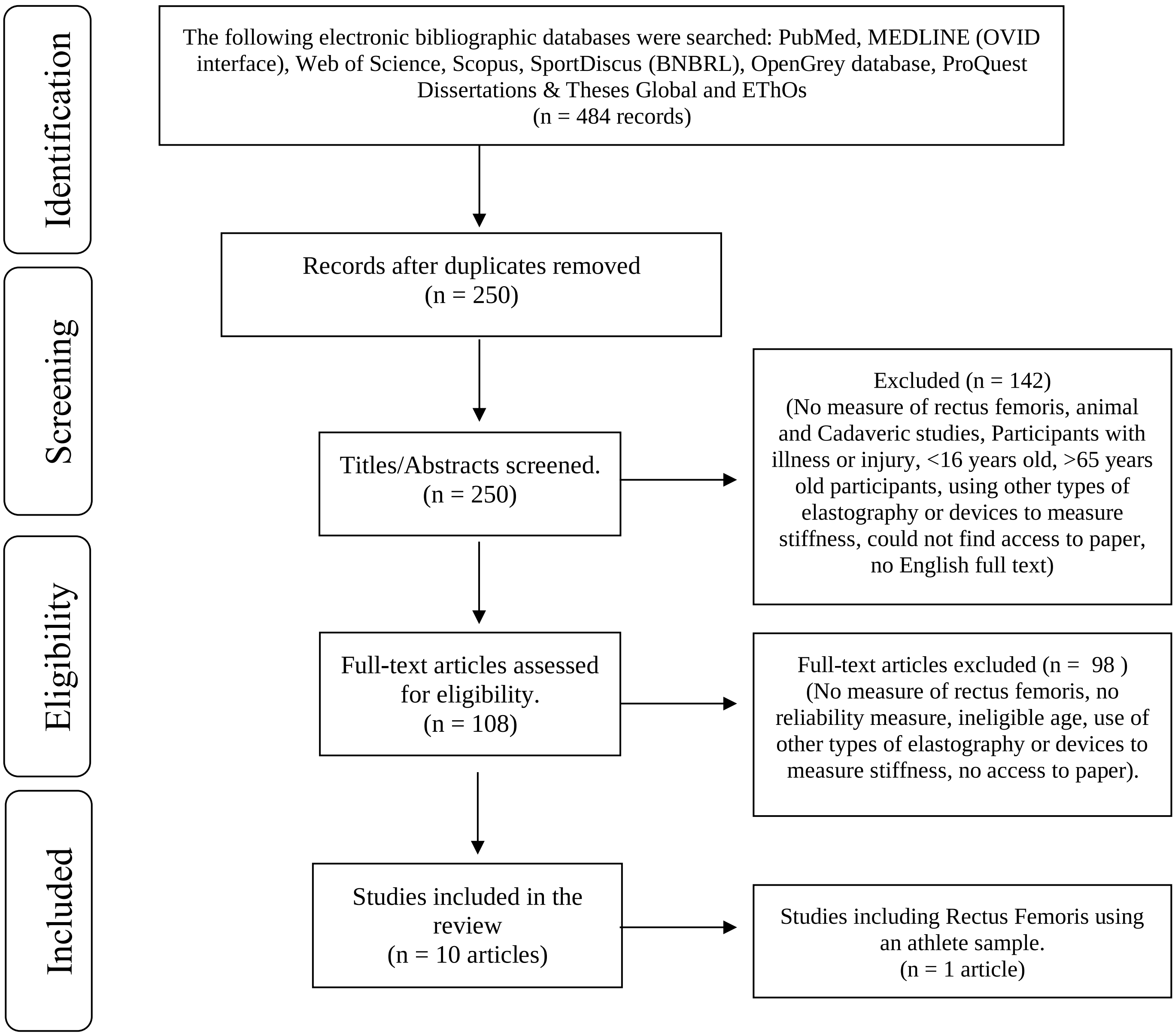


Figure 4.1 – PRISMA Flow Diagram of paper selection process in the current study.

There were 484 records identified during the initial search strategy, with 234 records being removed due to duplication. The remaining 250 records had their titles and abstracts screened to assess their eligibility, and a further 102 records were removed as they did not meet the inclusion criteria. The remaining 108 articles had their full-text articles assessed for eligibility, in which a further 138 full-text articles did not meet the inclusion criteria due to, no reliability measure (n = 53), illness or injury present in the study population (n = 18), no measure of rectus femoris (n = 15), ineligible age (n = 10), using a different type of elastography (n = 8), and no access to full-text article (n = 4). Following the paper selection process, 10 studies met the eligibility criteria for the systematic review. Of the 10 full-text articles 1 of these papers used an athletic population and this will be used for a further sub-group analysis. From the 10 full-text articles, there was a sample size of 227 participants comprised of 176 males and 51 females (Table 3). The age of the participants ranged from 21.8 ± 1.4 years (Coombes et al. 2018) to 43.1 ± 9.1 years (Andonian et al. 2016). The height of the participants ranged from 159.1 ± 1.5 cm (Wu et al. 2020) to 180 ± 6.7 cm (Bouillard et al. 2012). The weight of the participants ranged from 51.5 ± 1.2 kg (Wu et al. 2020) to 95 ± 1.0 kg (Tas et al. 2017).

Author	Title of Paper	Sample Size	Gender	Participant Demographics
Andonian et al. 2016	Shear-Wave Elastography Assessments of Quadriceps Stiffness Changes prior to, during and after Prolonged Exercise: A Longitudinal Study during an Extreme Mountain Ultra- Marathon	N=50	46 Male / 4 Female	age: 43 ± 9.1 years, height, 175 ± 6.2 cm; body mass, 72.2 ± 8 kg
Bouillard et al. 2012	Effect of vastus lateralis fatigue on load sharing between quadriceps femoris muscles during isometric knee extensions	N=16	15 Male / 1 Female	age: 24.6 ± 2.6 years, height: 180 ± 6.7 cm, weight: 73.9 ± 9.8 kg
Coombes et al. 2018	Heterogeneity of passive elastic properties within the quadriceps femoris muscle-tendon unit	N=19	10 Males and 9 Females	age: 21.8±1.4 years; height: 171.5±9.4 cm and weight: 66.1±10.3 kg)
Dubois et al. 2015	Reliable protocol for shear wave elastography of lower limb muscles at rest and during passive stretching	N=10	Males	(age: 25.5 + 2.8 y, height: 176 + 11.0 cm, weight: 68 + 13.3 kg, body mass index [BMI]: 21.7 + 2.0 kg/m)
Freitas et al. 2019	Does epimuscular myofascial force transmission occur between the human quadriceps muscles in vivo during passive stretching?	N= 12	12 Males	age: 23.7 ± 3.6 yrs; height: 1.76 ± 0.08 m; body mass: 71.2 ± 8.9 kg
Nakamura et al. 2021	The comparison of different stretching intensities on the range of motion and muscle stiffness of the quadriceps muscles	N=18	Males	age: 22.7 + 2.8 years; height: 169.1 + 4.2 cm; body mass: 63.6 + 6.6 kg
Otsuka et al. 2019	Dependence of muscle and deep fascia stiffness on the contraction levels of the quadriceps: An in vivo supersonic shear-imaging study	N=14	Males	(age, 26 ± 4 years: height, 171.5 ± 6.2 cm: body mass 65.5 ± 11.0 kg; means ± SDs)
Ren et al. 2021	Changes in muscle hardness from resting to mid-range lengthened positions detected by shear wave elastography (SWE) with a novel protocol of ultrasound probe placement	N=26	14 Males and 12 Females	Age: 33.5 ± 5.9 years, height: 1.66 ± 0.10 m, weight 60.3 ± 10.8 kg, BMI 21.4 ± 2.6
Tas et al. 2017	Shear wave elastography is a reliable and repeatable method for measuring the elastic modulus of the rectus femoris muscle and patellar tendon	N=12	Males	ranged in age from 19 to 33 years (mean age 25.33 ± 4.56 years). The mean body mass index, weight, and height were 25.57 ± 3.48 kg/m2 (range, 19.10–30.32 kg/m2). 78.96 ± 10.88 kg (range, 61.90–95.00 kg), and 1.76 ± 0.05 m (range, 1.70–1.86 m), respectively.
Wu et al. 2020	In vivo assessment of material properties of muscles and connective tissues around the knee joint based on shear wave elastography	N=50	25 Males and 25 Females	Age groups ranging from 23.6 ± 0.6 to 61.2 ± 1.4 years, 159.1 ± 1.5 to 173.4 ± 1.2 cm and 51.5 ± 1.2 to 69.7 ± 1.7 kg

Table 4.3 – Eligible Article Information and Study Details

Author	Identification of Where to Scan	Participant Position	Joint Angles	Equipment Used	Scanning Condition	Sets/Repetitions of Scanning
Andonian et al. 2016	Line drawn 15 cm above the patella, perpendicular to the patella-ASIS axis and parallel to the longitudinal axis of the center of the RF.	Supine Position	All subjects were examined in the supine position with their knees passively flexed at 20°	Feet holder to ensure that the muscle remained at rest. An articulated arm ensures that no contact occurs between the transducer and thigh	Relaxed	Three successive SWE acquisitions were performed per scanning session
Bouillard et al. 2012	a region with a muscle thickness of at least 1.5 cm, avoiding hypoechoic regions related to dense connective tissues	Seated	With their trunk and right leg flexed at 85° and 80°	Isokinetic Dynamometer	Active. Isometric Knee Extension	Each muscle was scanned 2 x 10 seconds in random order
Coombes et al. 2018	50-75% thigh length for RF	Seated	Backrest declined by 30° from upright. Test angles of 25°, 40°, 55°, 70° and 85° of knee flexion.	Isokinetic dynamometer	Knee was passively moved to the following angles 25°, 40°, 55°, 70° and 85°	N/A
Dubois et al. 2015	The anterosuperior iliac spine and the apex of the patella were identified by palpation, and the inferior third of their distance was marked.	Seated and supine positions	Passively stretched: supine knees hanging outside the table. Rest: Sat upright with the heels outside the table; hip angle 90°.	None	Relaxed and Stretched	3 operators x 6 measurements x 2 positions
Freitas et al. 2019	At 20–40% and 60–80% of each muscle's proximal-to-distal length, according to the fascicle orientation	Seated and supine positions	Participants assumed two positions: seated with the hips at 8°; and lying supine with the hips at 0°.	Isokinetic dynamometer and Probe Holder	Stretched	x 2 regions x 2 repetitions
Nakamura et al. 2021	Measured at the midpoint, 60 and 80% distal between the anterior superior iliac spine and the proximal end of the patella, respectively	Supine	The participants were lying on the treatment bed at neutral hip joint position with 90° flexed hip and knee joint	None	Stretched	Long-axis elastographic images were obtained two times.
Otsuka et al. 2019	RF muscle belly (midpoint of the greater trochanter and lateral condyle of the femur)	Seated	Hip flexed 80° and knee flexed 70°	Isokinetic dynamometer	Rest and during isometric knee extension contractions at 20%, 40% and 60% of the MVC	The SWV was measured at two sites and two directions during contractions separately in a random order
Ren et al. 2021	The ASIS and the superior border of the patella, with the transverse line drawn in the middle of the line between landmarks.	Supine	For the RF the knee extended and then passive flexed at 90°.	None	Rest and mid-range lengthened	N/A
Tas et al. 2017	3 cm along the longitudinal axis and along the ASIS line with the patella proximal to the plumpest section of the muscle	Seated	Knee extended and hip at 90°	None	Relaxed	Mean of 6 (2 x 3) measurements was recorded. Repeated 3 times at 20 minutes and 1 week after the first measurements
Wu et al. 2020	The kinesiology of the musculoskeletal system (Neumann, 2006) and atlas of human anatomy (Netter, 2018) were used as references to determine where to scan.	Supine	Supine position on a bed with their knee fully extended and ankle in a neutral position.	None	Relaxed	For each observed muscle SWE measurements were performed three times by three operators

Table 4.4 – Methodological Details of each Eligible Study

Author	Ultrasound Machine Used	Probe Size	Probe Orientation	Region of Interest (ROI)
Andonian et al. 2016	An Aixplorer ultrasound system (version 8.0; Supersonic Imagine, Aix-en-Provence, France)	linear transducer (4–15 MHz, SuperLinear 15–4; Vermon, Tours, France)	Transducer parallel to the skin plane and when several thin fascicles could be traced across the image without interruption	A fixed-size square region of interest (1.2 cm2) delimiting the elastographic field-of-view with a 5mm diameter ROI
Bouillard et al. 2012	Aixplorer ultrasound scanner (version 4.2, Supersonic Imagine, Aix en Provence, France)	a linear transducer array (4–15 MHz; SuperLinear 15-4, Vermon, Tours, France)	The probe was aligned to the muscle fiber direction for vastus muscles	A region of interest (ROI) was the largest rectangular muscular region available that avoided aponeuroses, tendon, and bone
Coombes et al. 2018	Aixplorer Ultrasound Scanner (version 9.3; Supersonic Imagine, Aix-en-Provence, France)	linear transducer (4–15 MHz, Vermon, Tours, France)	For RF, the angle of the probe was tilted relative to the skin, to align with the three dimensional fascicle arrangement .	An area within the elastogram that avoided overlying facia or areas void of colour was selected. Mean ± SD ROI areas (cm2) were .04±0.29 respectively.
Dubois et al. 2015	Aixplorer ultrasound scanner (Version 4.2, Supersonic Imagine, Aix-en-Provence, France)	4- to 15-MHz ultrasonic probe	Acquisitions were performed with the probe in a plane parallel to the muscle fibers and perpendicular to the skin.	The ROI was defined in the square region of shear modulus measurement as the largest square between fasciae.
Freitas et al. 2019	Aixplorer, v11; Supersonic Imagine, Aix-en-Provence, France	A linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France	Scan was measured according to the fasicle orientation	Only pixels of the color map containing the muscular region of interest were selected by avoiding aponeurosis areas or other non-muscular structures
Nakamura et al. 2021	Aixplorer Supersonic Imagine, Aix-en-Provence, France	a SL10-2 linear probe	N/A	The size of the region of interest was 10 x 20 mm and set near the each muscle center, with an analysis area of a 5-mm-diameter circle at the center of the stiffer region
Otsuka et al. 2019	(Aixplorer version 6.4, Supersonic Imagine, Aix-en-Provence, France)	L15–4, Supersonic Imagine, Aix-en-Provence, France	N/A	The region of interest (ROI) for the muscles was set manually.
Ren et al. 2021	An Aixplorer multiwave ultrasound scanner (version 10.0; Supersonic Imagine, Aixen-Provence, France)	A linear transducer array (4–15 MHz, Super Linear 15-4, Vermon, Tours, France)	The probe was align with the muscle contraction direction.	The location of the region of interest (ROI) and the diameter of Q-Box was adjusted to avoid the artificial part, with a minimum diameter of 6 mm.
Tas et al. 2017	Acuson S3000 ultrasound system (Siemens Medical Solutions, Mountain View, CA)	9L4 (4–9-MHz)	The rectus femoris muscle was assessed by placing the ultrasound transducer longitudinally in relation to the muscle fibers	N/A
Wu et al. 2020	(Supersonic Imagine, Aix-enProvence, France)	a linear transducer array (10-2 MHz, SL 10–2, Aix-en-Provence, France)	The probe was controlled to maintain an operating orientation that was parallel to the muscle fibers.	10 mm x 10 mm squared-shaped ROI and 10 mm x 20 mm rectangular-shaped ROI

Table 4.5 – Ultrasound Specific Details of each Eligible Study

Author	Reliabilty Measure	Reliability Test Used	ICC	CV	SEM
Andonian et al. 2016	Reliability	ICC and SEM	Pre - 0.89 (0.84-0.93), Mid - 0.92 (0.88-0.96), Arrival - 0.92 (0.87-0.95), Recovery 0.91 (0.85-0.95)		Pre - 0.16 kPa. Mid - 0.15 kPa Arrival - 0.15 kPa. Recovery - 0.14 kPA
Bouillard et al. 2012	Reliability	ICC and SEM	0.962		3.4 kPa
Coombes et al. 2018	Within-day reliability	ICC and CV	0.88 (0.77 - 0.94)	4.80%	0.10 m/s
Dubois et al. 2015	Inter-operator reproducibility and intra-operator repeatability.	CV and ICC	0.92 (0.91 - 0.94)	Inter-Operator - 9% Intra-Operator - 8%	
Freitas et al. 2019	Repeatability for each muscle.	ICC	0.73 - 0.99		
Nakamura et al. 2021	Test-retest reliability	ICC and CV	0.876	6.2 ± 3.2 %	
Otsuka et al. 2019	Intrasession reliability	CV, SEM, and ICC	Longitudinal - (Rest) 0.91, (20%) 0.91, (40%) 0.62, (60%) 0.75. Transverse - (Rest) 0.95 (20%) 0.65, (40%) 0.83, (60%) 0.83	Longitudinal - (Rest) 3.4, (20%) 6.0, (40%) 10.2, (60%) 6.8. Transverse - (Rest) 4.2 (20%) 8.8, (40%) 9.3, (60%) 6.7	Longitudinal (m/s) - (Rest) 0.02 (20%) 0.10, (40%) 0.37, (60%) 0.24. Transverse (m/s) - (Rest) 0.04 (20%) 0.12, (40%) 0.15, (60%) 0.41
Ren et al. 2021	Intra-rater and inter-rater reliability	ICC	Intra Rater - (Rest) 0.786, (Mid-Length) 0.781 Inter-rater - (Rest) 0.732, (Mid-Length) 0.711		
Tas et al. 2017	Interobserver, intraobserver, and interday reliability	ICC, CV, and SEM	Intraobserver - 0.93 (0.83 - 0.97) & 0.94 (0.84-0.97). Interday - 0.91 (0.78-0.96) & 0.81 (0.54-0.92). Interobserver - 0.95 (0.32-0.88)	Intraobserver - 8.7% & 7.8%. Interday - 9.8% & 11.7%. Interobserver - 6.3%	Intraobserver - 0.18 & 0.16 m/s Interday - 0.2 & 0.24 m/s Interobserver - 0.17 m/s
Wu et al. 2020	Repeatability	ICC	0.987 (0.979 - 0.992)		

Table 4.6 – Reliability Measurements of each Eligible Study

Variance in Methodological Details of the Studies

The methodological details for each eligible study, as hypothesized, are generally inconsistent when considering the identification of where to scan, participant position, the joint angle in which the scan took place, and scanning condition (Table 4.4). 3 of the 10 studies identified the mid-point of the muscle (Nakamura et al. 2021, Otsuka et al. 2019 & Ren et al. 2021) for the USWE scan, whereas Coombes et al. 2018 and Freitas et al. 2019 used a percentage of muscle length, 50-75% and 20-40% to 60-80% respectively. Andonian et al. 2016 used a set measurement of 15cm above the patella, and Bouillard et al. 2012 and Tas et al. 2017 used a slightly different method where they used a position in the muscle where there was at least 1.5cm muscle thickness or searched for the “plumpest section” of the rectus femoris muscle.

Of the 10 articles, 3 papers assessed the rectus femoris in only a relaxed condition (or at rest)(Andonian et al. 2016, Tas et al. 2017, & Wu et al. 2020), 3 papers assessed the muscle in only a passively stretched position (Coombes et al. 2018, Freitas et al. 2019, & Nakamura et al. 2021), and 1 paper assessed the muscle using only an isometric contraction (Bouillard et al. 2012). 3 papers measured the rectus femoris in more than one condition. Dubois et al. 2015 and Ren et al. 2021 measured the rectus femoris in both a relaxed and passively stretched position, and Otsuka et al. 2019 measured the muscle in a relaxed condition and during an isometric contraction. 4 of the 10 papers (Bouillard et al. 2012, Coombes et al. 2018, Freitas et al. 2019, & Otsuka et al. 2019) used an isokinetic dynamometer to maintain participant position and to fix in specific joint angles and Andonian et al. 2016 used a custom-made foot holder to ensure that the muscle remained at rest and an articulated arm to ensure that no contact occurred between the transducer and thigh. Freitas et al. (2019) also used a probe holder to place the USWE probe on to remove the probability of human error due to the sensitivity of the device.

Of the 10 articles, 5 used a seated position to measure the rectus femoris muscle stiffness, and 6 used a supine position (Freitas et al. 2019 used both a seated and supine position during their study). Of those who measured the rectus femoris in a seated position, both Tas et al. 2017 and Dubois et al. 2015 had the participant seated in an upright position with knees extended, hips at 90° and heels outside the table and Freitas et al. 2019 had the participant seated with the hip at an 8° angle. Bouillard et al. 2012 and Otsuka et al. 2019 both used a similar participant position with the hip and knee flexed at 85° and 80°, and 80° and 70° respectively. Coombes et al. 2018 used several joint positions during his seated assessments, measuring the stiffness at a 25°, 40°, 55°, 70° and 85° all with the backrest declined by 30° from upright. From the participant positions and joint angles in a supine position, the methodologies seemed to have more consistency than when compared with seated. Freitas et al. 2019 and Wu et al. 2020 laid supine with knees fully extended and ankles in neutral position. Andonian et al. 2016 followed a similar methodology but with a small 20° joint angle of the knees in a passively flexed position whilst laid in a supine position. Dubois et al. 2015, Nakamura et al. 2021 and Ren et al. 2021 all laid supine with knees flexed at 90° hanging over the edge of the bed.

The methodological variable which displayed the most variation was the scanning procedure and the sets and repetitions performed to measure the muscle stiffness. Not only were methodologies inconsistent, but there was also an inconsistency in the amount of detail provided for this specific information. Two of the ten papers did not provide any information regarding the scanning protocol (Coombes et al. 2018, & Ren et al. 2021). Of the 8 papers that did report scanning procedures, 2 completed three scans (Andonian et al. 2016, & Wu et al. 2020), although Wu et al. 2020 did repeat this procedure with three different operators. 4 papers completed 2 repetitions to measure muscle stiffness. Bouillard et al. 2012 completed 2x10sec videos, Freitas et al. 2019 completed x 2 reps in 2 different regions of the muscle, Otsuka et al. 2019 completed scans on 2 sites in 2 different directions and Nakamura et al. 2021 just acknowledged that two scans had been obtained. Dubois et al. 2015 and Tas et al. 2017 both completed 6 scans. Dubois et al. 2015 completed 6 measurements in 2 positions measured by three different operators, and Tas et al. 2017 took a mean of 6, after recording 2 x 3 measurements.

Variance in Ultrasound Specific Details for each Study

The ultrasound specific details for each eligible study, however, did show more consistency when considering the ultrasound machine used, probe size and probe orientation (Table 4.5). 9 out of the 10 papers used an Aixplorer Ultrasound Scanner (Supersonic Imagine, Aix-en-Provence, France), albeit with different versions depending upon the year in which the research took place (ranging from version 4.2 to version 11). Of these 9 papers, 7 also used the same probe size to measure the muscle stiffness utilising a linear transducer array (4–15 MHz; SuperLinear 15-4, Vermon, Tours, France). The two outliers (Nakamura et al. 2021, & Wu et al. 2020) used a different probe size during their studies, with a linear transducer array (10-2 MHz, SL 10–2, Aix-en-Provence, France). The only paper which did not use an Aixplorer Ultrasound Scanner (Tas et al. 2017), used an Acuson S3000 ultrasound system (Siemens Medical Solutions, Mountain View, CA) with a 9L4 (4–9-MHz) probe. 8 out of the 10 papers also used the same probe orientation, measuring the rectus femoris in a longitudinal plane, aligned to the muscle fibre direction. The 2 other papers did not disclose their probe orientation information, but it is hypothesized that Otsuka measured in both a longitudinal and transverse plane based upon reliability measurements in the results section.

Though most ultrasound specific details showed a greater degree of consistency, the region of interest (ROI) used to measure the exact anatomical location of muscle stiffness did have a larger variation in detail provided. Only 3 of the 10 papers highlighted that they used a set ROI which was consistent throughout their respective studies. Wu et al. 2020 used a 10 mm x 10 mm squared-shaped ROI and 10 mm x 20 mm rectangular-shaped ROI, and both Andonian et al. 2016 and Nakamura et al. 2021 used a 10 x 20 mm rectangle, which was sat near the muscle centre, with an analysis area of a 5-mm-diameter circle at the centre of the stiffer region. 6 of the 10 papers, stated that their ROI was manually set to the largest region available that avoided aponeuroses, tendon, and bone but only Ren et al. 2021 included a measurement value, stating the minimum ROI had to have a diameter of 6-mm. Coombes et al. 2018 did state that the mean ± SD of the ROI areas (cm2) were .04 ± 0.29 during their study, which does emphasize that each participant had a different sized ROI. Only Tas et al. 2017 did not include any information in relation to the ROI.

Reliability Outcomes from the Systematic Review

The reliability measures collected were within-day reliability, inter-operator reproducibility, intra-operator repeatability, test-retest reliability, intrasession reliability, intra-rater/inter-rater reliability and interobserver / intraobserver / interday reliability. 7 of the 10 papers stated two or more reliability measures, whereas 3 papers only displayed one. All 10 papers displayed an intra-class correlation coefficient (ICC) for a reliability measure (Table 4.6). The ICC from the selected studies ranged from moderate reliability (ICC=0.62, Otsuka et al. 2019) to an excellent standard of reliability (ICC=0.99, Wu et al. 2020). 5 papers displayed a coefficient of variation (CV), and 5 papers displayed a standard error of measurement (SEM). The CV from the selected studies ranged from 3.4% (Otsuka et al. 201) to 11.7% (Tas et al. 2017) rated very good, and good respectively. The SEM from the selected studies ranged from 0.02 m/s to 0.41

m/s (Otsuka et al. 2019) and 0.14 kPa (Andonian et al. 2016) to 3.4 kPa (Bouillard et al. 2012) depending upon the measurement used to assess the muscle stiffness. The study from Otsuka et al. 2019 clearly highlighted how the intensity of a MVC influenced the reliability in comparison to measuring the muscle stiffness at rest. When assessing muscle stiffness at 40% MVC, the ICC decreased to 0.62 in comparison to 0.91 at rest and at 20% MVC, which shows the decrease in reliability when the intensity of the contraction increases. In comparison to the rest of the studies which assessed muscle stiffness in a relaxed or passive state, the intra-rater and inter-rater reliability assessed by Ren et al. (2021) did show a slightly lower ICC (0.711 to 0.786), and the interday and interobserver reliability measured by observer 2 by Tas et al. (2017) did show a range of ICC down to 0.54, and even 0.32.

DISCUSSION

This systematic review emphasizes the inconsistency and variation when considering using USWE for measuring muscle stiffness, even when assessing the same muscle group. As USWE starts to be introduced into more practical and applied settings of professional sport, it is important to begin to derive a consistent protocol, based on current knowledge and literature, to ensure uniformity across future research. The aim of this review was to identify what aspects of the USWE methodology consists of more variation, and which aspects are more constant throughout the literature, in order to develop a framework of what elements of a methodology should exist when designing a protocol including USWE to measure muscle stiffness.

Identification of where to scan the assessed muscle and Region of Interest

During the aforementioned studies, there is a clear difference when considering which region of the rectus femoris is measured, the process used in which they identify the location to scan and the ROI which is utilised to measure the muscle stiffness. As USWE only measures the muscle stiffness of a small region and cannot take a measurement which describes the whole muscle, it must be considered that if scans were taken on different locations of the muscle that there could potentially be different stiffness outcomes, due to the inhomogeneity of muscle tissue. This review accentuates the variation in the ROI used to assess muscle stiffness, but also stresses how miniscule the area of muscle which is being scanned is, according to our findings, the most used ROI is between 10 x 10 and 10 x 20mm. This is emphasized further, as 2 of the papers measure muscle stiffness at multiple sites across the muscle, in order to provide more context of the stiffness across the whole muscle rather than a single value. In the literature, studies investigating differences in regional rectus femoris muscle stiffness are less prevalent and report conflicting findings. A study from Kodesho and colleagues (2021a) displayed a higher degree of muscle stiffness at proximal locations in comparison to central or distal locations of the rectus femoris. It is hypothesized that muscular tension from a connecting tendon may cause increased stiffness, which may explain the greater shear modulus at a proximal location due to its proximity to the rectus femoris tendon. However, one of the articles in the systematic review, Freitas et al. (2019) displayed no significant differences based on location of the USWE scan. The inconsistency of measurement location is a limitation of the current research, and it is clear that more research must be done, specifically in the rectus femoris, to validate where the most effective position is to measure based upon the research question being asked.

Participant Position and Joint Angle

During this review, there was a divide in which studies performed the scan in a seated or a supine position. This was largely dependent upon whether the aim of their respective study was to measure the rectus femoris in a relaxed (or at rest) state, a passively stretched (lengthened) state or whether to examine the muscle during an isometric contraction. This review demonstrates that the reliability of performing a USWE can be influenced by the condition of the muscle when being scanned. For example, Ren et al. (2021), albeit marginal, did display a decrease in ICC when comparing the rectus femoris in a relaxed and lengthened position, and Otsuka et al. (2019) emphasized this further by a more significant decrease in ICC, when comparing measurements at rest to when performing different intensities of an MVC. Although measuring muscle stiffness during an isometric contraction may be the most applicable and relevant action to a sporting task or act of physical performance, it is noticeable that an increase in intensity can lead to a significant drop in reliability as illustrated by Otsuka et al. (2019). That being said, Bouillard et al. (2012) highlighted excellent reliability (ICC = 0.962) when only measuring the MVC at 20% intensity, which may present the threshold of contraction that ensures quality and accuracy of the scan.

Access to an isokinetic dynamometer is fundamental not only for performing an isometric contraction like Otsuka et al. (2019) and Bouillard et al. (2012), but also if the researcher would like to determine specific joint angles and restrict participant movement in studies such as Freitas et al. (2019) and Coombes et al. (2018). The only exception is Andonian et al. (2012) who built a custom-built foot holder to ensure that the knee stayed at 20°, which demonstrates an alternative way to quantify stiffness in a standardised way, in the absence of specialised equipment during an extreme mountain ultra-marathon. When comparing the reliability measurements of the studies that used equipment to fix participant positions, and those who simply used the participant sat or laid on the bed, there does not seem to be a difference in the reliability outcomes. However, this may be a result of measuring the rectus femoris muscle, and the ease and access of manipulating a therapy bed to achieve a hip and knee angle of 90° or full extension without requiring an external stimulus. For example, to assess the biceps femoris muscle with a flexed knee, there is a necessity for a piece of equipment to lock the leg into the correct joint position, or an examiner is required to manually hold the limb which will influence the reliability and accuracy of the scan. An increasingly common methodological approach used by Freitas et al. (2019) was to use a probe holder to fix the transducer on the desired location. Due to the sensitivity of the USWE technology, a probe holder removes the probability of human error. When trying to perform more advanced studies involving USWE with different methods of contraction or movement, a probe holder becomes essential, as the possibility of error and difficulty for the examiner increases significantly when trying to maintain a position with the probe when the muscle is contracting.

Examiner Experience

If a probe holder is not used, examiner experience to reduce the possibility of human error becomes even more significant. Of the 9 other papers, only 4 papers mention how much experience the ultrasound examiner had. Andonian et al. (2016) and Wu et al. (2020) used a radiologist with 4 years and 3 years of experience respectively, Coombes et al. (2018) used an examiner with 18 months experience, and Tas et al. (2017) used two examiners, one with 5 years of experience and one with 1 year of experience. The study by Tas et al. (2017) put emphasis on the importance of examiner experience, as their interday reliability between both observers shows an ICC difference of 0.1 (0.91 – 0.81) when comparing the examiner with 5 years of experience in relation to the examiner with 1 year of experience. That being said, as the examiners during these studies are not using the imaging for anything other than measuring muscle stiffness, the examiner only needs a general understanding of identifying anatomical locations and being able to correctly position and handle the ultrasound probe. However, this is a procedure that cannot be performed by an untrained individual, as it does require a level of expertise to perfect the technique of applying minimal probe pressure, keeping the hand as still as possible and ensuring the probe is correctly orientated. If the study includes any further assessment such as diagnosis of an injury, or a clinical evaluation of an image then a more extensive background and education in ultrasound and imaging will be essential.

Scanning Procedure

The scanning protocol itself was also very inconsistent across the review, with all studies performing an array of different sets and repetitions to attain their final measurement. 4 of the studies only completed 2 repetitions of the scan, whereas 4 studies completed 3 repetitions for each scan to achieve an average value for each scanning session. From the review, it did not seem that there was much justification or rationale for the scanning procedure, apart from referring to previous studies or creating a methodology to fit their study based upon the number of muscles assessed, number of observers, and time constraints for example. 3 studies used multiple observers during their study to assess inter-observer reliability to ensure the accuracy of the USWE scans and to reduce the likelihood of error. Bouillard et al. (2012) also highlighted collecting a 2 x 10 second clip of each USWE scan to retrieve an average muscle stiffness value rather than a still image, which presents as a useful idea especially when measuring during an isometric contraction, which as this review shows, can significantly decrease reliability.

When considering ultrasound-specific details, there did tend to be more consistency, especially when considering the ultrasound device used, probe size and probe orientation. It seems to be a standardized approach from the articles within this review to use an Aixplorer ultrasound system (Supersonic Imagine, Aix-en-Provence, France) with a linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France) with the scans being performed with the probe in a plane parallel to the muscle fibres and perpendicular to the skin, with only a few exceptions to the outlines listed above. When reviewing the USWE literature, it does seem as though the Aixplorer ultrasound system is the most used in research-focused studies, but different types of ultrasound devices have been introduced to the literature as the technology becomes more readily available.

Athlete Demographic – Sub-Group Analysis

In this review, there was one article which used an athlete demographic and assessed muscle stiffness of participants before (4 days before), during (mid-point), upon finishing (1 h after finish) and post- (48-72 hours after the race) a 330km mountainous extreme ultra-marathon (Andonian et al. 2016). Andonian and colleagues used novel methods during their scanning processes in order to maximise accuracy and reliability of scans, especially when having to collect scans as quickly and efficiently as possible during a competitive race situation. They used a custom-made system which was developed to enable transducer position-locking once optimal locations and orientations were obtained, a foot holder to ensure that the quadriceps femoris muscle remained at rest, and an articulated arm to ensure that no contact occurred between the transducer and the thigh. Andonian et al. (2016) was one of only three studies to use an intervention within their study and assess the response of an exercise stimulus. Bouillard used a 6 x 10-s knee extension protocol at 20% of maximal voluntary contraction (MVC) followed by a sustained submaximal isometric knee extension at 20% of MVC until task failure, and Nakamura et al. (2021) measured muscle stiffness pre- and post- 3 different stretching protocols.

The study by Andonian et al. (2016) was the only study in this review to perform a pre- and post- study to assess the muscle stiffness response following a bout of exercise, similar to the prospective study in this thesis assessing the muscle stiffness response following a Premier League academy football match. Another methodological consideration for this type of study involves the timing of USWE measurements for examining the effect of an exercise bout on muscle stiffness. In the current literature, the research from Lacourpaille and colleagues (2014, 2017), Guilhem and colleagues (2016), and Siracusa and colleagues (2019) exhibit changes to muscle stiffness 1hr- post exercise intervention before soon returning to baseline levels, yet other studies (Sadeghi et al., 2018, Chalchat et al., 2022, & Xu et al., 2019) have reported more sustained changes to muscle stiffness which last longer than 1hr. When considering the research from Andonian and colleagues, the quadriceps muscle stiffness had a significant decrease immediately post-competition and did not return to baseline for some of the ultra-marathon competitors for up to 72hr. As an ultra-marathon is a cause of extreme supraphysiological stress, fluid accumulation due to oedema, could also be a reason for the sustained decrease in muscle stiffness in comparison to the other studies, as the body provides a protective mechanism to combat the extreme load. The extreme tissue stress experienced by the ultra-marathon is also demonstrated by the significant increases in plasma creatine kinase (CK), myoglobin, lactate, and C-reactive protein (CRP). The inconsistency in the muscle stiffness response between these research studies is likely due to the variance in exercise stimulus, especially when comparing an ultra-marathon to the prospective Premier League academy football match in this thesis for example, with entirely different physiological and functional requirements. From the literature, it seems as though the intensity and volume of exercise bouts influences the length of time which the stiffness is altered for, which is hypothesized to be related to the amount of tissue stress experienced. Therefore, it seems as though measuring muscle stiffness within 1hr post-intervention seems useful to encompass all types of exercise stimulus in measuring a response to exercise.

Conclusion

When taking into consideration the above information, it is clear that there is no established, evidence-based protocol to collect USWE scans in the current research. As the popularity of USWE increases, and interest from within professional sports teams starts to rise, it is imperative to consider the current literature in order to develop a framework of what elements of a methodology should exist when designing a protocol including USWE to measure muscle stiffness. From this systematic review, the practical recommendations for implementing a USWE methodology to measure rectus femoris muscle stiffness in a Premier League football club are as follows:

Practical Applications

1. Using the midpoint of the muscle (between the ASIS and the superior border of the patella, with the transverse line drawn in the middle of the line between landmarks) for the anatomical location of the USWE scan (Nakamura et al. 2021, Otsuka et al. 2019 & Ren et al. 2021). This will avoid interference from the tendon when measured distal or proximal to the muscle belly and will help to standardize muscle stiffness values across future research. The exception would be if the study is analysing regional

differences within a muscle and has multiple scans across the muscle length, but it is still advised that one of the scans would be at the midpoint of the muscle.

2. The gold standard for fixing a participant into the correct joint angle and position is to use an Isokinetic Dynamometer (Bouillard et al. 2012, Coombes et al. 2018, Freitas et al. 2019, & Otsuka et al. 2019) to avoid unnecessary movement which may affect scan accuracy. However, this review establishes when measuring the rectus femoris, the reliability outcome does not decrease when only a treatment bed is used due to the accessibility and ease of scanning the rectus femoris muscle. As a treatment bed can manipulate both knee and hip angle to 90° by simply sitting upright, or lying supine with knees overhanging the bed, quality data can still be attained in a relaxed and passively stretched state without the use of an IKD. The use of an IKD is hypothesized to be of more significance for other muscle groups, that cannot be controlled as easily.

3. Retrieving USWE scans in a relaxed condition is more reliable than assessing muscle stiffness in a passively stretched condition or during an isometric contraction. This review shows that there could be a minor drop-off in reliability for passively stretched conditions (Ren et al. 2021) and a progressive decrease in reliability dependent upon the intensity of the isometric contraction (Otsuka et al. 2019). As there is only a minor drop-off in reliability during passively stretched scans, and the muscle in a stretched condition could be characterised as a more relevant state to an athletic performance task, this may be the preferred condition of the muscle during the scan to infer the most useful conclusions.

4. Due to the sensitivity of the USWE readings, it is recommended that 3 scans are taken per measurement Andonian et al. 2016, & Wu et al. 2020), especially if there is only one examiner. Although studies within this review only repeat 2 scans, as scans can be performed quickly once the examiner is skilled and experienced in this area, performing 3 scans can be deemed to be beneficial. If an isometric contraction is being performed, a video analysis may be more useful than collecting still images.

5. The standardized approach analysed within this review is to use an Aixplorer ultrasound system (Supersonic Imagine, Aix-en-Provence, France) with a linear transducer array (4–15 MHz. Super Linear 15-4, Vermon, Tours, France) with the scans being performed with the probe in a plane parallel to the muscle fibres and perpendicular to the skin.

6. Based on the review, the ROI measured should be a 5mm diameter circle within a 10 x 10mm or 10 x 20mm Q-Box, utilising the colour map to select the region of the muscle which avoided aponeurosis areas, or non-muscular structures. It is recommended that the ROI remains consistent throughout all measurements to increase accuracy and reliability of scans (Nakamura et al. 2021, Wu et al. 2020).

7. If performing a study in response to an exercise stimulus or intervention (Andonian et al. 2016), ~60mins post- exercise should be used to assess the muscle stiffness response to ensure that the response to exercise is truly being measured.

CHAPTER 5

THE RELATIONSHIP BETWEEN MUSCLE STIFFNESS AND PHYSICAL PERFORMANCE MARKERS IN PREMIER LEAGUE ACADEMY FOOTBALLERS

INTRODUCTION

As the systematic review has established a methodological approach for the use of USWE within a Premier League football setting, the practical recommendations can now be utilised to start examining the application of USWE.

It is important to state that stiffness is not a stable factor and can be influenced by a range of factors such as age and maturation. To the authors knowledge, no researcher has investigated the effect of muscle stiffness and physical performance between different age groups in a Premier League academy football club. Based on the conflicting findings in the literature review, more research is required to establish if there are age-related changes to stiffness, especially muscle stiffness, and the effect that this may have on performance (Granata et al, 2002, Korff et al. 2009, Laffaye et al. 2016, Di Giminiani and Visca, 2017, Lehnert et al. 2020). We hypothesize that there will be age-related changes as a function of training exposure, and the potential musculoskeletal adaption which may take place when athletes increase their training volume and intensity as they get older. This may be even more relevant in a study population of Premier League academy footballers where football-specific stimuli and strength and conditioning exposure are carefully and strategically periodised to increase as they progress through the age groups. Establishing these stiffness characteristics for Premier League academy footballers may help in areas such as talent identification, injury prevention and exercise programme prescription.

The existing research associated with whole lower extremity stiffness accentuates the importance of designing a strength and conditioning programme which optimises stiffness to maximise performance in high-speed running (Bret et al., 2002), change of direction speed

(Serpell et al, 2014), and jump performance (Arampatzis et al, 2001) in the athletic population. However, in team sports such as professional football, where actions are comprised of fast stretch shortening and slow stretch shortening contractions, specific tasks may benefit from more compliant muscles to increase the storage and release of elastic energy to increase concentric force output (Brughelli and Cronin, 2008). The conflicting research of the effects of muscle stiffness on different athletic tasks, such as running at different speeds (Ueno et al., 2018), and different types of contractions (Laffaye, et al, 2005; Pruyn et al, 2014; Wu et al. 2010; Kubo et al., 2007), causes difficulty for practitioners in professional football to programme effectively to ensure the players are ultimately prepared for all movements and actions associated with a professional football match. Previous research has also stated that relatively high levels of stiffness have been related to repetitive stress and soft-tissue injuries such as hamstring strains (Watsford et al. 2010), so this must be accounted for during the prescription of training modalities such as strength training and plyometric training to minimise the injury potential. Therefore, a more comprehensive understanding of muscle stiffness and the influence on physical performance would allow practitioners in professional football to make more informed decision-making on programming and exercise prescription.

Despite the literature highlighting that stiffness can be an important factor for performance, the methods utilised tend to measure stiffness across multiple structures, meaning it is difficult to identify what structure requires stiffness in order to improve performance. It is yet to be established if performance is improved due to stiffness of individual muscles or tendons working in isolation or whether stiffness of the combined lower body structure working together increases performance. Measuring the stiffness an individual unit, such as the rectus femoris, and comparing this against a holistic lower body stiffness measurement, might give insight into the contribution and importance of each specific component of stiffness against the whole unit, which may be able to provide meaningful information on the relationship between the optimal levels of stiffness and performance in Premier League football.

Therefore, the aims of this study will be: (1) to quantify rectus femoris muscle stiffness values across different age groups in Premier League academy footballers, (2) to determine the relationship between rectus femoris muscle stiffness and measures of physical performance, (2) and to (3) assess the differences in relationship between a holistic lower body stiffness measurement (vertical stiffness derived from a hop test) in comparison to the USWE method which measures muscle stiffness only. It is hypothesised that there will be significant differences in muscle stiffness, with the participants who display higher stiffness values, demonstrating superior physical performance due to an increased volume and intensity of training exposure throughout the academy system, which would subsequently lead to an increased level of musculoskeletal adaptation.

METHODS

EXPERIMENTAL APPROACH TO THE PROBLEM:

This study utilised a cross-sectional design, where all data was collected in a single test session for Under 23, Under 18 and Under 16 teams in a category 1 football academy at a Premier League football club. Different physiological capabilities such as vertical power, horizontal speed, change of direction and maximal strength are all key physical characteristics in professional football. The performance testing included proxy measurements of all the physical characteristics listed above. The testing battery consisted of a bilateral countermovement jump (CMJ), vertical hop test, 5m, 10m and 20m sprint test, 5-0-5 change of direction test, and a maximal isometric squat test. Muscle stiffness testing included measurements of the rectus femoris in both a relaxed and passively stretched position using the USWE. The quadriceps muscle group, specifically the rectus femoris, is one of the prime movers for all of the performance tests selected. All athletes were familiar with testing protocols prior to testing, with a standardised warm-up protocol being completed before beginning the testing battery. Moreover, players refrained from completing strenuous physical activity for 48 hours before testing.

PARTICIPANTS:

Forty-six Premier League academy male football players volunteered to participate in this study across 3 age groups: Under 23 (n=15; age:19.4±1.2; height:182±7.1cm; mass:74.6kg±8.4kg), Under 18 (n=19; age:17.3±0.4; height:180±8.2cm; mass:68.8kg±6.3kg) and Under 16 (n=12; age:16.5±0.4; height:176±6.1cm; mass:64.3kg±6.3kg). The sample size has been determined as a result of a priori power analysis using G*Power-2 (Version 3.1.9.6, Heinrich-Heine-Universitat, Dusseldorf, Germany). Using previous research analysing lower-body stiffness in netballers (Pruyn et al., 2014), a large effect size (0.75) was anticipated for the between-group differences for stiffness. Therefore, with a power level of 1-β = 0.8 (Beck, 2013) and a level of 0.05., the minimum sample size per group was deemed to be twenty-one participants. All players were uninjured at the time of testing, with each player training a minimum of 3 times a week, with 1 match per week, as well as 2 structured strength and conditioning sessions individual to each athlete. Written informed consent (Appendix 9.2) and a Participant Information Sheet (Appendix 9.3) was given to all players in line with the club's academy policy with written consent for all players younger than 18 provided by a parent/guardian. Ethical approval was granted by the ethics committee at University of Birmingham, Birmingham, United Kingdom.

PROCEDURES:

All players undertook the same protocols before fitness testing, to ensure a standardized approach would be adopted across all age groups. Before testing, all players performed a standardised warm up consisting of 5 minutes cycling followed by ankle, hip and thoracic mobility, core and glute activation and dynamic stretches. A series of progressively harder practise tests were then performed at perceived intensities of 50%, 70% and 90% of maximum for each test. After the warmup, all participants completed 3 trials on each test, with the maximum score taken for statistical analysis. All speed tests were performed indoors on an artificial pitch at the club's training facility wearing moulded football boots. All jump and strength tests were performed indoors in the club's gym facility wearing running trainers.

ULTRASOUND SHEAR WAVE ELASTOGRAPHY:

All ultrasound shear wave elastography (USWE) measurements were examined using a Canon Aplio i800 ultrasound system (Canon Medical Systems Corp., Otawara, Tochigi, Japan) with a high-frequency linear transducer (frequency range, 7.2 – 14 MHz). Practical recommendations from the outcome of the systematic review were used for the methodology of the USWE. The entire probe was covered with ultrasonic gel that was 3-4mm thick to ensure optimal image quality and to minimize the transducer pressure on the skin. The ultrasound examiner had three years' experience of USWE, and five years' experience of ultrasound scanning, more specifically on the rectus femoris muscle. While obtaining the images, consistent minimal pressure was applied to the probe and care was taken to ensure the examiner's hand was as still as possible. Transducer pressure was avoided when performing USWE as applied pressure can compress the muscle tissue below the surface. Barr et al. (2012) highlighted that a 10% increase in compression of the transducer can double muscle stiffness measurements. The ultrasound machine was set to a split screen mode, with the 2D-SWE map (left side) and quality mode (right side) displayed and examined for every scan. The quality mode, which is identified as the propagation mode, is a visual feedback function in which reliable data is obtained when the lines are parallel and smooth, and the increase in distance between the lines is parallel to the increase in stiffness. An example of a high-quality and low-quality USWE image is shown below for reference (Figure 5.1, Figure 5.2). The speed of ultrasound waves was measured in metres/second (m/s) for velocity, and within seconds the results were displayed on the ultrasound machine. During stabilization of USWE images for 5 seconds, USWE images were frozen and saved. This process was repeated three times, and three measurements were collected for each condition for a total of six scans per scanning session. The velocity range was set to 0-8m/s on a standardized “musculoskeletal pre-set mode.” Subsequently, a 5mm diameter “region of interest” (ROI) was used to take measurements. Mean velocity values were automatically calculated by averaging the three values for each scanning session.

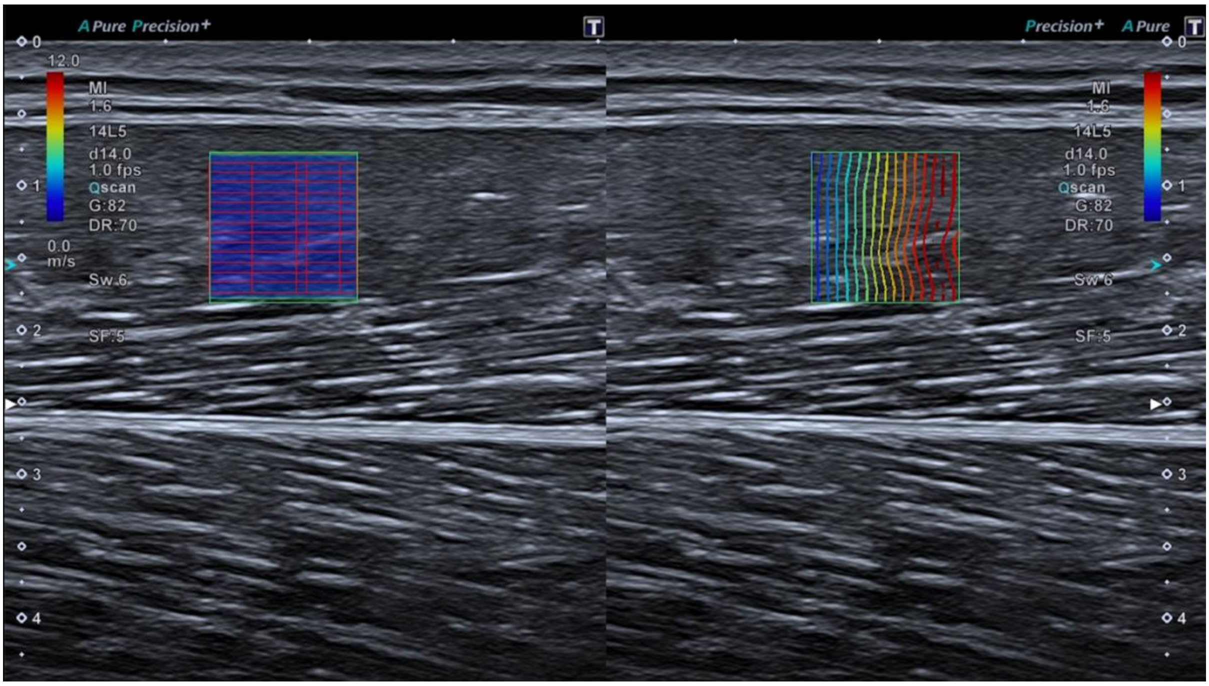


Figure 5.1 – An example of a high-quality scan using the USWE split screen 2D-SWE Map and Quality Mode function. The right side of the image displays parallel and smooth lines, which illustrates there are minimal artifacts, that could make the results harder to interpret, in the muscle tissue being measured. A high-quality ultrasound scan for the Rectus Femoris should be clear, with a distinct boundary of the superficial and deep aponeurosis between the thickness of the muscle.

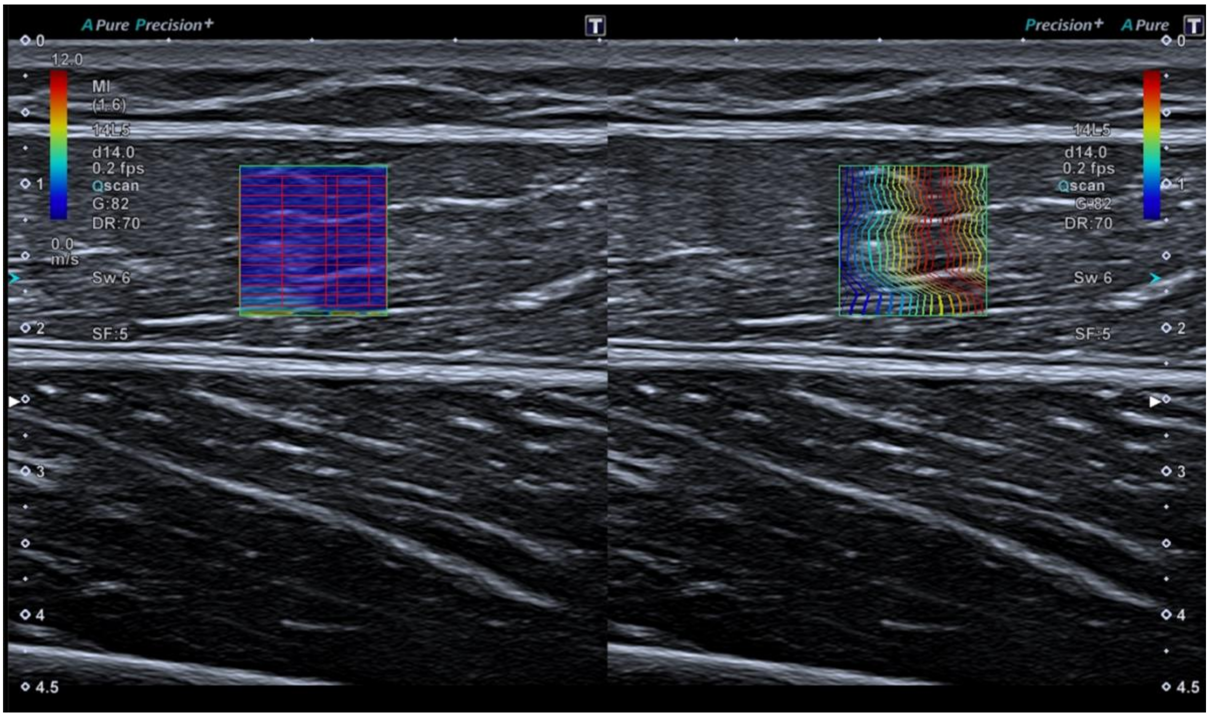


Figure 5.2 – An example of a poor-quality scan using the USWE split screen 2D-SWE Map and Quality Mode function. The right side of the image displays lines which are uneven and curved in the ROI, which raises concern of the quality of muscle tissue being measured due to the artifacts which may be present.

The USWE measurements in the musculoskeletal system are presented in shear-wave speed (SWS) rather than tissue elasticity (kPa) based on the findings from Davies et al, 2019. The calculation of tissue elasticity is based upon the assumptions that the soft tissues are elastic, incompressible, homogeneous, and isotropic. The SWS measured during USWE is only a portion of the calculation of the Young modulus, which is needed to determine tissue elasticity, as $E = 3\mu = 3pcT^2$, where E = the Young's modulus, μ = the shear modulus, p = tissue density, and cT = the speed of transversely propagated waves, or shear waves. With this relationship, unless the tissue density is 1 g/mm^3, the SWS does not correspond exactly with the Young's modulus. The musculoskeletal system, with its viscoelastic, heterogeneous, anisotropic tissues, presents inherent challenges to calculating tissue elasticity using the Young's modulus, and therefore SWS provides a more applicable measurements of muscle stiffness.

Prior to the onset of this study, a prior methodological pilot study was performed to compare muscle stiffness when measured across different sites of the rectus femoris muscle. Similarly to the work by Kodesho and colleagues (2021a), the rectus femoris was measured at a proximal, mid-point and distal location. The proximal and distal locations were measured at 10% and 90% of the muscle length respectively, and the mid-point was measured at 50% of the muscle length between the anterior inferior iliac spine (AIIS) and the patella tendon (PT). 8 non-athletes (age:27.2±1.2; height:182±7.1cm; mass:78.6kg±8.3kg), volunteered to take part in the pilot study. Please see above for a comprehensive overview of the *methods* for the USWE scan. The rectus femoris was measured in two different conditions for all participants; relaxed (Figure 5.3) and passively stretched (Figure 5.4). For the relaxed condition, participants were sat upright on a medical bench with their hip angle at 90° and their lower body fully outstretched. In the passively stretched position, participants were laid supine on a medical bench with their knee angle at 90° with their feet hanging over the bench. For both conditions, the verbal communication provided by the ultrasound examiner was to “stay relaxed as possible.”

Figure 5.3 – Example of participant during a relaxed scan of the Rectus Femoris.

Figure 5.4 – Example of participant during a passively stretched scan of the Rectus Femoris.

Player	Relaxed Proximal (m/s)	Relaxed Mid-Point (m/s)	Relaxed Distal (m/s)	Passively Stretched Proximal (m/s)	Passively Stretched Mid-Point (m/s)	Passively Stretched Distal (m/s)
1	1.82	1.53	1.67	3.05	2.79	2.98
2	1.98	1.85	1.89	2.91	2.69	2.84
3	1.67	1.52	1.65	2.72	2.63	2.73
4	1.52	1.45	1.5	2.32	2.13	2.18
5	1.84	1.52	1.78	3.07	2.82	2.99
6	2.02	1.83	1.95	2.56	2.3	2.43
7	2.11	1.89	2.05	2.54	2.29	2.40
8	1.62	1.48	1.61	2.92	2.62	2.83
Average	1.82	1.63	1.76	2.76	2.53	2.67
SD	0.21	0.19	0.19	0.27	0.26	0.30

Table 5.1 – Pilot USWE measurements in a proximal, mid-point and distal location in a relaxed and passively stretched condition.

In agreement with the results attained by Kodesho and colleagues, the average proximal measurements in both a relaxed (1.82 ± 0.21 m/s) and passively stretched (2.76 ± 0.27 m/s) conditions were greater than the measurements at the mid-point (1.63 ± 0.19 m/s, 2.53 ± 0.26 m/s) and the distal location (1.76 ± 0.19, 2.67 ± 0.3 m/s) in a relaxed and passively stretched condition respectively. That being said, the distal measurement was also shown to be display greater muscle stiffness than the measurement at the mid-point of the rectus femoris, which is hypothesized to be because of the tension from the connecting tendon at both the AIIS and PT (Kodesho et al. 2021a).

Therefore, during this study, muscle stiffness was only measured on the right rectus femoris, at the mid-point between the AIIS and PT in line with SENIAM guidelines as this was the furthest point from any tendinous structure to ensure the measurement was transposed of predominantly muscle with minimal interference (Freriks, Hermens, Disselhorst-Klug, & Rau, 2000). The muscle was located with the transducer oriented longitudinally before the probe was oriented perpendicular to measure the muscle stiffness reading. The mid-point of the rectus femoris was measured prior to the scanning with a measuring tape with a permanent marker pen, with the participant positioned supine on a medical bench with their knees overhanging the bed. The rectus femoris was measured in two different conditions for all participants; relaxed (Figure 5.3) and passively stretched (Figure 5.4) as was completed in the pre-pilot methodological study.

COUNTERMOVEMENT JUMP:

A countermovement jump (CMJ) was performed on a force plate (Model FDLite; Vald Performance, Queensland, Australia) sampling at 1000Hz. Athletes were firstly weighed barefoot (Seca 875 flat weighing scales, Seca, Birmingham, UK) with the weight inputted into the force plate manufacturer's software. Force plates were zeroed before the athletes stepped on. Athletes then stepped on, standing motionless to be weighed given the instruction to be as still as possible. The athletes then stood upright with hands on hips to reduce alterations in centre of mass (Gutiérrez Dávila, Amaro, Garrido and Rojas, 2014). Athletes then lowered themselves into a self-selected squat depth (Petronijevic et al., 2018) before jumping as high as possible, keeping knees extended during the flight phase of the jump. For each jump, verbal encouragement was provided to ensure that maximal effort was given during each attempt. They completed 3 reps with a 30 second rest between trials. If the hands left the hips or the knees became flexed during the flight phase, the jump was asked to be retaken. Using the manufacturer's software, values of jump height, reactive strength index modified, countermovement depth, eccentric peak force, concentric peak force, flight time, contraction time and flight time to contraction time ratio were provided and exported to Microsoft Excel for further analysis.

VERTICAL HOP TEST:

Participants performed a bilateral hopping test consisting of 15 consecutive vertical hops on a force plate (Model FDLite; Vald Performance, Queensland, Australia) sampling at 1000Hz. Participants were instructed to keep their hands on their hips for the duration of the test and were verbally encouraged to jump as high possible on the first jump. Following the first jump, the participants were verbally encouraged to jump as quickly as possible for the remaining fourteen jumps. Participants completed three efforts with two minutes rest between attempts. Five acceptable consecutive hops were used for analysis (typically 6th to 10th jump) (Hobara et al., 2013), with the average of contact and flight time data from these hops then used to calculate stiffness based on Dalleau's et al. (2004) equation, a method that has been shown to be a reliable and valid way of measuring leg stiffness (Dalleau et al., 2004).

$$K_N = \frac{M \times \pi(T_f + T_c)}{{T_c}^2\left(\frac{T_f + T_c}{\pi} - \frac{T_c}{4}\right)}$$

Where M was total body mass, Tc the ground contact time and Tf the flight time

5 / 10 / 20M SPRINT TEST:

Electronic timing gates (Brower Timing Systems, UT, USA) were placed on the artificial pitch at the club's training ground, at 0, 5, 10 and 20m to measure sprint performance. Distances were chosen to match the demands of sprints within a Premier League academy football match, with the ability to sprint over these distances a main indicator of performance level (Haugen, Tønnessen, Hisdal, and Seiler, 2014). Timing gates were placed at a height of 1m. Participants began with a 2-point stance 0.3m behind starting gates, and after the count of 3-2-1, the athlete accelerated through the timing gates enabling split times to be recorded to the nearest 100th of a second. This method of measuring sprint performance has been shown to have excellent within- and between-session reliability in male athletes (McMahon et al., 2017).

5-0-5 AGILITY TEST:

Agility was measured using 5-0-5 agility test, a method previously reported to have high reliability and acceptable coefficient of variation (Stewart, Turner, and Miller, 2012). To complete the test, electronic timing gates (Brower Timing Systems, UT, USA) were placed on the starting line. Participants began in a 2-point stance 0.3m behind the starting mark. They then sprinted 5m, performed a 180° turn and sprinted 5m back through the same timing gates. The time was recorded from when the participants crossed the timing gates at the start line and finished after passing the timing gates following the 180° turn. During each trial, the participant changed direction using alternating limbs to reduce impact of fatigue, with the fastest time on each leg recorded after 2 trials. For a trial to be successful, the players foot must have fully crossed the line during the turn.

ISOMETRIC BELT SQUAT:

Isometric squats have shown to provide a valid and convenient way of measuring lower limb strength (Brady et al., 2018; Bazyler, Beckham and Sato, 2015; Blazevich, Gill, and Newton, 2002). The isometric belt squat was utilised in this study due to the constraints of the environment and participant familiarity. The isometric belt squat was performed on a force plate (model FDLite; Vald Performance, Queensland, Australia) sampling at 1000Hz. Force plates were zeroed before the subject's stepped on. Subjects then stepped onto the Force Plates wearing an Integral Harness (Desmotec, Italy) before standing motionless to be weighed. The harness was then attached to a chain affixed to the platform, between the subject's feet, with the height of the squat controlled by adjusting the length of the chain. The subject lowered themself into position, with the knee angle approximately 90-120° and hip flexed to approximately 120-140°, replicating the power position of a clean which produces the greatest amount of force (Chavda et al., 2020; Nuzzo, McBride, Cormie and McCaulley, 2008). Participants were then instructed to push the ground away as hard as possible and will continue to do so until told to stop. Participants completed 3 tests with 2-minute rest between each test. This test lasted 5 seconds, with the highest peak force (N) out of the 3 tests recorded and analysed using ForceDecks software. Relative strength scores were calculated based on the athletes previously weighed body mass.

STATISTICAL ANALYSIS:

All data was recorded as mean and standard deviation (SD) in Microsoft Excel and was then transferred to SPSS for further analysis. The normality of the data distribution was verified with the Shapiro-Wilk tests. Within session reliability was calculated using a 2-way random model intraclass correlation coefficients (ICCs) for absolute agreement with 95% confidence intervals. The ICC was interpreted based on research from Koo and Li (2019) where values >0.9= excellent, 0.75–0.9= good, 0.5–0.75 = moderate, and <0.5 = poor. Coefficient of variation was calculated by (SD/Mean)*100, with values <10% considered acceptable; a value Joseph et al (2013) used as a threshold for determining good reliability. The standard error of measure (SEM) was calculated using the equation SD*SQRT(1-ICC).

A One-Way ANOVA was then conducted to analyse the differences between age groups for muscle stiffness values and mean performance scores, with statistical significance set at p<0.5. The magnitude of difference between the groups was also calculated using Cohen's d effect size (Mean of group1- Mean of group2)/SDpooled) where scores were interpreted based on (Cohen, 2013) interpretation with >0.19 = trivial, , 0.2– 0.49 = small, 0.5–0.79 = moderate, >0.8 = large.

To analyse the relationship between muscle stiffness and performance testing score, a Pearson's r correlation was calculated, with the statistical significance set at p<0.5 with results interpreted as negligible if <0.1, weak= 0.1–0.4, moderate =0.4–0.7, strong =0.7–0.9, and very strong >0.9 (Hopkins et al., 2009).

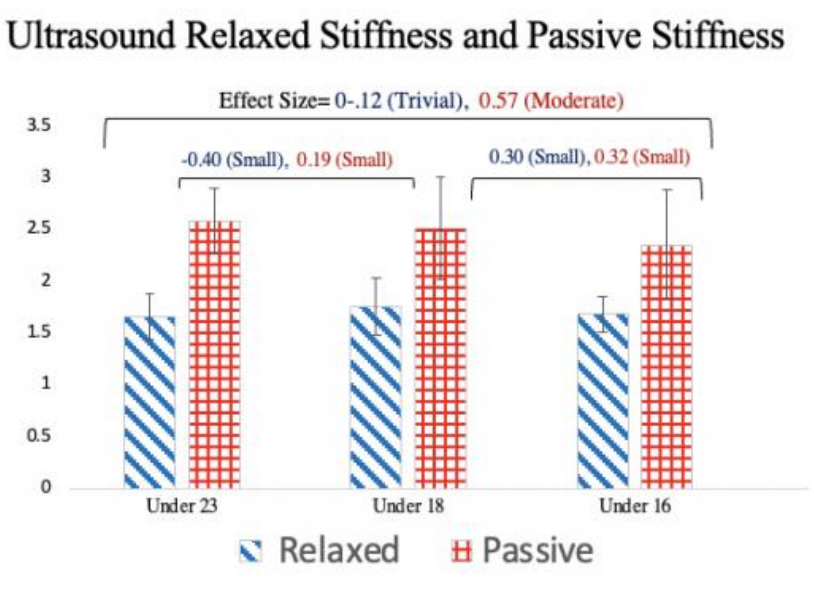
RESULTS:

NORMALITY/RELIABILITY ANALYSIS:

All data were deemed to be normally distributed ($p=0.05$), with no outliers, as assessed by boxplot. Coefficient of variation scores were deemed acceptable for all performance tests (< 6.5%), and all performance tests also showed moderate reliability (ICC = 0.50–0.99). The reliability of all stiffness measures was also deemed acceptable with test variability < 5.95% and all stiffness measures showing excellent reliability (ICC = 0.94 – 0.98).

DIFFERENCE BETWEEN UNDER 23, UNDER 18, & UNDER 16 AGE GROUPS

The average muscle stiffness values from the USWE were different between age groups within the academy age groups (Figure 5.1). We hypothesized that the older academy players would possess the highest muscle stiffness due to a number of factors such as superior training age, increased maturity and greater athleticism and physical development. That being said, the Under 18 team exhibited the highest muscle stiffness in a relaxed condition (1.75 m/s ± 0.31 m/s) and the Under 23 team exhibited the highest muscle stiffness in a passive condition (2.59 m/s ± 0.31 m/s).



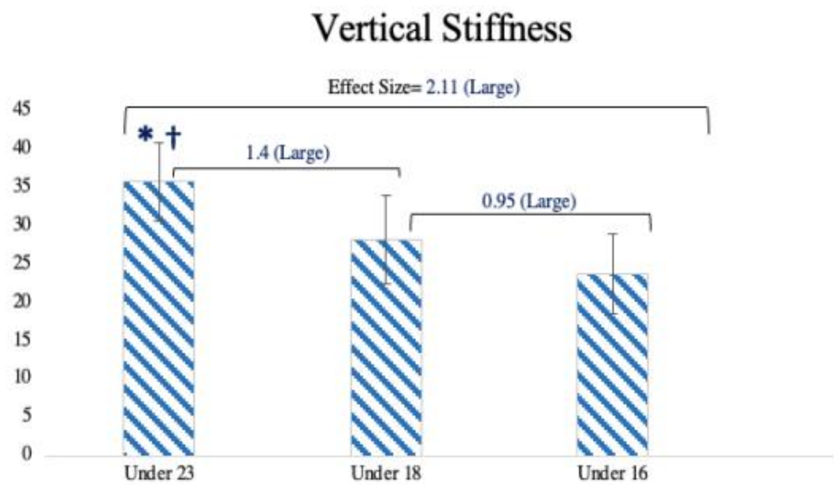


Figure 5.5

Mean Stiffness values from
Ultrasound (relaxed and passive) and Vertical Hop test.
*= Statistically greater stiffness compared to U18 Team ($p<0.05$)
†= Statistically greater stiffness compared to U16 Team ($p<0.05$)

Performance Measure	U23 Mean ± SD	U18 Mean ± SD	U16 Mean ± SD
<u>CMJ</u>			
Jump Height [cm]	45.45±4.09	43.41±4.09	42.40±5.11
Reactive strength index	0.64±0.10	0.62±0.10	0.59±0.12
Countermovement Depth	31.90±3.89 †	29.68±3.89	24.81±9.63
Eccentric peak force [n]	2011.47±359.4†	1855.84±359.48	1629.75±292.84
Concentric peak force [n]	2043.80±283.86	1879.95±283.65	1883.83±279.90
Flight Time [ms]	607.80±19.88	594.42±19.88	587.17±36.45
Contraction Time [ms]	747.33±94.59	737.47±94.59	760.50±118.0
Flight time:contraction time	0.87±0.12	0.87±0.12	0.84±0.14
<u>Hop test</u>			
Flight time [s]	0.45±0.03	0.45±0.03	0.43±0.05
Ground contact time [s]	0.16±0.02 †*	0.18±0.02	0.19±0.04
<u>Speed Test</u>			
5m [s]	0.94±0.04†	0.97±0.04	1.00±0.06
10m [s]	1.65±0.04	1.69±0.05	1.71±0.08
20m [s]	2.84±0.07†	2.93±0.08	2.96±0.08
505 L [s]	2.37±0.07	2.43±0.10	2.44±0.08
505 R [s]	2.39±0.07	2.43±0.08	2.44±0.09
<u>Isometric Squat</u>			
Strength [N/M]	5495.50±910.79 *†,**	4621.47±624.63 *†*	2891.3±43.96
Relative strength [N/M/Kg]	73.49±11.04 *†*	64.11±8.39 *†*	63.23±17.03

†= Significantly better than Under 16 Team at 0.05 level
**= Significantly better than Under 18 Team at 0.05 level*

Table 5.2 – Mean Performance Testing for each age group

The performance testing measures also agreed with our hypothesis of the older academy players being superior in their physical development and athleticism in speed, strength and jump assessments (Table 5.1). The Under 23 squad were significantly faster than the Under 16s over 5m (0.94s ± 0.04s vs. 1.00s ± 0.05s), 10m (1.65s ± 0.04s vs. 1.71s ± 0.08s) and 20m speed tests (2.84s ± 0.07s vs. 2.95 ± 0.08, $p<0.05$). Both the Under 23 squad (5495.50 N ± 73.48 N) and Under 18 squad (4621.47 N ± 64.10 N) were significantly stronger than the Under 16 group (2891.30 N ± 863.23 N, $p<0.01$) in the Isometric Belt Squat assessment, whilst the Under 23 (2011.46 N ± 367.17 N) also had a significantly larger eccentric peak force compared to the Under 16 group (1883.83 N ± 279.89 N, $p<0.01$) in the CMJ.

RELATIONSHIP BETWEEN STIFFNESS MEASURES AND PERFORMANCE TESTING

Table 5.2 displays the relationships between performance measures and stiffness measures, with all of the age groups' data merged together. Several significant correlations were found, which show that stiffness from the USWE seemed to correlate with indicators of force and power in the CMJ (Please refer to Table 5.2). These findings may potentially indicate that athletes with higher values of rectus femoris muscle stiffness are able to load more effectively concentrically and eccentrically in the CMJ, with a faster contraction time. These findings also suggest that higher values of rectus femoris muscle stiffness is potentially linked to flight time in the vertical hop test.

The vertical stiffness from the hop test was correlated to 5m, 10m and 20m sprint testing ($p<0.05$), which agrees with previous research, displaying that vertical power and vertical stiffness can be attributed to greater success in horizontal speed and force production tasks

(Carr et al, 2015, Smirniotou et al, 2008). Vertical stiffness was also correlated to peak force from the Isometric Belt Squat ($p<0.05$) and numerous jump measures including CMJ eccentric peak force ($p<0.01$) and concentric peak force ($p<0.05$) displaying that vertical stiffness characteristics are also potentially related to strength and other jumping activities.

Performance Test	Ultrasound Relaxed	Ultrasound Passive	Vertical
CMJ			
Jump Height [cm]	0.12	0.01	0.07
Reactive strength index	0.26	0.16	-0.01
Countermovement Depth	-0.01	0.18	-0.35*
Eccentric peak force [n]	0.44	0.29*	0.44 †
Concentric peak force [n]	0.36 *	0.33*	0.36 *
Flight Time [ms]	0.09	0.00	0.08
Contraction Time [ms]	-0.33 *	-0.13	0.07
FT:CT	0.26	0.23	-0.08
Hop test			
Flight time [s]	0.25	0.42 †	0.04
Ground contact time [s]	-0.05	0.10	-0.88 †
Speed Test			
5m [s]	0.11	0.12	-0.37 *
10m [s]	0.19	0.12	-0.33 *
20m [s]	0.19	0.02	-0.41 *
505 L [s]	0.09	0.22	-0.19
505 R [s]	0.18	0.26	-0.22
Isometric Squat			
Strength [N/M]	0.17	0.05	0.38 *
Rel. strength [N/M/Kg]	0.10	0.00	0.26

CMJ = countermovement jump; L = left; R = right.
*Significantly significant at 0.05 level
†Significantly significant at 0.01 level

Table 5.3 – Pearson's r correlations between Ultrasound stiffness measurements, Vertical Stiffness derived from the Hop test and performance measurements across the whole group.

DISCUSSION:

The aims of this study were to: (1) to quantify rectus femoris muscle stiffness values across different age groups in Premier League academy footballers, (2) to determine the relationship between rectus femoris muscle stiffness and measures of physical performance, and to (3) assess the differences in relationship between a holistic lower body stiffness measurement (vertical stiffness derived from a hop test) in comparison to the USWE method which measures muscle stiffness only. The results exhibited several agreements to our predicted hypothesis, with the older academy players possessing superior athletic prowess in the athletic performance tasks and displaying higher rectus femoris muscle stiffness in a passive condition when measured using the USWE. However, the Under-18 age group displayed the highest muscle stiffness in a relaxed muscle condition which contradicts the older academy players displaying the higher levels of muscle stiffness. When considering all of the age groups, the study also exhibited several significant correlations between muscle stiffness derived from the USWE and select jumping metrics from the CMJ and vertical hop test, but muscle stiffness had no correlations with any speed, strength, or agility performance markers. The vertical stiffness measure was used as a stiffness measure of the lower limb unit rather than a performance measurement, to correlate against the stiffness measure of the individual muscle unit from the USWE. The correlations between stiffness measurements and athletic performance tests were more evident with the vertical stiffness calculated during the vertical hop test. Unlike muscle stiffness with the USWE, vertical stiffness was significantly different between age groups, with the Under-23 squad being significantly higher in stiffness than both the Under 18 squad and the Under 16 squads. Vertical stiffness was also significantly correlated to 5m, 10m and 20m speed testing, peak force from the Isometric Belt Squat, and CMJ eccentric and concentric peak force. Therefore, the vertical stiffness measure may provide a better indication of the athlete's physical performance potential and could suggest that individual muscle stiffness itself does not necessarily predict performance in an athletic performance task.

It was hypothesized that the Under-23 age group would have higher values of rectus femoris muscle stiffness due to an increased volume and intensity of training exposure throughout the academy system, which would subsequently lead to an increased level of musculoskeletal adaptation. Despite the Under-23 age group on average having higher levels of muscle stiffness in a passive muscle condition, the Under-18 age on average had higher levels of muscle stiffness in a relaxed condition. This may be explained by the little age difference between the Under-23 and Under-18 age group (~2.1 years), as the Under-23 age group during this specific study were quite young. That being said, despite the age difference only being on average ~2.1 years, this would be two years of training exposure in a professional football environment where the players on average would be subject to 5 x training sessions (~45-90mins), 5 x themed movement preparation/pre-activation sessions (~15-30mins), 3 x strength and conditioning focused sessions (~30-60mins) as well as 1 x 90minute football match within a weekly microcycle. This microcycle would be repeated on average for 46 weeks of the year, so despite the years in age being seemingly small, the additional strength and conditioning stimulus a player would receive in a 2-year window would be significant which may indicate there is other variables which effect stiffness other than age and physical development. Another factor which may have influenced the Under 18's possessing a higher muscle stiffness could be an acute adaptation to recent training exposure rather than their training age. As the Under 18 squads at an academy level follow a regimented programme to allow for their educational needs and to maximise their physical development, their weekly schedules usually allow for more training and strength-related gym sessions as the priority during this phase of the academy is development. Yet, at Under 21 level, as the player approaches a more realistic schedule of a professional footballer, where the match schedule becomes more congested and less structured, the focus turns more orientated around performance rather than development, which can subsequently decrease their training exposure to increase their freshness and readiness to compete. The performance testing from this study however does support the additional training stimulus and superior physical development from the older academy players. The Under 23 squad were significantly faster and stronger than the Under 16s, in the 5m, 10m, and 20m speed tests and the Isometric Belt Squat assessment respectively. The Under 23 age group also had a significantly larger eccentric peak force capacity in comparison to the Under 16 group in the CMJ.

When considering all of the age groups together, the study exhibited several significant correlations between muscle stiffness derived from the USWE and the jump profiling metrics from the CMJ and vertical hop test. The study reported that concentric peak force in a CMJ was found to be significantly higher in stiffer individuals when muscle stiffness was measured on the USWE in both a relaxed and passive position. Eccentric peak force from the CMJ and hop test flight time from the vertical hop test was also found to be higher in the players with a higher muscle stiffness when measured in a passive position. The USWE relaxed stiffness measurement was also negatively correlated to CMJ contraction time. The extent of these findings may potentially indicate that academy football players with higher values of rectus femoris muscle stiffness may be more effective in jumping tasks such as the CMJ and the vertical hop test.

However, when considering jump height, a metric that can be used to quantify success in a range of sports from diving to football (Baker, 1996), no significant correlations were found with muscle stiffness. This is in line with previous research looking at joint stiffness (Kubo et al., 2007) and vertical stiffness (Pruyn, Watsford, & Murphy, 2014) which also found no correlations between stiffness and CMJ performance. This can potentially be attributed to the slow stretch shortening cycle (SSC) nature of a CMJ, where a more compliant muscle may be beneficial in increasing the storage of elastic energy and consequently the duration over which force can be applied (Mohammadian, Sadeghi, Khaleghi Tazji and Maloney, 2021). However, with numerous researchers finding relationships with stiffness and CMJ performance (Bojsen-Møller et al. 2005; Mohammadian, Sadeghi, Khaleghi Tazji and Maloney, 2021), the influence of stiffness in slow SSC activities, specifically the CMJ, is still unclear.

What remains more evident is the relationship between stiffness and fast SSC actions, with increased stiffness of the muscles altering the interaction between the muscular and tendinous lengthening. This increased muscle stiffness facilitates lengthening of the tendons, providing muscles the opportunity to function in their preferred quasi-isometric state. This in turn increases the tendon contribution which can increase elastic energy storage and release, increasing power output and enhancing performance (Lai, Schache, Lin and Pandy, 2014; Brazier et al., 2019), which may support the outcome of this study with flight time in the vertical hop test being greater for stiffer individuals as measured by USWE in a passive position. Numerous other researchers have supported this argument (Mohammadian, Sadeghi, Khaleghi Tazji and Maloney, 2021; Rabita, Couturier, & Lambertz, 2008; Arampatzis, Bruggemann, & Klapsing, 2001) with all studies finding increased stiffness improved fast SSC jump performance over several different jumping tasks. This is further support by Pruyn, Watsford, & Murphy (2014) who found increased rectus femoris stiffness improved drop jump performance. Given these results, it would suggest that increased muscle stiffness can help improve performance output in fast SSC jump activities such as a drop jump, and hopping exercises.

As discussed above, the correlations between stiffness measurements and athletic performance tests were more evident with the vertical stiffness calculated during the hop test than the USWE measurement. Previous studies have also found correlations between stiffness and physical performance markers such as strength and force production (Alfuraih et al 2019; Bojsen-Møller et al., 2005). Wilson et al., (1994) suggests the stiffer muscle will have a larger length of the muscular contractile component coupled with a smaller contractile shortening velocity. This in turn facilitates an increase in force production by optimising the force velocity and length tension relationships in the muscle. Similar to the vertical hopping assessment, previous research has focused on a holistic stiffness measurement (vertical stiffness, leg stiffness and joint stiffness) where the whole musculotendinous unit stiffness is reflected in the stiffness value. As the tendons are large contributors of force velocity and modulate force length relationships, it may explain why other studies have experienced more significant relationships with strength, speed, and agility, and why USWE measurements of an individual muscle may not detect any significant relationships with these physical performance markers.

Higher levels of stiffness have also previously been associated with success in both acceleration speeds and maximum velocity running in holistic stiffness assessments (Nagahara and Zushi, 2017; Takahashi et al., 2018; Bret et al., 2002). Young, Benton and Pryor (2001) hypothesised that due to the quadricep being a primary force contributor in sprint performance, increased stiffness in the quadricep will improve the initial transmission of force from the contractile components to the skeletal system, thus enhancing sprint performance by increasing rate of force production during push off. However, the results of this study found that whilst the Under 23s were significantly quicker than the Under 16s, no significant differences were found in rectus femoris stiffness between the Under 23 and Under 16 team, and no whole squad correlations were found between muscle stiffness and sprint performance. This is likely because only sprints under 20m were performed, where success in this is determined on the ability to produce a large horizontal ground reaction force impulse (Kawamori, Nosaka, and Newton, 2013), decreasing the importance of quick ground contact times and therefore muscle stiffness. That being said, when looking at stiffness across a limb in the vertical stiffness measure rather than within a muscle with the USWE, there was a significant correlation to 5m, 10m, and 20m speed which emphasizes a holistic lower limb stiffness assessment may be a more effective method to draw any conclusions from acceleration and maximum velocity activities.

A limitation of this study was that muscle stiffness was only measured using the rectus femoris muscle, therefore, the study discounted the contributions of other significant muscle groups for the athletic performance tasks, especially in tasks such as sprinting aforementioned. To provide a more comprehensive overview of where muscle stiffness may be required for these performance tasks, multiple muscular structures could be measured. If the USWE was used to measure the muscle stiffness at more individual muscle sites, it would be able to provide a more comprehensive understanding of individual muscle stiffness relevant to a sporting task, which may be able to provide further insight into an athlete's physical profile by isolating individual muscle stiffness inside of the MTU. This information alongside data from the vertical hop test would be able to illustrate which structures within the MTU of the lower body are designed to be stiff, and which structures are required to be stiff to enhance performance. This information could then allow practitioners to make more informed decisions in their programme design in relation to muscle stiffness and improving performance in their specific sporting demands.

The findings of this study suggest that correlations between stiffness measurements, athletic performance tests and age of athlete are more evident with the vertical stiffness calculated during the hop test, in comparison to the passive and relaxed USWE scans. Unlike muscle stiffness with the USWE, vertical stiffness was significantly different between age groups, with the Under-23 squad being significantly higher in stiffness than both the Under 18 squad and the Under 16 squads. Rectus femoris muscle stiffness was only correlated to select jump-related metrics, whilst increased vertical stiffness levels appear to be beneficial across a range of performance tasks including acceleration, maximal strength and jump performance, and seems to be a more effective way of identifying higher achievers in athletic performance tasks. This is significant for Premier League academy practitioners to understand, as measuring vertical stiffness via a hop test represents a movement which is relevant to sporting performance in comparison to the passive or relaxed condition used for measuring muscle stiffness. The study also only measured the stiffness of the rectus femoris in a relaxed and passively stretched condition. To make the study more representative of an athletic task (similar to a hopping test), an isometric contraction at a different range of intensities of maximal voluntary contractions (MVC) could have been administered. Utilising the USWE with the muscle in an active condition could display the muscle architecture in a more accurate condition to the athlete's sport and could help to supply more information about muscle stiffness and the relationship with athletic performance characteristics which could help to bridge the gap closer to a more realistic sporting task, such as the vertical hop test. Further research into the muscle stiffness of other individual muscle sites with the USWE may also help to profile where in the lower limb that stiffness is effective for athletic performance tasks to help gain a more comprehensive understanding. As a result, this study provides potential evidence that detailed testing and screening of whole-body and individual muscle stiffness units alongside a training programme to enhance stiffness levels may be recommended to contribute to the success of sporting performance.

CHAPTER 6

MUSCLE STIFFNESS ASSESSED BY USWE EXHIBITS NO SIGNIFICANT CHANGE FOLLOWING PREMIER LEAGUE ACADEMY MATCH-PLAY

INTRODUCTION

The previous chapter confirmed, although not as effective as the vertical hopping assessment in relating to physical performance, that rectus femoris muscle stiffness measured by USWE has potential relationship with specific metrics of CMJ performance. In the next distinct phase of research, we aim to investigate the application of USWE for readiness and response to exercise through Premier League academy match-play.

Ultrasound Shear-Wave Elastography (USWE) is becoming increasingly popular in sports medicine as practitioners attempt to discover more effective and efficient ways to assess muscle condition without the need for a biopsy. This approach is not admired with athletes in elite sport due to the disturbance of muscle tissue and the time commitment to gather results from a laboratory setting when feedback is required instantaneously. As such, the non-invasive USWE technology could be useful for objectively quantifying muscle tissue properties and providing immediate feedback for elite sport practitioners. If technologies such as this are exploited alongside other markers of load and recovery status, the muscle stiffness quantification could provide meaningful insight into injury risk and recovery status since increasing muscle stiffness alongside load may indicate that the muscle is struggling to cope with the demand.

A developing body of research has demonstrated that the observed changes in muscle stiffness measured via USWE may be sensitive to changes in muscle damage. Lacourpaille et al. (2017) presented a relationship between an increase in muscle stiffness following eccentric exercise in the elbow and knee extensors with subsequent force loss in a muscle function assessment after 48 hours. Guilhelm et al. (2016) and Lacourpaille et al. (2014) further emphasise the potential relationship to muscle damage, as they also display a rise in muscle stiffness within 1 hour of eccentric exercise in the elbow flexors and knee extensor muscles respectively. These research findings provide some evidence to suggest that USWE may have potential to measure how the mechanical properties of the muscle respond to exercise thereby providing a proxy measurement of the adaptive response within the tissue.

The large quantity of Premier League football clubs will perform a battery of tests two days following a match (commonly known as M+2 screening), to examine each individual players physical status and level of recovery following a game (Thorpe et al. 2012, Buccheit et al. 2014, Carling et al. 2018). The results of this screening process will help to establish a players' physical condition following a game and give evidence as to how well a player may have recovered from a match. For example, if a player shows signs of significant fatigue or loss of strength in these given tests, additional recovery strategies may be employed to minimize the risk of injury. Thus, if a technology such as USWE is able to predict a loss of force 48 hours post- match-play as supported by Lacourpaille et al. (2017) it would allow sport science practitioners and coaching staff to make informed decisions on training modifications and player status two days before the traditional M+2 screening analysis.

That being said, research thus far suggests that changes in muscle stiffness following exercise are dependent on the type of exercise modality prescribed. In support of Lacourpaille et al (2017), there is a growing body of research which implies that eccentric exercise causes an increase in muscle stiffness (Guilhelm et al. 2016, Jones et al., 2021, Chalchat et al., 2022, Chalchat et al., 2023, & Xu et al., 2019). However, research investigating the effects of endurance and aerobic-based exercise on muscle stiffness have implied that muscle stiffness decreases post-exercise (Andonian et al., 2016, Morales-Artacho et al., 2017 & Sadeghi et al., 2018). While the exact physiological mechanisms that influence muscle stiffness post-exercise are still debated within the literature, research has outlined Ca^{2+} disturbances post-exercise could be a potential reason as to why eccentric-based exercise can cause an increase in muscle stiffness (Lacourpaille et al. 2017; Herzog et al, 2014) and current hypotheses suggest the immediate decrease in muscle stiffness following endurance exercise may be a result of cross-bridge attachment release induced by aerobic-based exercise.

As elite footballers are expected to have the endurance and aerobic capacity to play at a high level for 90+ minutes (Allen et al. 2023), whilst being able to tolerate maximal eccentric contractions in decelerating and sprinting mechanisms (Harper et al. 2019) during match-play, the muscle stiffness response following match-play is unknown based on the nature of a football match compromising of both endurance and eccentric capabilities. Therefore, the aim of this research is to perform a preliminary investigation to provide information on the response of muscle structural properties, more specifically muscle stiffness, following a 90-minute Premier League academy football match, and the potential use of USWE to provide useful information in a “real-world” football monitoring program. By measuring the adaptive response of the shear modulus following soccer-specific stimuli a picture of the impact of these different exercise patterns on the demands placed on the neuromuscular system may be able to be obtained. This data may provide a more detailed understanding of the load placed on these tissues than can be obtained using more traditional external physical outputs provided by MEMS or other typically used wearable sensors in elite football.

METHODS

Twelve Premier League academy footballers (age:17.3±0.4; height:180±8.2cm; mass:68.8kg±6.3kg) volunteered to participate in this study. All players were full-time with the Under-18 squad at a premier league academy and were uninjured at the time of testing. All participants regularly train a minimum of 4 times a week, with 1 match, as well as 2 structured strength and conditioning sessions during their weekly microcycle. Written informed consent was given by all players in line with the club's academy policy with written consent for all players younger than 18 provided by a parent/guardian. Ethical approval was granted by the ethics committee at University of Birmingham, Birmingham, United Kingdom.

The sample size required in order to show significance was determined as a result of a priori power analysis using G*Power-2 prior to the start of the study (Version 3.1.9.6, Heinrich-Heine-Universitat, Dusseldorf, Germany). Using previous research analysing the time-course profile of MVC knee extensions on a quadricep muscle, a medium effect size of (0.5) was anticipated for the pre- and post- differences for stiffness. Therefore, with a power level of $1-\beta = 0.8$ (Beck, 2013) and a level of 0.05, the minimum sample size per group was deemed to be 33 participants. As the study examined an Premier League academy match stimulus in a "real-world" experiment, there was consequently no flexibility on including more participants, due to only 11 players being able to start a match, with 3 substitutions allowed as by Premier League regulations. This, re-iterates, the challenges and complexity of acquiring research with Premier League academy athletes during competition.

The Premier League academy footballers were scanned on average ~75mins pre- and ~75mins post- a Premier League academy Under-18 football match, which comprised of 2 x ~45min halves (Figure 6.1). From the twelve players, only seven players played the full 90-min match, two players played 75mins, two players played 45mins, and one came off injured after 15mins. Please refer to the *Ultrasound Shear Wave Elastography* section in the *Methods* of Chapter 6 for a comprehensive overview of the USWE methodology used for muscle stiffness quantification through this thesis. Before the completion of the study, a reliability assessment was performed on 8 participants imitating the experimental procedure of this study without the intervention, (3 hours between measurements) to assess for repeatability and within-subject variation (Appendix 9.4). The coefficient of variation (CV) was deemed acceptable for within-subject variation (7.8%), and the intra-class coefficient exhibited good reliability (ICC = 0.87). During this study, the players were only scanned in a passively stretched condition, rather than a relaxed position and were scanned in a randomised order. Based upon the findings in Chapter 5, only a passively stretched condition was selected as it can be characterised as a more relevant state to an athletic performance task with only a negligible drop in reliability in comparison to the relaxed condition (ICC=0.87 vs. ICC=0.92). This can be supported by further relationships to physical performance in a passively stretched condition than a relaxed condition in Chapter 6.

Figure 6.1 – Methodological Schematic of the Experimental Protocol

Players' physical demands were monitored during the match-play using a portable 18 Hz GPS unit and 600 Hz triaxial accelerometer (APEX pod accelerometer, MAPPS Technology and Bluetooth LE; STATSports; North Ireland). Randers et al. (2010) shown the validity, reproducibility and reliability of GPS devices. Each unit was introduced into an adjustable neoprene vest, inside a back pocket, positioned on the upper part of their backs, between the scapulaes. The GPS unit measures time motion parameters represented by the distance covered and number of efforts at different running velocities throughout an activity. During the statistical analysis we used the following physical metrics, which have been previously used in elite football (Anderson et al, 2016 & Dwyer et al, 2012): Total Distance (m), High Metabolic Load (m), High-Speed Running (>19.8km/h), Explosive Distance (m), Accelerations (>3ms^2), Decelerations (>3ms^2) and Sprint Distance (>25.2km/h). Post-match, the data from the GPS unit was downloaded and analysed using a customized software package (Apex, STATSports, Irlanda, Version 1.2). Dwell time or minimum effort duration (MED) used in our variables were of 0.5 s in Accelerations and Decelerations, and 1 s in Sprint Distance and High-Speed Running.

For the statistical analysis, a Paired Sample T-Test was conducted to analyse the differences between the Pre- and Post- muscle stiffness measurements, and a Pearson's r correlation was calculated to assess the relationship between muscle stiffness values and the MEMS external output from the match play, with the statistical significance for both analyses set at $p<0.05$. This analysis was repeated twice, once for the full eleven players who featured in the game, and once for only the seven players who played 90mins. The magnitude of difference between the groups was also calculated using Cohen's d effect size (Mean of group1- Mean of group2)/SDpooled) where scores were interpreted based on (Cohen, 2013) interpretation with >0.19 = trivial, , 0.2– 0.49 = small, 0.5–0.79 = moderate, >0.8 = large. The standard error of measure (SEM) was calculated using the equation SD*SQRT(1-ICC) to evaluate if any changes pre- to post- match were meaningful and outside the measurement of error, and the minimal detectable change (MDC) was calculated to assess the magnitude of change in shear modulus that would exceed the threshold at measurement error at the 95% confidence interval (Sarabon et al. 2019) using the equation 1.96*SEM*√ 2.

RESULTS

Individual physical outputs measured by MEMS during the Premier League academy match-play are displayed in Table 6.1. The average physical output for those who played 90mins was: 10483m Total Distance, 2213m High Metabolic Load, 649m High Speed Running (>19.8km/h), 1459m Explosive Distance, 79 Accelerations (>3ms^2), and 91 Decelerations 91 (>3ms^2), and 188m Sprint Distance (>25.2km/h).

No significant difference was found between the pre-scan (2.56 ± 0.40 m/s) and post-scan (2.39 ± 0.38 m/s) for muscle stiffness (p = 0.07) though a mean decrease of -0.17 ± 0.29 m/s was observed for all players who featured in the match, including those who did not play the full 90min (Figure 6.1). When considering only the players who played 90min, the outcome is similar with no significant difference being found between the pre-scan (2.67 ± 0.46 m/s) and post-scan (2.50 ± 0.42 m/s) (p = 0.254), with a mean decrease of -0.17 ± 0.35 m/s, but there was a moderate effect size of 0.45 (Figure 6.2). Despite no significant difference between scans for all players and for those who played the full 90mins, the average change in muscle stiffness was greater than the SEM (0.09m/s) which indicates the difference was outside of the measurement error, yet the MDC, which might be regarded as the minimum amount of change that needs to be observed for it to be considered real change, was 0.25m/s. The results established from this study agree with previous research supporting that endurance and aerobic-based exercise causes a decrease in muscle stiffness immediately post-exercise. Although there are maximal eccentric actions in tasks such as sprinting, COD and jumping in Premier League academy football, the activity as a whole can be categorised as a sub-maximal, aerobic and intermittent activity, and not to a high enough intensity to induce an increase in muscle stiffness.

The Pearson's r correlation performed on the difference in muscle stiffness and the physical output measured via MEMS revealed no significant correlations. Although no correlations of significance were discovered, a negative, strong association was found with Total Distance (r = -0.6291) (Figure 6.3) and a positive, moderate association was found with Number of Sprints (r = 0.5745) (Figure 6.4) with players who played 90mins. Further research into more Premier League academy football match stimulus is required, but these relationships may support previous literature with their muscle stiffness response, as the correlations imply that those who completed more Total Distance had a higher decrease in muscle stiffness and those that completed a greater number of sprints experienced less of a decrease in muscle stiffness. This may suggest that those who completed more Total Distance (a task of endurance) decreased more in muscle stiffness, and those who completed a greater number of sprints (a maximal task with eccentric actions) had less of a decrease in muscle stiffness

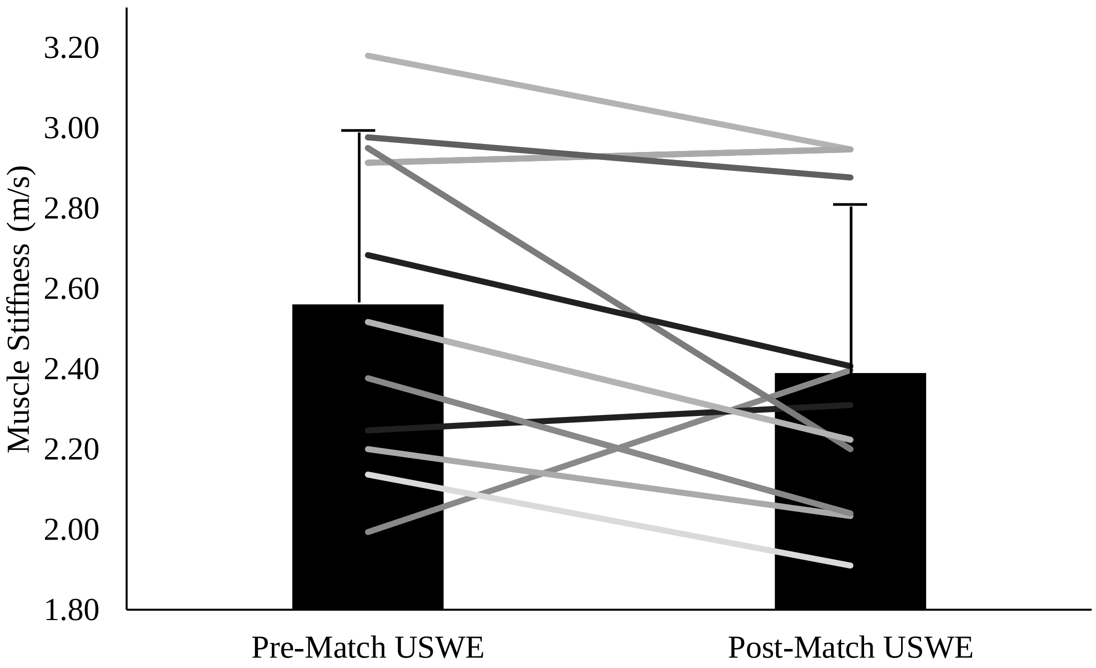


Figure 6.2 – Individual Pre- and Post- Muscle Stiffness Measurements following Elite Academy Match-Play for all players who featured in the match.

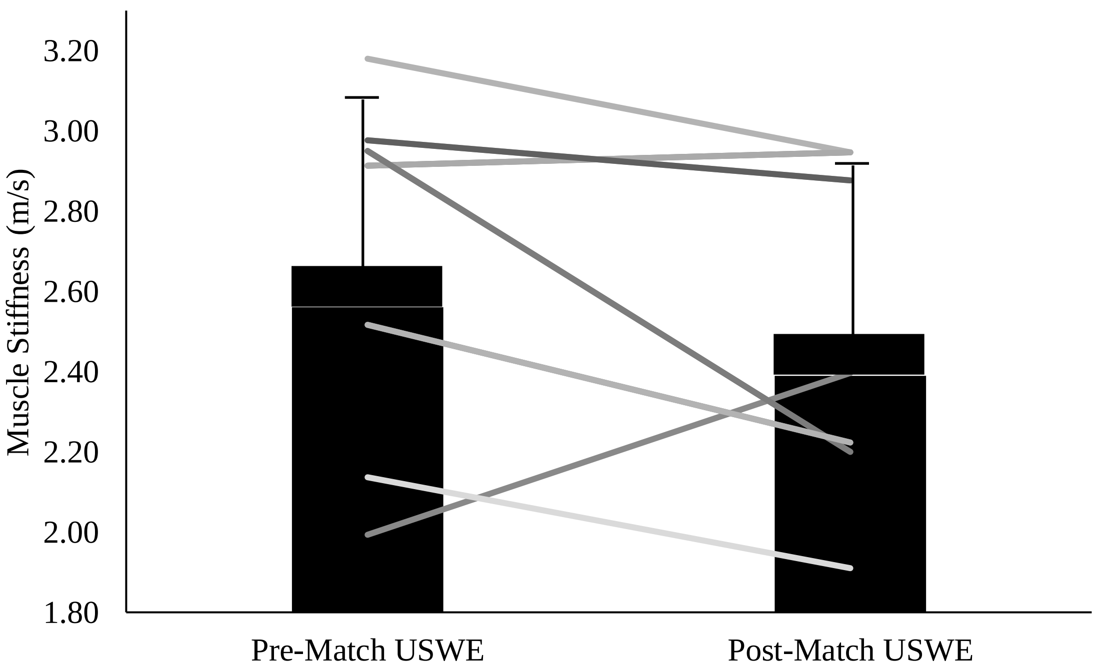


Figure 6.3 - Individual Pre- and Post- Muscle Stiffness Measurements following Elite Academy Match-Play for all players who only played 90-min.

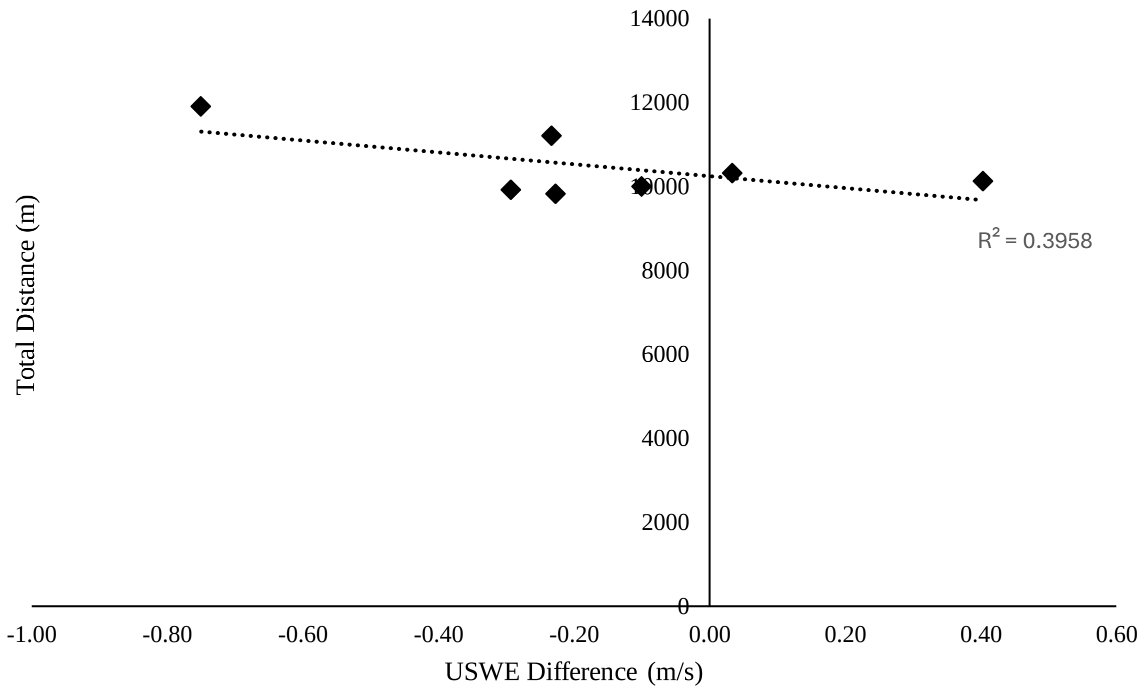


Figure 6.4 – Total Distance (m) from the players who played 90min correlated against the difference in muscle stiffness (m/s) pre- and post- match.

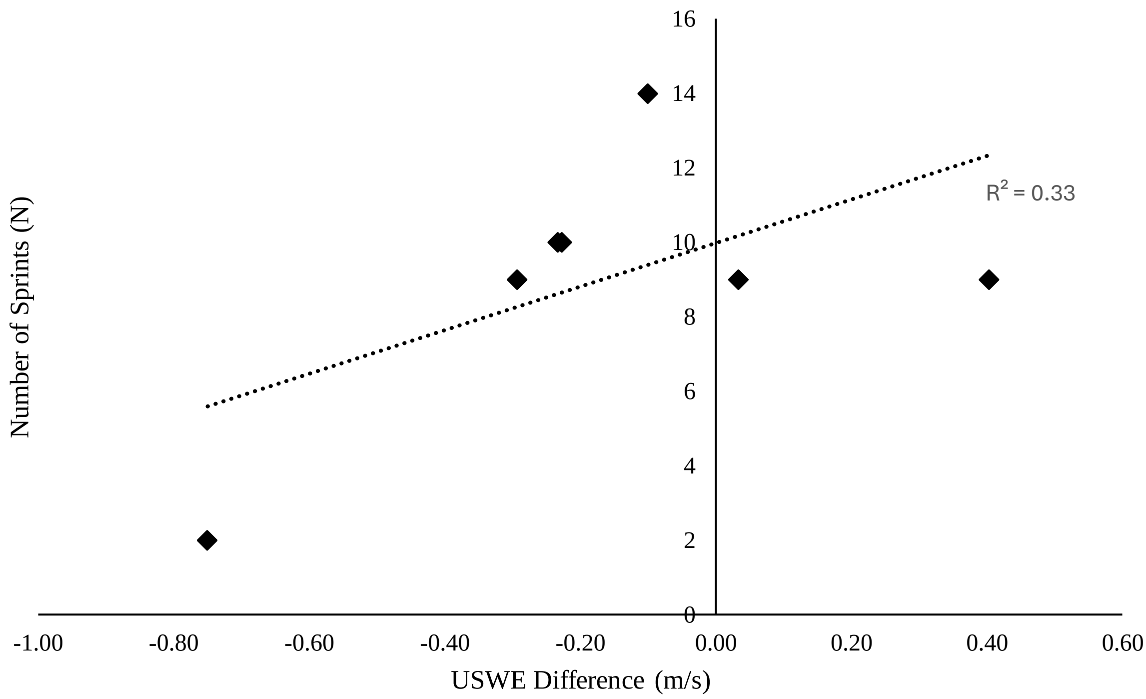


Figure 6.5 – Number of Sprints (n) from the players who played 90min correlated against the difference in muscle stiffness (m/s) pre- and post- match.

Player	Minutes Played	Total Distance	High Metabolic Load	High Speed Running (>19.5km/h)	Explosive Distance	Accelerations (>3m/s ^{2})	Decelerations (>3m/s ^{2})	Sprint Distance (>25.2km/h)	Max Speed (km/h)	Playing Position
1	90	11223	2592	1012	1517	82	86	346	28.91	CAM
2	90	10141	2168	619	1377	81	96	130	30.12	RB
3	76	9105	2198	610	1406	91	82	288	30.67	CM
4	90	9829	2215	562	1458	104	95	218	32.81	CB
5	45	5357	1129	254	725	49	43	74	31.14	CDM
6	90	10325	1719	472	1118	44	39	164	33.16	CB
7	90	11922	2661	803	1872	74	131	16	29.29	CM
8	90	10003	2383	593	1595	92	122	277	30.78	LW
9	90	9937	1756	483	1273	78	70	164	29.81	ST
10	83	10228	2444	839	1606	100	105	91	30.42	LB
11	45	5067	1404	316	744	54	52	170	32.08	CM
Average	90	10483	2213	649	1459	79	91	188		

Table 6.1– Individual physical output measured by MEMS during Elite Academy Match-Play

DISCUSSION

To our knowledge, there has been no known research conducted to assess muscle stiffness following football match-play. Despite no significant changes in muscle stiffness following match-play, the decrease was consistent at an individual level (~81% participants decreased in muscle stiffness) and changes at an individual level seem to be greater than the SEM. There was also a moderate effect size of 0.45, despite no level of significance. This may indicate that if the sample size was larger, a decrease in muscle stiffness could have been significant. While the exact physiological mechanisms that influence muscle stiffness are still debated within the literature, the current hypotheses suggest that muscle stiffness may decrease as a result of cross-bridge attachment release induced by the endurance nature of the match-play. This may show that although a Premier League academy football match is comprised of eccentric actions through rapid changes of direction, decelerations, and sprinting, that the aerobic and intermittent activity profile of the game dominates the muscular stiffness response.

Although Premier League academy football is predominantly an aerobic activity, decrease in muscle stiffness can also be linked to an increase in maximum shear strain during mechanical loading (Loerakker et al. 2013) such as in maximal sprinting or COD, as the shear strain causes deformation, which plays a major role in the aetiology of deep tissue injury. As a result of an increase in internal strain during mechanical loading, the properties of the muscle change, causing an increase in lipid content and a decrease in muscle tone (Scelsi et al. 2001), which can contribute to a decrease in shear modulus (Sadeghi et al. 2018). The research from Andonian and colleagues (2016) however attributed their decrease in muscle stiffness to supra-physiological stress, and the protective mechanism of the muscle tissue for the development of inflammation and muscle swelling. Further research is required to see whether Premier Leagur academy football provides enough of an eccentric and muscle-damaging stimulus to cause this type of protective response, but with the current literature, a decrease in muscle stiffness is more likely because of the cross-bridge attachment induced by endurance activity.

Despite no correlations of significance being found, a negative, strong association was found with Total Distance and a positive, moderate association was found with Number of Sprints with players who played 90mins. This may suggest that the physical output of individual metrics during match-play may influence the muscle stiffness response, with more aerobically demanding outputs such as Total Distance causing a decrease in muscle stiffness, and more eccentrically demanding outputs such as number of sprints causing the adverse response. That being said, the participant who completed the most Total Distance, Decelerations and the 3rd most High-Speed Running had the greatest decrease in muscle stiffness showing there may be concurrent effect with these actions as they are not completed in isolation during match-play, which may indicate that aerobic activity dominates the acute response. Research studies support that there are positional differences in fatigue and muscle damage post-match play due to the different physical demands. For example, a wide midfielder in the Premier League may complete ~1127m of High-Speed Running (Allen et al. 2023), in comparison to a centre back who may only complete ~572m, which places a far greater stress and demand on the hamstrings and high-speed running capacity of a player which will cause a different muscular response. It is hypothesized that positional demands and physical output from the match-play alongside other variables such as injury history, physical load from the previous weeks, strength and tolerance to fatigue will all influence a players' muscle stiffness response, and this further emphasises the individual nature of their response to exercise.

Although previous research has shown that elite football match-play can incur muscle damage responses (Romagnoli et al. 2016, Nedelec et al. 2012, Thorpe & Sunderland, 2012), the moderate effect size in muscle stiffness post- Premier League academy match-play shows a conflicting muscular stiffness response in comparison to Lacourpaille et al. (2017), who exhibited an increase in muscle stiffness immediately post- eccentric exercise during his suggested relationship with muscle damage. This questions whether a Premier League academy football match is intense enough to incur sufficient muscle damage that muscle stiffness is consequently affected, or whether the additional aerobic demands of a football match may disrupt the muscular stiffness response. That being said, the literature evaluating muscle damage following a soccer-specific stimulus does predict that muscle damage is caused. Romagnoli et al (2016) illustrated an increase in Creatine Kinase (strongly associated with a potential relationship to muscle damage) 30min, 24hr and 48hr post- an elite Serie A Football Match, and Thorpe et al (2012) displayed an 84% increase in Creatine Kinase levels following a semi-professional football match. To understand this relationship further and to investigate the true extent of the muscular response to Premier League academy football, further experimental studies are needed that use techniques such as muscle biopsies, which are regarded as the gold standard method of quantifying muscle damage. If muscle biopsies were utilised alongside USWE following an Premier League academy football match, stronger and more accurate inferences would be able to be drawn on muscle stiffness and what it truly means in relation to muscle damage.

A limitation of this study was the small sample size. The sample size required in order to show significance was determined as a result of a priori power analysis using G*Power-2 prior to the start of the study (Version 3.1.9.6, Heinrich-Heine-Universitat, Dusseldorf, Germany). Using previous research analysing the time-course profile of MVC knee extensions on a quadricep muscle, a medium effect size of (0.5) was anticipated for the pre- and post- differences for stiffness. Therefore, with a power level of $1-\beta = 0.8$ (Beck, 2013) and a level of 0.05, the minimum sample size per group was deemed to be 33 participants. As the study examined an Premier League academy match stimulus in a “real-world” experiment, there was consequently no flexibility on including more participants, due to only 11 players being able to start a match, with 3 substitutions allowed as by Premier League regulations. This, re-iterates, the challenges and complexity of acquiring research with Premier League academy athletes during competition.

Another methodological consideration involves the timing of USWE measurements following exercise for examining muscle stiffness. A limitation of this study was the timing of USWE scans, as logistics did not allow for all participants to be measured on exactly 75mins due to only having access to one USWE machine and one examiner. Also, as not all participants completed the full 90min match, the timing of post-match scans for players who were substituted off will be further away from the cessation of their match-play stimulus. For example, the participant who was substituted at half-time would have received his post-match scan closer to ~135mins. To our knowledge, there has been no research which has investigated the time-course response of muscle stiffness against different exercise modality to understand the difference that timing has on the muscle stiffness value. When considering the current literature, the research from Andonian and colleagues (2016) which evaluates the impact of an ultra-marathon on muscle stiffness shows a sustained decrease for up to 48hr – 72hr, yet when considering the research from Lacourpaille and colleagues (2017), who completed a bout of maximal eccentric exercise, the muscle stiffness returned to baseline after 1hr. The inconsistency in the muscle stiffness response between these research studies is likely due to the variance in exercise stimulus, but as a Premier League academy football match possesses both aerobic demands and explosive, eccentric actions, it is unknown as to whether the specific timing of USWE post-match will make an influence on the stiffness outcome. Further research should be conducted to establish whether the muscle stiffness alteration following an Premier League football match is sustained for a period of time by completing a time-course profile, to analyse how sensitive muscle stiffness is to time of scan. Assessing the effect of multiple football matches over a larger time frame with more regular assessments would allow us to receive a more holistic understanding of the effectiveness of USWE to assess changes in muscle stiffness associated with soccer-specific stimuli.

Regional muscle stiffness differences must also be considered when utilising USWE, as only a small region of the tissue is measured during USWE scans, and this cannot describe the stiffness of the whole muscle. Therefore, despite the mid-point of the RF being assessed during this study, it may not illustrate the true condition of the whole muscle tissue. Especially because it is the furthest point from any tendinous structures, which is likely to have a higher degree of stiffness due to tension from the connecting tendon. Studies investigating differences in regional quadriceps muscle stiffness are less prevalent than other muscle groups and report conflicting findings. Kodesho and colleagues (2021a) displayed a higher degree of muscle stiffness at proximal locations in comparison to central or distal locations of the rectus femoris. However, one of the articles assessed in the systematic review, Freitas et al. (2019) displayed no significant differences based on location of the USWE scan. The inconsistency of measurement location is a limitation of the current research in this field as outlined in the systematic review, and it is clear that more research must be done, specifically in the rectus femoris, to validate whether there is an effective position to measure or whether multiple positions across a muscle are required.

The findings of this study suggest that Premier League academy football does not provide enough of a high-intensity or eccentric stimulus to cause an increase in muscle stiffness, with the muscle stiffness response following a similar pattern to previous research studying aerobic-based or endurance exercise. Similar to the research of Lacourpaille et al (2017), the next phase of this research should be to examine the relationship between the muscle stiffness response and force loss 48hours- post the Premier League academy football stimulus in strength diagnostic testing which would be performed two days post- match-play. By measuring this relationship, it would be able to provide information on whether muscle stiffness values derived from the USWE could offer an insight into fatigue, recovery status or "muscle damage" and whether the immediate decrease in muscle stiffness post-exercise is solely an acute response to the stimulus. Despite weak evidence of relationships between Total Distance and Number of Sprints and the response to muscle stiffness, further research with a larger sample size of players over a greater number of games is necessary to determine whether these relationships are significant. If these relationships were deemed to be significant, it would justify the use of USWE post-game to assess the acute response of muscle stiffness and emphasize the muscle stiffness response is dependent upon the type of actions a player does during a game. When considering the practical application of USWE to measure the adaptive response of muscle stiffness post- Premier League academy match-play, future research would have to show meaningful insight, as the time constraints of examining all players pre- and post- match-play may prove to be demanding. A more plausible option may be to focus on specific players following match-play, whether this be players returning from injury focusing on their specific injury location (i.e., hamstring or quadriceps muscles), or specific players who may have illustrated poor recovery or greater fatigue following match-play and require an additional layer of screening following a match.

CHAPTER 7

A CASE SERIES APPROACH:

CHANGES IN MUSCLE STIFFNESS FOLLOWING

CONSEUCTIVE DAYS OF SOCCER-SPECIFIC

TRAINING

Case Study 1

INTRODUCTION

The previous chapter addressed the muscle stiffness response of Premier League academy soccer players following a Premier League academy soccer match. From a soccer-specific stimulus, this is the greatest stress that a Premier League academy player can be exposed to, and often a training stimulus is designed around this external physical output in order to adequately prepare players for the demands of a match (Buchheit et al, 2024). As illustrated in the prior chapter, a football match stimulus is uncontrolled, and there can be substantial individual variability in external output due to tactical, positional and physical factors during match-play. As football matches happen infrequently in comparison to training, measuring a one-off muscle stiffness response to a match stimulus might not provide sufficient information on an individual’s readiness and risk of injury. As soccer-specific training happens more regularly, this stimulus can still lead to injury and performance improvement, therefore, understanding the influence of the repeated stress of training over a period of time is also practically useful for sport scientists and practitioners in elite football.

To build upon the interpretation of the previous study, during this chapter we aim to analyse the influence of a series of soccer-specific training, to be able to assess the muscle stiffness response in a more controlled and prescriptive manner. As soccer-specific training is designed to initiate a specific response in players, whether that be physical or tactical, it is expected that the physical output will be more uniform across those who are experiencing it. By examining soccer-specific training over a period of time, we are able to assess how consecutive soccer-specific stimulus influences the response of muscle stiffness. Being able to understand these training demands is important, as a Premier League academy footballer is not just expected to perform a single bout of exercise but is expected to repeat a soccer-specific training or match stimulus at least 5 times a week. We have previously established in the review of the literature that the volume and intensity of exercise stimulus can strongly influence how long it takes the muscle stiffness to return to baseline, but we are yet to consider how daily consecutive stimulus effects the muscle stiffness measurement.

During the previous study investigating the effects of Premier League academy match-play, 81% of the soccer players displayed an immediate decrease in muscle stiffness to some degree. However, individual variation is apparent, with differing responses such as a 0.75 m/s decrease and a 0.4 m/s increase in muscle stiffness observed. This inherent individual variability might obscure what is actually happening at a muscular level. Often the screening and monitoring programmes in Premier League football teams specifically operate at an individual level, by understanding each individual players thresholds in their testing and understanding which tests are more sensitive to detecting fatigue. Therefore, from a scientific perspective, and also thinking about the practical application, it might be useful to use an approach which focusses on the individual. To account for the inherent individual variability, it was therefore logical to investigate this further research as individual case studies. The aim of this case series approach was to establish the individual response of a Premier League academy footballer performing consecutive days of singular soccer-specific stimulus to help to understand the muscle stiffness response to exercise.

To the author's knowledge, there is no current research which investigates the muscular stiffness response to consecutive soccer-specific stimulus. Therefore, the objective of this study was to measure muscle stiffness over a 5-day period, and to identify the effect of a Premier League academy footballer performing different soccer-specific stimuli performed back-to-back on continuous days. From this case study, we will be able to interpret the muscle stiffness response to training sessions in two ways, (1) the change in response to exercise (pre- to post-training), and to (2) assess whether the baseline value from the pre- training sessions can represent a readiness value as a response from the stimulus performed the day previous.

METHODS

A Premier League academy footballer (age: 17 yrs; height: 1.82m; mass: 76.8 kg) volunteered to participate in this case study. The participant was full-time with the Under-18 squad at a premier league academy and was uninjured at the time of testing. The participant trained a minimum of 4 times a week, with 1 match, as well as 3 structured strength and conditioning sessions during his weekly microcycle. The participant during this study was an attacking midfielder, who had been at the club for 8 years, and a current England youth international. During the current season, his average 90-minute match data was as follows: 11123m Total Distance, 1198m High-Speed Running (>19.8km/h), 228m Sprint Distance (>25.2km/h), 78 Accelerations (>3ms^2) and 90 Decelerations (>3ms^2). During the current season, his average weekly GPS loadings were: 26782m Total Distance, 2456m High-Speed Running (>19.8km/h), 503m Sprint Distance (>25.2km/h), 328 Accelerations (>3ms^2) and 318 Decelerations (>3ms^2). The player has a maximum speed recorded of 32.9km/h during his time at the club and was ranked in the top 5% across the Under-18 squad during the MAS 1500m time-trial assessment. Written informed consent was given to the players in line with the club's academy policy and written consent was provided by a parent/guardian. Ethical approval was granted by the ethics committee at University of Birmingham, Birmingham, United Kingdom.

Th Premier League academy footballer was scanned on average ~75mins pre- and ~75mins post- 5 days of soccer-specific stimulus to perform a 5-day longitudinal analysis of muscle stiffness pre- and post- interventions. Please refer to the Ultrasound Shear Wave Elastography section in the Methods of Chapter 6 for a comprehensive overview of the USWE methodology used for muscle stiffness quantification through this thesis. As per the previous chapter, the player was only scanned in a passively stretched condition. During the case study we assessed the muscle stiffness response in two ways: (1) evaluating the pre- to post- stiffness of each individual training session by examining the change in muscle stiffness within the same day, and (2) assessing the daily pre-measurement for fluctuation in muscle stiffness in response to the stimulus or consecutive stimulus from the previous days between days.

Over the 5-day longitudinal analysis, the researcher had no influence on the content or design of the soccer-specific stimulus within the interventions in an attempt to analyse the "real-world" training regime of a Premier League academy footballer in a "natural experiment" type study. The period of training we investigated was as follows: a 90min Premier League academy football match on Day 1, followed by 2 days of soccer-specific training with the men's senior team on Day 2 and Day 3, an indoor recovery session on Day 4 and a further training session on Day 5 with the Under-18's (Figure 1). The indoor recovery session consisted of 10min spin bike (90rpm @ comfortable resistance), 5min myofascial release with a foam roller focusing on all major lower limb muscle groups and 5min ground-based mobility focusing on all major lower limb muscle groups. For each soccer-specific stimulus (match-play or training), the participant worn a portable 18 Hz GPS unit and 600 Hz triaxial accelerometer (APEX pod accelerometer, MAPPS Technology and Bluetooth LE; STATSports; Northern Ireland). Please refer to the GPS section in the Methods of Chapter 7. The external output from each soccer-specific stimulus was correlated to the muscle stiffness responses to training to analyse whether external output influenced the response to the soccer-specific stimulus.

To use as an interpretative framework, the coefficient of variation (CV) and smallest worthwhile change (SWC) were calculated to help interpret the impact of stiffness. The SWC was measured from the pre-match muscle stiffness measurements of the 11 players in the study from the previous chapter to assess between-subject variation. The SWC was measured using the equation 0.2*SD of the muscle stiffness from the sample (Hopkins et al. 2004). The CV was calculated during a previous reliability study (Appendix 9.4) to assess within-individual reliability, and this was expressed in m/s rather than a percentage to compare with the SWC. These values were used for categorisation of meaningful change (Table 7.1), and described as no change, trivial change, possible change, and certain change to assess the magnitude of change in shear modulus that would exceed the threshold at measurement error.

Banding for Categorisation	Shear Modulus (m/s)	Likelihood of Change	Colour Key
< smallest worthwhile change	< 0.05	No Change	
Between SWC and CV	0.05 - 0.08	Trivial Change	
Between CV and 2 x CV	0.08 - 0.16	Possible Change	
> 2 x CV	> 0.16	Certain Change	

Table 7.1 : Categorisation of meaningful changes and colour key

RESULTS

A timeline of the physical outputs measured by GPS during the longitudinal analysis are displayed in Figure 7.1. The greatest physical output was performed on Day 1 in the U-18 Premier League academy match play: 11223m Total Distance, 1012m High Speed Running (>19.8km/h), 82 Accelerations (>3ms^2), 86 Decelerations (>3ms^2), and 205m Sprint Distance (>25.2km/h). The subsequent three training sessions on Day 2, Day 3 and Day 5 had similar physical outputs, with an average of: 5046.67 ± 360.24m Total Distance, 81.00 ± 50.27m

High Speed Running (>19.8km/h), 60.67 ± 17.62 Accelerations (>3ms^2), and 47.67 ± 4.04 Decelerations (>3ms^2), and 0m Sprint Distance (>25.2km/h).

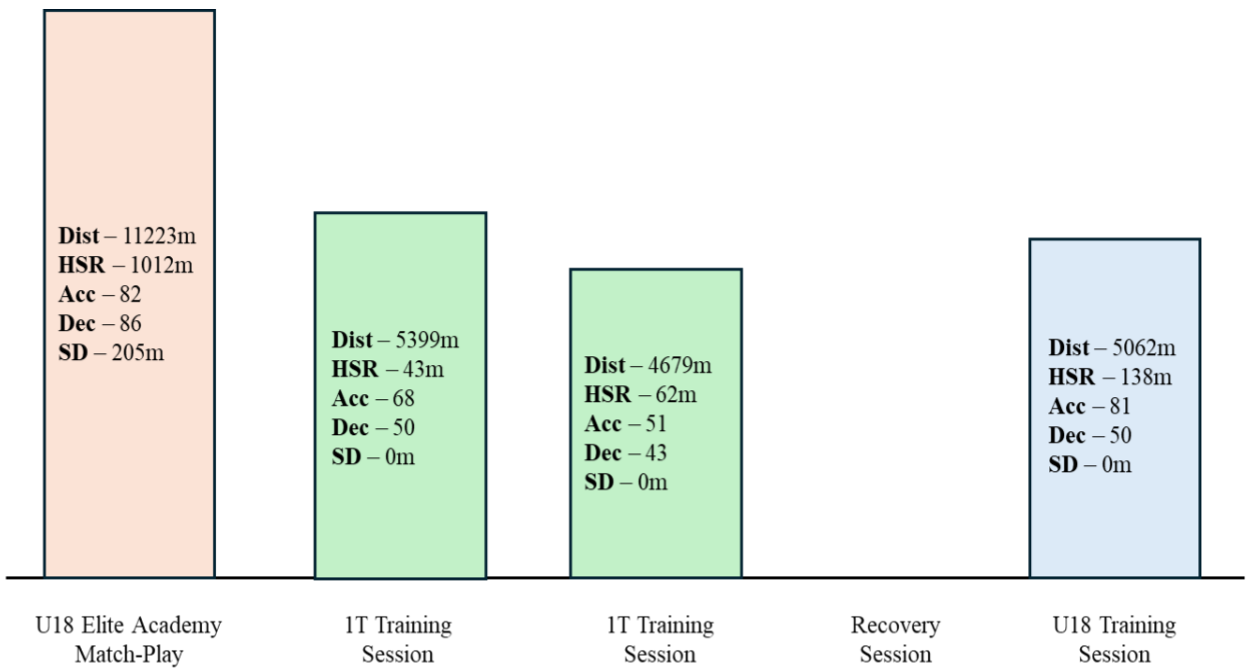


Figure 7.1 – Outline of match and training schedule, with physical GPS output.

When considering the muscular response to each daily stimulus, and assessing the pre- and post- measurements, the participant highlighted an immediate decrease in muscle stiffness following Premier League academy match play, however for the following four days highlighted an increase in muscle stiffness following training and recovery interventions (Figure 7.2). Following the match, there was a 0.23m/s (-7.2%, certain change) decrease in muscle stiffness with USWE, followed by a 0.17m/s (5.5%, certain change) increase on Day 2, 0.27m/s (8.4%, certain change) increase on Day 3, 0.10m/s (3.19%, possible change) increase on Day 4 and a 0.2m/s (6%, certain change) increase on Day 5. The greatest increase in muscle stiffness was following the training session two days following the game. Using the interpretative framework as a guide to assess meaningful change, all muscle stiffness responses apart from post- the recovery session, indicate certain change (Table 7.2). The recovery session, however, only highlights possible change. This may emphasize that the soccer-specific stimulus is producing a muscular response.

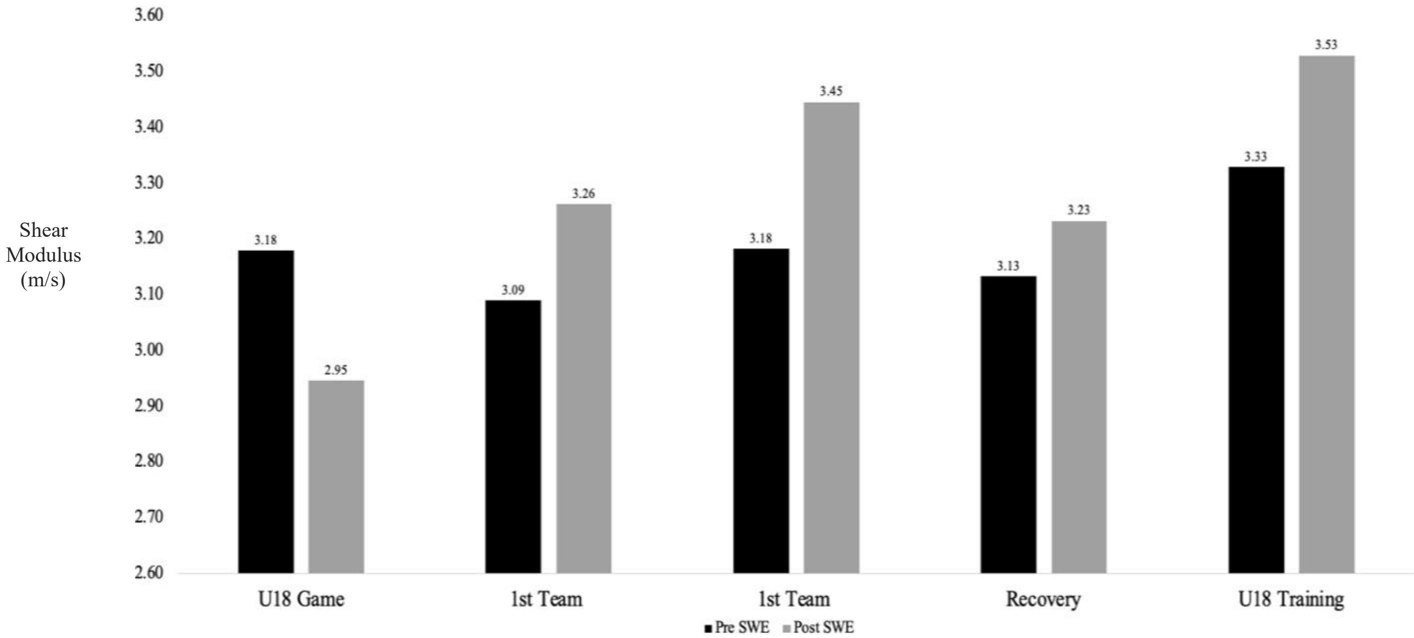


Figure 7.2 – Muscle stiffness response following soccer-specific match, training, and recovery stimulus.

Day	Soccer-Specific Stimulus	Pre- Muscle Stiffness (m/s)	Post- Muscle Stiffness (m/s)	Diff %	Magnitude
1	Match	3.18	2.95	-7.20%	
2	Training	3.09	3.26	5.50%	
3	Training	3.18	3.45	8.40%	
4	Recovery	3.13	3.23	3.19%	
5	Training	3.33	3.53	6.00%	

Table 7.2: Categorisation of meaningful change for pre- and post- soccer specific stimulus

When viewing the data of each daily pre-measurement, there is negligible change from day-to-day, with only a certain change being displayed on Day 5, with a 0.2 m/s increase (6.3 %) in comparison to the Day 4 pre-measurement. There is minimal fluctuation in the day-to-day between the other 3 days, with all differences being below 0.9 m/s (2.9%) and being categorized as possible or trivial change (Table 7.3)

Day	Soccer-Specific Stimulus	Previous Day Muscle Stiffness	Pre- Muscle Stiffness (m/s)	Diff %	Magnitude
1	Match		3.18		
2	Training	3.18	3.09	-2.80%	
3	Training	3.09	3.18	2.90%	
4	Recovery	3.18	3.13	-1.50%	
5	Training	3.13	3.33	6.30%	

Table 7.3: Categorisation of meaningful change for comparison of daily pre-measurements

Through analysis of GPS outputs from each individual soccer-specific stimulus (Figure 7.3, Figure 7.4, and Figure 7.5), it does appear that when Distance, High-Speed Running and Accelerations/Decelerations are higher in a soccer-specific stimulus, that a decrease in muscle stiffness occurs. The volume of physical work completed in the Premier League academy match may support the literature which signifies that endurance or aerobic-based exercise leads to a decrease in muscle stiffness (Morales-Artacho et al., 2017, Sadeghi et al., 2018, Fouré et al., 2022) as discussed in the previous chapter. The other three other training sessions, with similarly lower physical outputs (see Figure 7.1), all produce an increase in muscle stiffness.

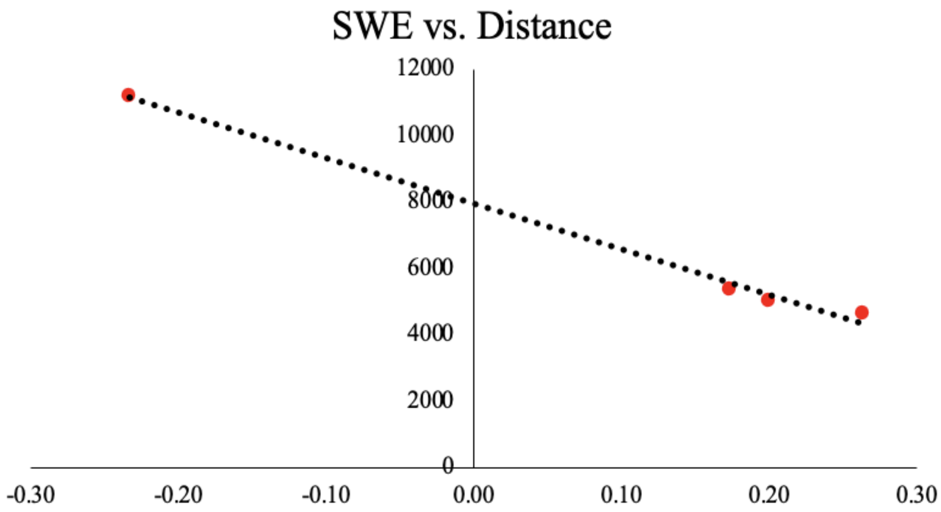


Figure 7.3 – Correlation of USWE Difference (m/s) and Total Distance (m) performed.

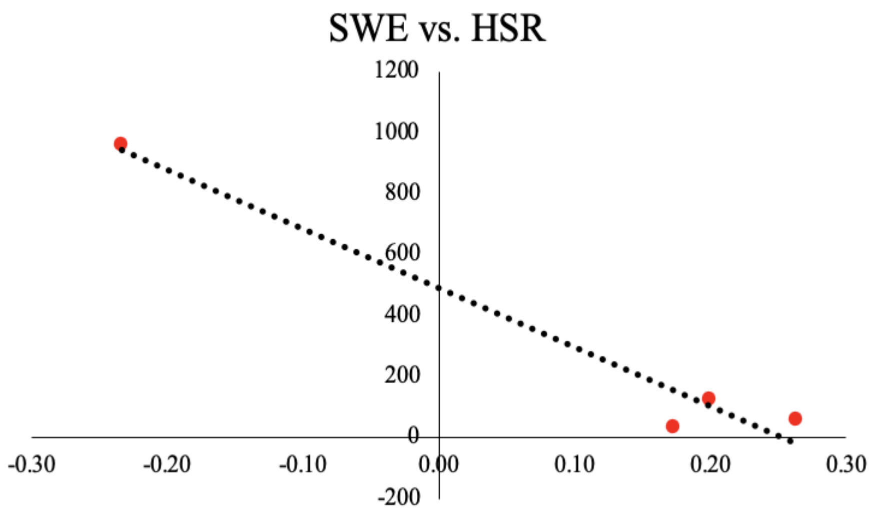


Figure 7.4 – Correlation of USWE Difference (m/s) and High-Speed Running (m) performed.

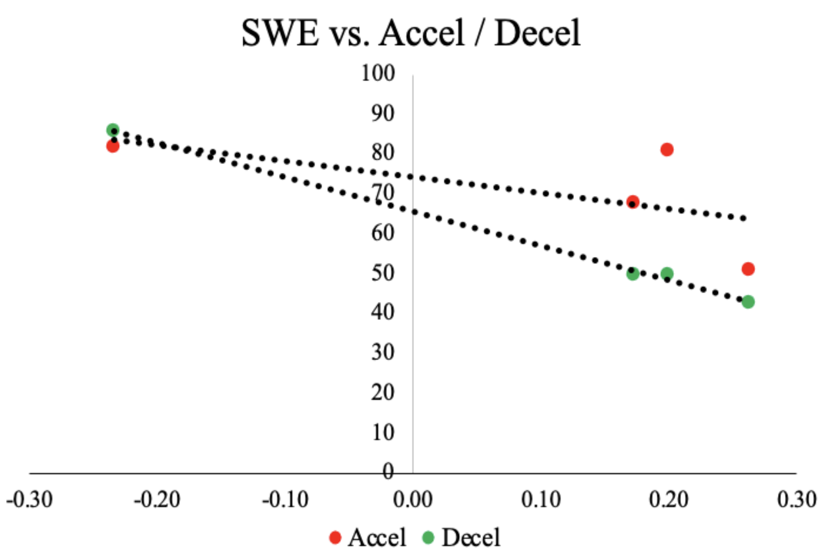


Figure 7.5 - Correlation of USWE Difference (m/s) and number of Accelerations and Decelerations performed.

DISCUSSION

This case study reveals that different types of soccer-specific stimulus can cause altered response in muscle stiffness, exhibiting that a Premier League academy match caused a decrease in muscle stiffness, whereas the subsequent 4 days of the longitudinal analysis, comprising of soccer-specific training sessions and a recovery session, produced a consistent response. All match and training interventions displayed certain changes in muscle stiffness post- stimulus, apart from the measurement following the recovery intervention, which only caused a possible change. When evaluating the between-day response and analyzing the effects of the repeated stimuli in the daily pre-measurements, there are only possible or trivial changes, apart from the certain change in muscle stiffness following the recovery session on Day 5, meaning that although muscle stiffness may have responded immediately post-activity, that it returned to close to baseline in the following day's pre-measurement.

When considering the muscular response to the exercise interventions in the case study, the current literature emphasizes that there is decrease in muscle stiffness following endurance or aerobic-based activity. The exercise interventions where decreases in muscle stiffness have been discovered vary from low-level 15min steady-state cycling (Morales-Artacho et al. 2017), to mountainous 330km ultra-marathons (Andonian et al. 2016). That being said, not all research agrees, as studies from Ohya and colleagues (2017) and Fouré and colleagues (2022) have shown the alternate response, with an increase in muscle stiffness following 30min treadmill running, and <60km ultra-marathon races, respectively. Interestingly, Fouré and colleagues (2022) displayed an increase in muscle stiffness following the shorter races, but a decrease in stiffness following the longer races which presents a similar muscle stiffness response to this current study. The research from Fouré and colleagues (2022) used a similar type of study design for collecting muscle stiffness, using a “real-life experiment,” as the exercise intervention was not standardised between participants, meaning all athletes ran at different speeds and intensities throughout, similar to that of a soccer-specific stimulus. When referring to the previous chapter, there was a similar trend present with a moderate relationship illustrated between Total Distance completed during the Premier League academy match and the severity of the decrease in muscle stiffness, which may support the finding from Fouré and colleagues, that a longer bout of activity, or a stimulus which causes a higher aerobic response may cause a greater degree of muscle stiffness change.

The only stimulus that did not cause a certain change in muscle stiffness was on Day 4 following the recovery intervention. The literature has shown that interventions such as static stretching, which was performed during the recovery session, can reduce passive stiffness measured by USWE (Ichihashi et al., 2016, Andrade et al., 2020, Umegaki et al., 2015). However, the post-measurement following the recovery intervention did still show a possible change, with a minor increase in muscle stiffness which goes against the current literature. This may show that the recovery intervention was either not effective in reducing the passive muscle stiffness, or that there was a concurrent effect from other activities such as foam rolling and low-level cycling which may have influenced the tissue response (Morales-Artacho et al. 2017, Schroeder et al. 2021, & Nakamura et al. 2021). The blunted muscle tissue response following the recovery session, though, does highlight that soccer-specific stimulus, albeit whether it is a training stimulus or football match, does cause a change in muscle stiffness. Further research is necessary to establish whether this tissue response to exercise is meaningful in a Premier League football monitoring system, but it may highlight the importance of a recovery intervention to diminish the muscle stiffness response after repeated bouts of training or match-play.

The training session 2-days post- the Premier League academy football match exhibits the greatest muscle stiffness change in comparison to the other soccer-specific stimulus. The current literature shows a trend of having increased muscle damage and fatigue levels at around ~48hr post- match play with an increase in creatine kinase (Scott et al. 2016, Perez-Castillo et al, 2023, Pooley et al. 2020, Thorpe et al. 2015, & Ispirilidis et al. 2008), cortisol and high-sensitivity interleukin 6 levels (Romagnoli et al. 2015) and decreased neuromuscular performance (De Hoyo et al. 2016), showing a Premier League footballer may still be recovering from the match-play at this time-point. However, the pre-measurement prior to the training sessions two-days following the match play (M + 2) has already returned to its baseline level of muscle stiffness from pre-match, so it is difficult to infer that the increase in stiffness on the M + 2 may be a consequence of the fatigue and muscle damage accumulated from the match-play, rather than just a response to the training stimulus,

As there is only negligible change between the pre-measurements in this case study, it may indicate that the assessment of USWE as a daily monitoring tool may not be useful in Premier League football to measure the consequence of repeating consecutive stimulus over multiple days. When referring to the current literature, most pre- and post- studies emphasize a change in muscle stiffness up to 1hr post-exercise before returning to baseline (Morales-Artacho et al., 2017, Ohya et al., 2017, Foure et al., 2022, Lacourpaille et al., 2014, Lacourpaille et al., 2017, Guilhem et al., 2016., Siracusa et al., 2019 & Chalchat et al., 2020), yet there is some instances in studies involving extreme exercise, such as ultra-marathons in the research from Andonian and colleagues (2016), where muscle stiffness changes are longer lasting, and can sustain for up to 72hr. This may further support that soccer-specific stimuli, despite the literature confirming that these stimuli induce fatigue and muscle damage (Nedelec et al. 2012 & Thorpe

& Sunderland, 2012), are not intense or physically damaging enough to affect muscle stimulus over a longer time frame than 75mins as performed in this study.

This case study has illustrated that different types of soccer-specific stimulus may provide different muscle stiffness responses, as the Premier League academy football match stimulus caused a decrease in muscle stiffness, whereas the training and recovery interventions displayed an increase in muscle stiffness. Based off the findings from Fouré and colleagues (2022), it could be hypothesized that the training and recovery sessions with a lower physical output do not provide a sufficient threshold of stimulus to cause a decrease in muscle stiffness. The current literature shows that interventions such as foam-rolling, cycling, and stretching decrease passive muscle stiffness (Ichihashi et al., 2016, Andrade et al., 2020, Akagi and Takahashi., 2014, Ikeda et al., 2021) and although the individual did not decrease in this case study, he did show a diminished response in comparison to the changes following a match and training stimulus. Despite the muscle stiffness having its greatest increase on the M+2, it is difficult to extrapolate any implications, as the pre-measurement had already returned to baseline. The pre-measurement of muscle stiffness only shows minor changes from day-to-day which may highlight that soccer-specific stimuli are not intense enough to incur longer-lasting changes in muscle stiffness, which makes it difficult to assess the consequence of repeating consecutive stimulus over multiple days.

Case Study 2

INTRODUCTION

In the first case study, we established that a Premier League academy match-play stimulus caused a decrease in muscle stiffness, and the subsequent training sessions had a consistent increase in stiffness post-activity for the specific player. We currently do not understand these implications for the athlete's ability to perform, based off whether their muscle stiffness increases or decrease post-exercise. Therefore, the objective of the 2nd case study was to repeat a longitudinal analysis to establish the individual muscle stiffness response during a consecutive training stimulus again, this time with the addition of a performance marker (countermovement jump) to help interpret the impact of these stiffness changes. The 2nd case study also presented the opportunity to measure the muscle stiffness response to repeated exercise over consecutive days with another Premier League academy footballer to assess the inherent individual variability.

METHODS

A Premier League academy footballer (age: 17 yrs; height: 177.5 cm; mass: 68.3 kg) volunteered to participate in this case study. The participant was full-time with the Under-18 squad at a premier league academy and were uninjured at the time of testing. The participant trained a minimum of 4 times a week, with 1 match, as well as 3 structured strength and conditioning sessions during his weekly microcycle. The participant during this study was a full-back, who had been at the club for 5 years, and also a current England youth international. During the current season, his average 90-minute match data was as follows: 10874m Total Distance, 1021m High-Speed Running (>19.8km/h), 198m Sprint Distance (>25.2km/h), 84

Accelerations (>3ms^2) and 97 Decelerations (>3ms^2). During the current season, his average weekly GPS loadings were: 25634m Total Distance, 2123m High-Speed Running (>19.8km/h), 399m Sprint Distance (>25.2km/h), 378 Accelerations (>3ms^2) and 384 Decelerations (>3ms^2). The player has a maximum speed recorded of 35.2km/h during his time at the club and was ranked average across the Under-18 squad during the MAS 1500m time-trial assessment. Written informed consent was given by the players in line with the club's academy policy with written consent for all players younger than 18 provided by a parent/guardian. Ethical approval was granted by the ethics committee at University of Birmingham, Birmingham, United Kingdom.

Prior to the case study, the player had ~48 hr of no prescribed activity and was encouraged to rest. The Premier League academy footballer was scanned on average ~75mins pre- and ~75mins post- 3 days of soccer-specific stimulus to perform a 4-day longitudinal analysis of muscle stiffness pre- and post- training interventions. During this case study, the individual performed an intensive-themed training session (focusing on smaller areas with an emphasis on overloading Accelerations and Decelerations), an extensive-themed training session (focusing on larger areas with an emphasis on overloading Total Distance, High-Speed Running and Sprint Distance), and a mixed-themed training session (an integration of both small and large area practices) interspersed by a recovery day. As opposed to the recovery session in Case Study 1, the recovery day within this case study had no specific session and was a day of complete rest. Please refer to the Ultrasound Shear Wave Elastography section in the Methods of Chapter 6 for a comprehensive overview of the USWE methodology used for muscle stiffness quantification through this thesis. As per the previous chapter, the player was only scanned in a passively stretched condition. As per the previous case study, we assessed the muscle stiffness response in two ways: (1) evaluating the pre- to post- stiffness of each individual training session by examining the change in muscle stiffness within the same day, and (2) assessing the daily pre-measurement for fluctuation in muscle stiffness in response to the stimulus or consecutive stimulus from the previous days between days.

Alongside each USWE scan, the participant completed 3 x CMJ on a force plate (Model FDLite; Vald Performance, Queensland, Australia) sampling at 1000Hz. The aim of using a performance marker was to assess a peak force attribute against the USWE scan, similar to the study of Lacourpaille and colleagues (Lacourpaille et al. 2014, & Lacourpaille et al. 2017) to see whether there was a distinct relationship between muscle stiffness and a performance task. Please refer to Countermovement Jump section in the Methods of Chapter 6 for a comprehensive overview of the CMJ methodology used throughout this thesis. The CMJ is one of the main tools used to examine neuromuscular status in elite sports (Garrett et al, 2019), due to its high repeatability and fatigue sensitivity to detect neuromuscular fatigue, which is essential for load monitoring in high-performance sport settings (Gathercole et al. 2015). As in the aforementioned chapter, values of jump height, reactive strength index modified (RSI-mod), countermovement depth, eccentric peak force and contraction time were provided and exported to Microsoft Excel for further analysis. These jump metrics were used to account for performance profiling, analysis of neuromuscular fatigue, and jump strategy to try and understand the stiffness change in more detail (Bishop et al. 2023). Jump height and eccentric peak force are output metrics which correspond to physical capacities, whereas RSI-mod, and contraction time are time-based metrics which correspond with neuromuscular fatigue. Countermovement depth is a jump strategy metric which helps to explain how they achieved their physical output, which can be modified is neuromuscular fatigue is present.

Please refer back to the previous methods section in Case Study 1 for a comprehensive overview of the GPS utilised during this study (APEX pod accelerometer, MAPPS Technology and Bluetooth LE; STATSports; Northern Ireland), and the interpretative framework used to assess for meaningful change. The interpretative framework approach was also repeated for the CMJ analysis, with the SWC and CV being measured from 19 Under-18 footballers in the study in Chapter 6 to assess for meaningful change. Using the classification of sensitivity from Hopkins (2004), as the SWC was greater than the CV for the CMJ, it shows that the CMJ is categorized as a marker of good sensitivity (Roe et al. 2016). Markovic and colleagues (2004) and Claudino and colleagues (2017) have both stated that the CMJ has a high reliability as a monitoring tool. Therefore, > 2 x CV was classified as certain change (1.54cm), and the difference between SWC and CW (0.81cm – 1.54cm), was classified as possible change.

RESULTS

A timeline of the physical outputs measured by GPS during the longitudinal analysis are displayed in Figure 7.6. On Day 1 in the intensive session the participant performed: 5955m Total Distance, 112m High Speed Running (>19.8km/h), 134 Accelerations (>3ms^2), 99 Decelerations (>3ms^2), and 52m Sprint Distance (>25.2km/h). On Day 2 in the extensive session the participant performed: 9941m Total Distance, 364m High Speed Running (>19.8km/h), 121 Accelerations (>3ms^2), 82 Decelerations (>3ms^2), and 121m Sprint Distance (>25.2km/h), and on Day 3 in the mixed session, the participant performed: 9057m Total Distance, 190m High Speed Running (>19.8km/h), 88 Accelerations (>3ms^2), 57 Decelerations (>3ms^2), and 29m Sprint Distance (>25.2km/h).

The participant highlighted a decrease in muscle stiffness following every training session (Figure 7.7), with the greatest decrease occurring following the extensive session (Day 2) with a 0.39 m/s decrease (-14.9%, certain change). The muscle stiffness response on the intensive (day 1) and mixed-theme (day 4) training session only displayed possible change (-0.14m/s, -6.09% and -0.1m/s – 4%, respectively). Despite the muscle stiffness decreasing after every training session, the pre-training muscle stiffness was seen to increase every day for the first three consecutive days (Day 1 – 2.3 m/s, Day 2 – 2.61 m/s, Day 3 – 2.74 m/s), which could infer a response to multiple bouts of exercise (Table 7.5). On Day 2, there was a 0.31 m /s (13.4%, certain change) increase in muscle stiffness from the previous day's pre-measurement. On Day 3, there was a 0.13 m/s (4.9%, possible change) increase in comparison to the pre-measurement on Day 2. However, on Day 4, there was a 0.28 m/s (-10.2%, certain change) decrease in muscle stiffness in relation to the pre-measurement of Day 3. The pre-training muscle stiffness finally decreases on the 4th day, which may be an outcome of the recovery day on Day 3.

Day	Soccer-Specific Stimulus	Pre- Muscle Stiffness (m/s)	Post- Muscle Stiffness (m/s)	Diff %	Magnitude
1	Training	2.3	2.16	-6.09%	
2	Training	2.61	2.22	-14.90%	
3	Recovery	2.74			
4	Training	2.46	2.36	-4.00%	

Table 7.4: Categorisation of meaningful changes for pre- and post- soccer-specific stimulus

Day	Soccer-Specific Stimulus	Previous Day Muscle Stiffness	Pre- Muscle Stiffness (m/s)	Diff %	Magnitude
1	Training		2.3		
2	Training	2.3	2.61	13.40%	
3	Recovery	2.61	2.74	4.90%	
4	Training	2.74	2.46	-10.20%	

Table 7.5: Categorisation of meaningful changes for comparison of daily pre-measurements

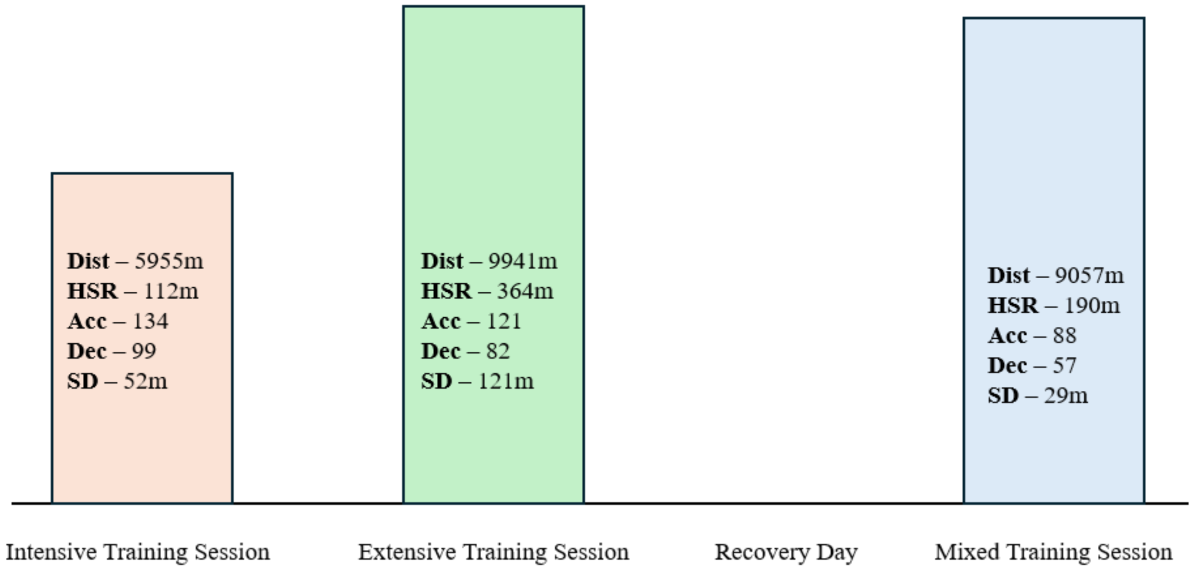


Figure 7.6 – Outline of match and training schedule, with physical GPS output.

The participant's CMJ jump performance is shown in Table 7.6. When assessing the CMJ jump height (Figure 7.8) following the intensive and mixed training session, there was a certain change (>2xCV) in jump height post-training (37.2cm – 40.4cm and 35 – 38.4cm respectively), which may highlight a potentiation effect. However, there was a drop-off in CMJ performance following the extensive training session (38.3cm to 36.8cmcm, possible change). From the GPS output, the extensive training session was the most physically challenging training session of the week, which may help rationalize the drop-off in jump height. The participants eccentric peak force is displayed in Figure 7.9. Unlike the jump height measurements, eccentric peak force did show a high negative correlation (r = -0.8087) against time in each CMJ assessment across the 4-day longitudinal analysis. This could indicate neuromuscular fatigue, and a reduced ability to load as effectively during the eccentric portion of the jump.

	Jump Height (cm)		RSI-mod (m/s)		Eccentric Peak Force (N)		Contraction Time (s)		Countermovement Depth (cm)	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	37.2	40.4	0.53	0.58	1500	1551	703	699	27.9	28.6
Day 2	38.3	36.8	0.49	0.49	1515	1492	788	703	31.2	27.9
Day 3	36.4		0.46		1495		800		30	
Day 4	35	38	0.48	0.48	1464	1394	736	799	29.2	29.7
Average	36.7	38.5	0.49	0.52	1493.5	1479.0	756.8	733.7	29.6	28.7
SD	1.4	1.8	0.03	0.06	21.4	79.3	45.3	56.6	1.4	0.9

Table 7.6 – Overview of CMJ performance over the 4-day longitudinal analysis

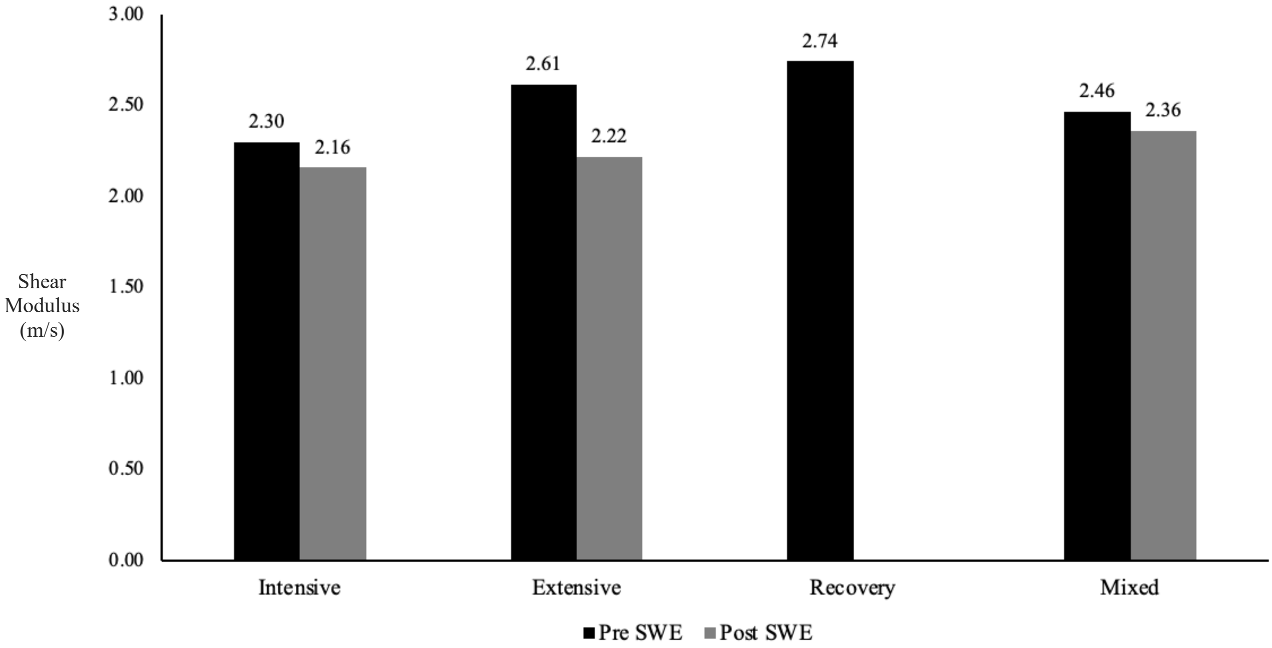


Figure 7.7 – Muscle stiffness response (m/s) following soccer-specific match, training, and recovery stimulus.

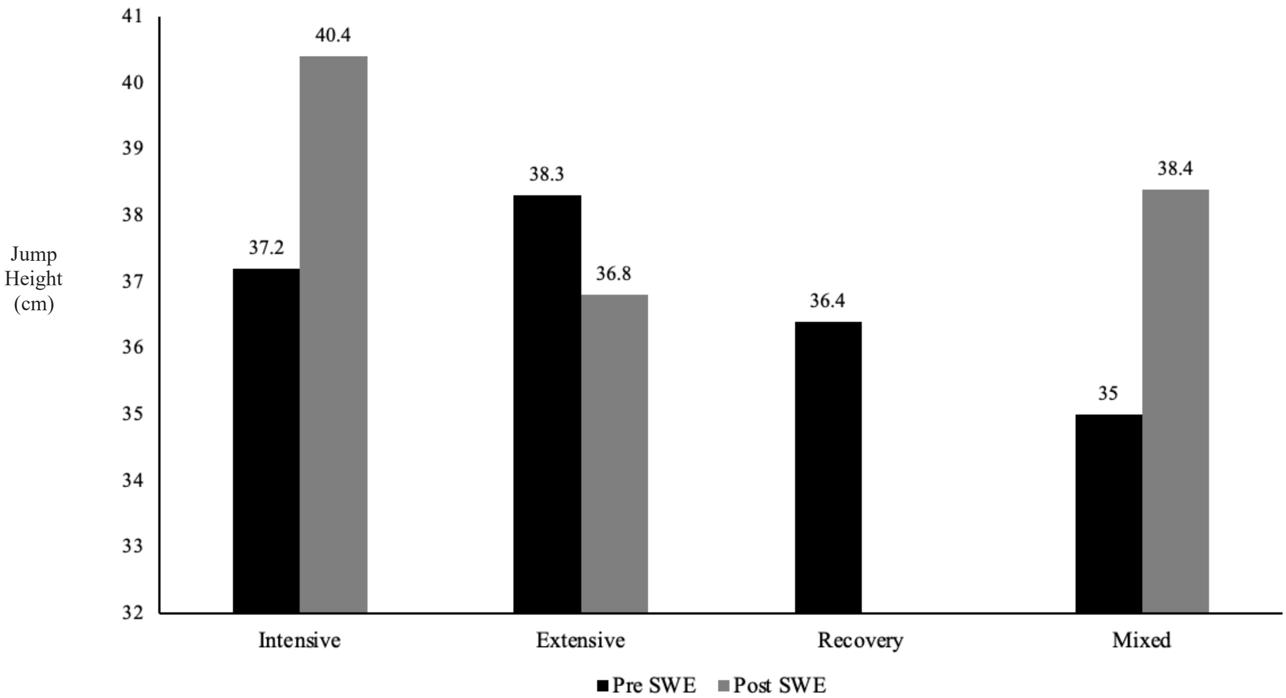


Figure 7.8 – Jump Height (cm) following soccer-specific match, training, and recovery stimulus.

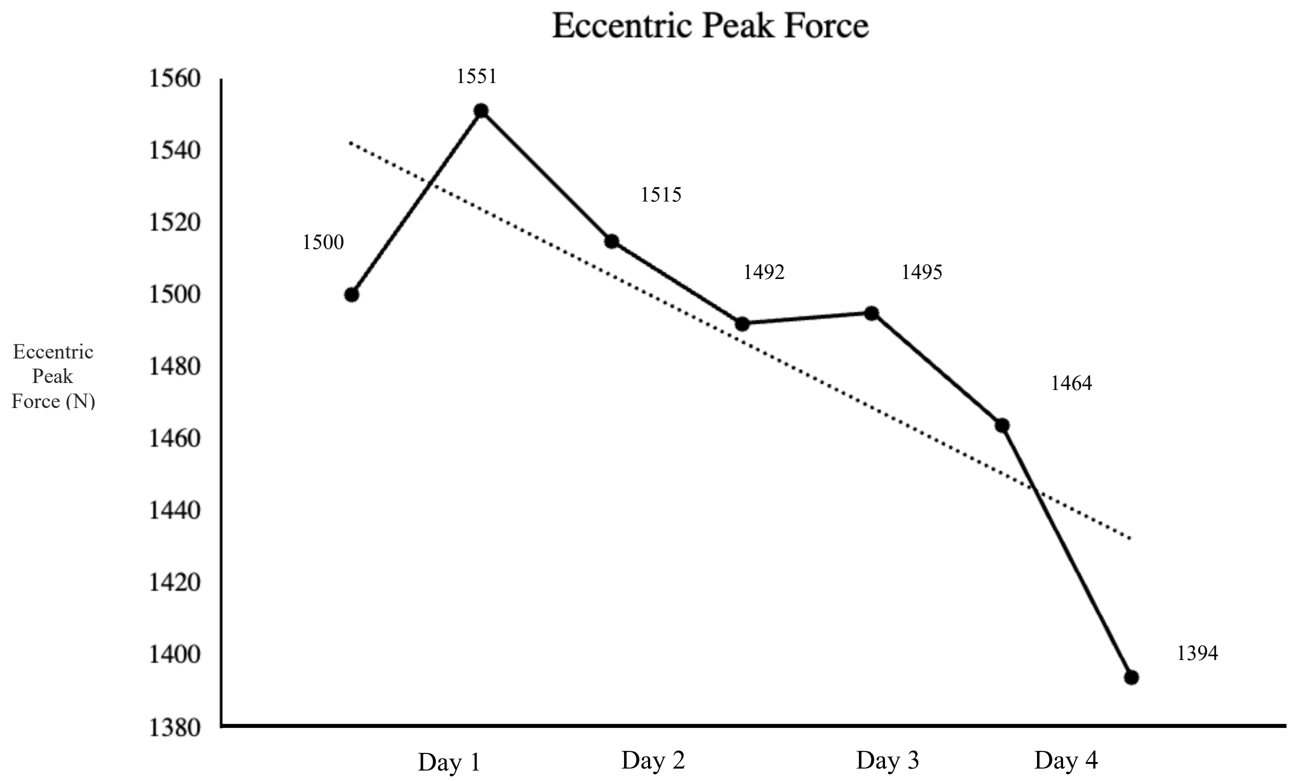


Figure 7.9 – Eccentric Peak Force (N) response following soccer-specific match, training, and recovery stimulus.

As in case study 1, and the previous chapter, there does seem to be a relationship with the highest Total Distance (Figure 7.10) and High-Speed Running (Figure 7.11) causing the highest decrease in muscle stiffness, which may further re-iterate that a longer bout of activity, or a stimulus which causes a higher aerobic response may cause a greater degree of muscle stiffness change. When assessing the relationship between muscle stiffness change and jump performance there does not seem to be any correlation, showing the consequence of muscle stiffness change does not seem to effect function of physical performance.

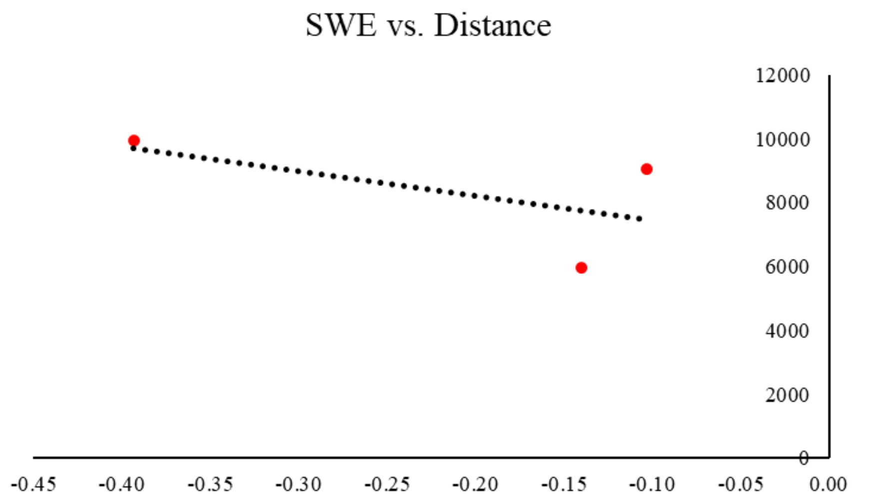


Figure 7.10 – Correlation of USWE Difference (m/s) and Total Distance (m) performed.

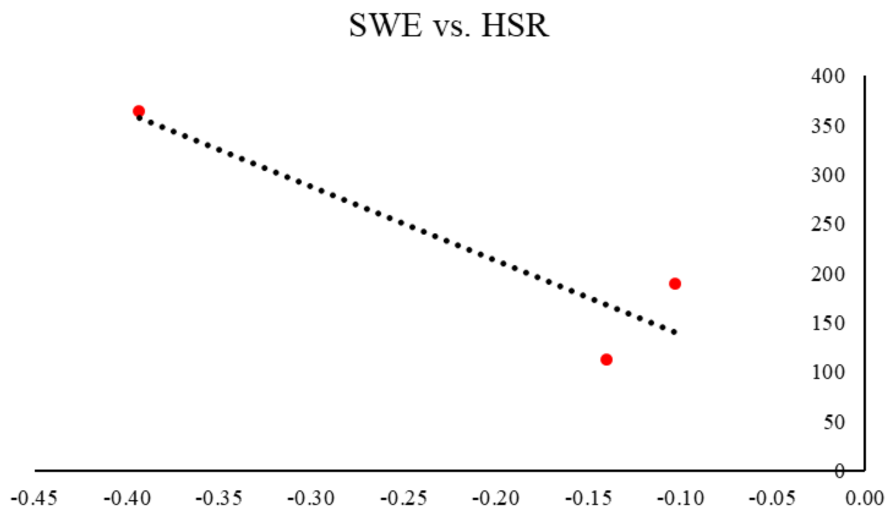


Figure 7.11 – Correlation of USWE Difference (m/s) and High-Speed Running (m) performed.

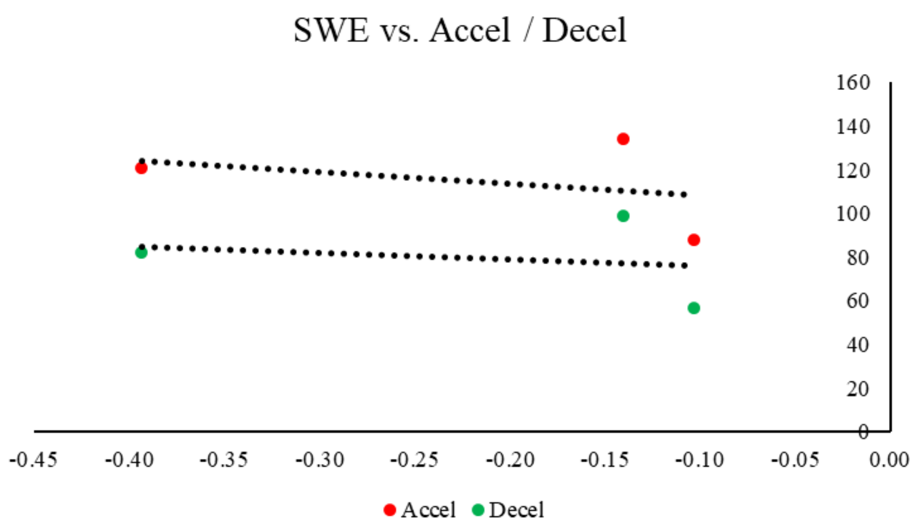


Figure 7.12 – Correlation of USWE Difference (m/s) and number of Accelerations and Decelerations performed.

DISCUSSION

During this case study, there was a decrease in muscle stiffness to all 3 soccer-specific training sessions, which shows conflicting results in comparison to case study 1, which may be a function of the individual variation. The extensive session on Day 2, where the participant completed the most Total Distance and High-Speed Running, instigated a 14.9% decrease in muscle stiffness (- 0.39 m/s) whereas the other two sessions (intensive and mixed) only caused <6.09% decreases in muscle stiffness (0.14 m/s and 0.10 m/s, respectively). When analysing the pre-measurements of muscle stiffness, there was a greater change in muscle stiffness between days, which may infer there was a response to completing repeated stimulus over consecutive days. On Day 2 and Day 4, muscle stiffness change was greater than 10.2% on both occasions (0.31 m/s increase and 0.28 m/s decrease respectively). The decrease in muscle stiffness on the Day 4 pre-measurement may be a consequence on the recovery day on the day preceding. Although there were no clear relationships between CMJ performance and muscle stiffness change, the CMJ did show some interesting findings. There was an increase in CMJ jump height post- two of the training sessions, which may highlight a potentiation effect, but there was a decrease following the extensive training session. Eccentric peak force displayed a gradual decrease across the four-days which indicates indicate neuromuscular fatigue, and a reduced ability to load as effectively during the eccentric portion of the jump. Therefore, this information may show that specific individuals would benefit from daily monitoring of muscle stiffness.

In comparison to case study 1, there is some conflicting results as each training stimulus during this study elicits a decrease in muscle stiffness. When comparing the external physical output of the intensive session in this case study, in comparison to the two first team training sessions and U18 training session in case study 1 there is little difference in total volume of Total Distance and High-Speed Running, yet different muscle stiffness responses have followed, which may be more of a function of the inherent individual variation discussed in the previous chapter. The only other difference between case-studies worth mentioning is the Premier League academy match-play preceding the training sessions in the previous case study. Although there is increases in muscle stiffness in the training sessions following the match, it is unlikely that this was a response from the match-play as the pre-measurement had already returned to baseline, hence strengthening the argument for individual variation in muscle stiffness responses.

However, there was some likenesses between the two case studies, as there does seem to be a correlation between the Total Distance and High-Speed Running performed and the extent of muscle stiffness change post-activity. Both case studies highlighted their greatest decrease in muscle stiffness following their soccer-specific stimulus which comprised of the highest Total Distance and High-Speed Running, in the Premier League academy match (0.23 m/s) and extensive session (0.39 m/s) respectively. This may potentially indicate that when a soccer-specific stimulus comprises of a certain threshold of endurance and aerobic stress that a muscle stiffness decrease occurs post-exercise, despite the eccentric and more explosive actions which the external output may encompass.

The extensive training session experienced the greatest muscular response, which was the day following the intensive session, may be a consequence of performing multiple stimulus over consecutive days and the compound effect of fatigue and muscular disruption incurred by both sessions. This can be supported by the rising pre-stiffness values from Day 1 to Day 3 when we analyse the readiness measurements, as the muscle stiffness values increased from 2.3 m/s to 2.61 m/s and 2.74 m/s over the course of the three days, which may reinforce the compound effect of completing the training sessions on consecutive days. Interestingly, on Day 4, the pre-muscle stiffness value decreases back to 2.46 m/s following a day of recovery. The gradual increase in muscle stiffness evident in the pre-stiffness scan shows a conflicting outcome to case study 1, which shown minimal change day-to-day with the pre- measurement. Unlike the previous case study, the results exhibit muscle stiffness increasing after being exposed to multiple stimulus over consecutive days and may signify the use of USWE in readiness monitoring process for specific individuals.

When assessing the CMJ performance in relationship with the muscle stiffness, there was no clear correlation. In the two training sessions with minor muscle stiffness responses (intensive and mixed), there was an increase in jump height post- the training session (37.2 cm to 40.4 cm and 35 cm to 38.4cm respectively), which could display a potentiation effect. This post-activation potentiation (PAP) refers to a short-term improvement in performance as a result of using a conditioning exercise to improve subsequent performance (Rahim et al. 2007, Ruben et al. 2010). For example, Weber et al. (2010) demonstrated how a squat protocol may improve performance in subsequent vertical jumps, due to the muscles being placed in a 'potentiated' or 'activated' state. Although PAP usually refers to a heavy-load exercise to potentiate the muscle, a football training session may have administered a similar response as a conditioning exercise for the CMJ. However, as the CMJ was not performed until ~75mins post the training session, there is evidence that the potentiation effect may have dissipated (Tilin et al. 2009).

Following the extensive training session however there was a decrease in jump height (38.3 cm to 36.8 cm), and this correlates with the largest decrease in muscle stiffness post- training session. A decrease in jump height could be anticipated due to the increase in neuromuscular fatigue following a physically exerting training session. Kalkhoven and Watsford (2018) has previously stated that muscle stiffness is thought to be a regulator of performance, especially in explosive actions such as jumping, whereby higher levels of stiffness appear advantageous. Thus, a decrease in jump height performance seems logical if there is a decrease in muscle stiffness. Following the extensive training session, the CMJ jump height goes on to decrease for the next 2 consecutive days which also accentuates the increase in neuromuscular fatigue over the training microcycle (38.3 cm pre-extensive, to 36.8cm post-extensive, to 36.4cm pre-recovery, and to 35cm pre-mixed session), which is reinforced when scrutinizing eccentric peak force from the CMJ, and the decrease which transpires across the longitudinal analysis. This could be an expected response to multiple bouts of exercise from a neuromuscular perspective, but this further accentuates the lack of relationship between the muscle stiffness measures and the functional performance task during this study.

CONCLUSION

This case series establishes that the type of stimulus, and the series in which soccer-specific stimulus is performed can affect the individual muscle stiffness response. In the 1st case study, there is a subsequent increase in muscle stiffness post- soccer specific stimulus following Premier League academy match play where muscle stiffness had originally decreased. Whereas, in the 2nd case study, there was a decrease in muscle stiffness post- all soccer specific stimulus. The contrasting muscular responses in each case study re-enforces the individual variation, meaning we cannot draw too many definitive conclusions. There seems to be an agreement across both case studies that the amount of Total Distance and High-Speed Running performed may influence muscle stiffness response, as both studies have the greatest decrease in muscle stiffness following the highest volume of Total Distance and High-Speed Running.

When assessing a performance task (CMJ) to muscle stiffness in the 2nd case study, there does not seem to be a clear relationship. This emphasizes that although the CMJ may be a clear indicator of neuromuscular fatigue, or a reduction in force through a training micro-cycle, that the muscle stiffness measurement needs to be looked upon through a different lens. To gain a more comprehensive understanding of how a series of soccer-specific stimulus influences muscle stiffness, it would be useful to repeat this study with a larger study group. This would help to investigate the individual variation in muscle stiffness response to the micro-cycle of soccer specific stimulus, and to help provide further information on the usefulness of USWE in a Premier League football monitoring programme.

CHAPTER 8

SYNTHESIS OF FINDINGS

The purpose of this following chapter is to consider the findings of this thesis in regard to the original aim and subsequent objectives of the research program. Practical recommendations to optimise the effectiveness of USWE to be utilised as a monitoring tool within a Premier League football club will be discussed based upon the synthesis of the major findings. Limitations and future recommendations for future research based on the findings will also be examined.

Completion of Objectives

The primary aim of this thesis was to evaluate the effectiveness of USWE to assess physical performance and the change in muscle stiffness in response to soccer-specific stimuli. This was met through the completion of four separate studies (Chapter 3, 4, 5 and 6) investigating the following objectives:

Objective 1 - The aim of the systematic review was to establish what parameters of the USWE methodology must be included when conducting and reporting shear-wave elastography studies focusing on the rectus femoris muscle. By having a greater understanding of the methodological processes used within the USWE literature, we will be able to create a framework of the necessary methodological requirements needed to employ this technology within a practical setting.

From the systematic review, it is clear that there is no established, evidence-based protocol to collect USWE scans in the current research. As the popularity of USWE increases, and interest from within professional sports teams starts to rise, it is imperative to consider the current literature in order to develop a framework of what elements of a methodology should exist when designing a protocol including USWE to measure muscle stiffness. From this systematic review, the practical recommendations for implementing a USWE methodology to measure rectus femoris muscle stiffness in Premier League football are as follows:

- Using the midpoint of the muscle (between the ASIS and the superior border of the patella, with the transverse line drawn in the middle of the line between landmarks) for the anatomical location of the USWE scan.

- The gold standard for fixing a participant into the correct joint angle and position is to use an Isokinetic Dynamometer to avoid unnecessary movement which may affect scan accuracy. However, this review establishes when measuring the rectus femoris, the reliability outcome does not decrease when only a treatment bed is used due to the accessibility and ease of scanning the rectus femoris muscle.

- As there is only a minor drop-off in reliability during passively stretched scans, and the muscle in a stretched condition could be characterised as a more relevant state to an athletic performance task, this may be the preferred condition of the muscle during the scan to infer the most useful conclusions.

- Due to the sensitivity of the USWE readings, it is recommended that 3 scans are taken per measurement, especially if there is only one examiner.

- Scans are required to be performed with the probe in a plane parallel to the muscle fibres and perpendicular to the skin.

- Based on the review, the ROI measured should be a 5mm diameter circle within a 10 x 10mm or 10 x 20mm Q-Box, utilising the colour map to select the region of the muscle which avoided aponeurosis areas, or non-muscular structures.

- If performing a study in response to an exercise stimulus or intervention (Andonian et al. 2016), ~60mins post- exercise should be used to assess the muscle stiffness response to ensure that the response to exercise is truly being measured.

Objective 2 - The aims of this study are: (1) to quantify rectus femoris muscle stiffness values across different age groups in Premier League academy footballers, (2) to determine the relationship between rectus femoris muscle stiffness and measures of physical performance, and to (3) assess the differences in relationship between a holistic lower body stiffness measurement (vertical stiffness derived from a hop test) in comparison to the USWE method which measures muscle stiffness only.

The findings of this study suggest that correlations between stiffness measurements, athletic performance tests and age of athlete are more evident with the vertical stiffness calculated during the hop test, in comparison to the USWE scans. Unlike muscle stiffness with the USWE, vertical stiffness was significantly different between age groups, with the Under-23 squad being significantly higher in stiffness than both the Under 18 squad and the Under 16 squads. Rectus femoris muscle stiffness was only correlated to select jump-related metrics, whilst increased vertical stiffness levels appear to be beneficial across a range of performance tasks including acceleration, maximal strength and jump performance, and seems to be a more effective way of identifying higher achievers in athletic performance tasks. This is significant for Premier League football academy practitioners to understand, as measuring vertical stiffness via a hop test represents a movement which is relevant to sporting performance in comparison to the passive or relaxed condition used for measuring muscle stiffness. Further research into the muscle stiffness of other individual muscle sites with the USWE may also help to profile where in the lower limb that stiffness is effective for athletic performance tasks to help gain a more comprehensive understanding. As a result, this study provides potential evidence that detailed testing and screening of whole-body and individual muscle stiffness units alongside a training programme to enhance stiffness levels may be recommended to contribute to the success of sporting performance.

Objective 3 - The aim of this study is to perform a preliminary investigation to provide information on the response of muscle structural properties, more specifically muscle stiffness, following a 90-minute Premier League academy football match, and the potential use of USWE to provide useful information in a "real-world" football monitoring program.

The findings of this study suggest that Premier League academy football does not provide enough of a high-intensity or eccentric stimulus to cause an increase in muscle stiffness, with the muscle stiffness response following a similar pattern to previous research studying aerobic-based or endurance exercise. Despite no significant changes in muscle stiffness following match-play, the decrease was consistent at an individual level (~81% participants decreased in muscle stiffness) and changes at an individual level seem to be greater than the SEM. This may indicate that if the sample size was larger, the decrease in muscle stiffness may have been significant. While the exact physiological mechanisms that influence muscle stiffness are still debated within the literature, current hypotheses suggest the immediate decrease in muscle stiffness may be a result of cross-bridge attachment release induced by the endurance nature of the match-play. Despite weak evidence of relationships between Total Distance and Number of Sprints and the response to muscle stiffness, further research with a larger sample size of players over a greater number of games is necessary to determine whether these relationships could potentially be significant. If these relationships were deemed to be significant, it would justify the use of USWE post-game to assess the acute response of muscle stiffness and emphasize the muscle stiffness response is dependent upon the type of actions a player does during a game.

Objective 4 – The aim of the case series was to (1) assess how consecutive soccer-specific stimulus influences the response of muscle stiffness measured by USWE, and (2) to establish how a consecutive training stimulus of different themed training sessions may influence the acute change of muscle stiffness with the addition of a performance marker (CMJ) to help interpret the impact of the stiffness.

This case series establishes that the type of stimulus, and the series in which soccer-specific stimulus is performed can affect the individual muscle stiffness response. In the 1st case study, there is a subsequent increase in muscle stiffness post- soccer specific stimulus following Premier League academy match play, whereas in the 2nd case study, with the training sessions performed in absence of a preceding football match, there was a decrease in muscle stiffness post- soccer specific stimulus. There also seems to be an agreement across both case studies that the amount of Total Distance and High-Speed Running performed may influence muscle stiffness response, as both studies have the greatest decrease in muscle stiffness following the highest volume of Total Distance and High-Speed Running. When assessing a performance task (CMJ) to muscle stiffness in the 2nd case study, there does not seem to be a clear relationship. This emphasizes that although the CMJ may be a clear indicator of neuromuscular fatigue, or a reduction in force through a training micro-cycle, that the muscle stiffness measurement needs to be looked upon through a different lens.

GENERAL DISCUSSION

The general objective of this thesis was to see whether muscle stiffness was an important parameter to monitor within Premier League academy football, and to see whether USWE could be a useful technology to employ to measure it. Muscle stiffness has historically been neglected in the research, due to there (1) not being a general consensus of what muscle stiffness actually is, and the role it plays as well as (2) not having the proficient equipment and technology which can measure it. Within elite sports performance, a whole battery of testing is regularly performed in order to predict how the athlete is responding to the training and competition schedule. Yet this information is generally an estimate of the internal muscular response, rather than objective data, as most elite sports organisations either do not have the facilities to perform procedures such as muscle biopsies, or do not want to cause any disruption to the athlete during season. That being said, even gold standard procedures such as Z-band streaming from muscle biopsies to quantify muscular disruption is not ideal, as this is not a procedure that can be performed regularly and as part of a daily or weekly monitoring system. Therefore, gaining a greater understanding of technologies such as USWE and its ability to monitor mechanical muscle properties could be revolutionary in the sport science industry.

The systematic review illustrated that there is major variation in the methodology used to collect muscle stiffness information via the USWE. This review provided practical applications to collect muscle stiffness in the rectus femoris during this study, but a general consensus of an evidence-based methodology in the applied and research setting needs to be agreed upon.

Until an agreement is reached, it will be very difficult to make informed decisions on the muscle stiffness derived from USWE, without reference of normative data, and knowing what can be characterised as high or low levels of muscle stiffness for each individual muscle group. Until published research starts to become more uniform, and more research is carried out in elite sport organisations to establish normative values, it is difficult to see how USWE will make an impact, especially at this early stage of its research and development of use within a Premier League football team.

That being said, the literature review also emphasized that measuring muscle stiffness can be useful from a performance standpoint, due to it being an important characteristic to aid football performance, due to its relationship with other performance tasks (Faude et al., 2012, Kalkhoven and Watsford., 2018, Pruyn et al., 2014, Miyamoto et al., 2019). In the 2nd study, the muscle stiffness of 46 Premier League academy football players was correlated against a battery of strength, power, and speed testing markers to evaluate whether there was a crossover in muscle stiffness and athletic performance task outcome. The conclusion of this study seems to show that rectus femoris muscle stiffness from the USWE does correlate with several metrics of the CMJ, but more holistic lower-limb stiffness tests (for example, the vertical hop test used in this study) seem to be more effective in predicting physical performance. The practical implications from this study, show the vertical hop test, should be the preferred mode of measuring muscle stiffness in its link to physical performance. Measurements of USWE could still be proved to be useful within a physical testing and screening battery in Premier League football for profiling and physical performance identification, but this study does not provide clear enough evidence of links to physical performance to be used as a predictor of performance yet from measuring the rectus femoris. From a profiling perspective, receiving an insight into individual muscle stiffness from the USWE may be useful at less frequent time points, for example in pre-season testing to get a baseline reading, but it is recommended that methodologies such as the vertical hop test should utilised for measuring stiffness over time with more regular tests across a season, due to its evidential link to physical performance. That being said, despite the outcomes of this study, the efficacy of this study cannot wholly reject the hypothesis of the relationship between muscle stiffness and physical performance, due to methodological factors, player characteristics and contextual football specific factors which can all influence the data and the relationships within the study. An example of a methodological consideration would be that the study only measures one location of the rectus femoris muscle. If this study was repeated utilising more individual muscle sites (such as Biceps Femoris or Gastrocnemius Medialis) there would have been more confidence in the work to accept or reject the efficacy and hypothesis that had been set. Furthermore, when considering player characteristics that could influence the result, there are several variables such as age, prior injury and acute : chronic training load which could all impact the outcome of the study due to only monitoring muscle stiffness at one time point with one set of athletes. Another consideration that seems important are contextual football specific factors, such as stage of the season, which could play a major role in the athletes overall physical capacity. Therefore, further research is required to investigate whether there is a relationship between physical performance and muscle stiffness derived from the USWE.

Despite the limitations of the 2nd study, the value of the applied context provides direction for further fundamental efficacy work that should be done going forward to address whether this relationship exists. In a lab-based setting, it would be useful to investigate the chronic physiological adaptation over time of an individual muscle (such as the Rectus Femoris) with a specific isolated exercise (such as a Leg Extension) rather than global, more athletic movement tasks following a structured, progressive training programme which provides a regular exposure to the specific isolated exercise over a set amount of time. This would allow practitioners and researchers alike to observe the muscle stiffness changes over time in response to the stimulus, and also provide information on the re-modelling of tissue and how the muscle has adapted to the additional stress and load.

In study 3, and the individual case series in Study 4, there does seem to be an agreement that the amount of Total Distance performed may influence muscle stiffness response. In Study 3, there was a negative, strong association found with Total Distance (albeit not categorised as significant), and in both case studies the greatest decrease in muscle stiffness was following the highest volume of Total Distance. This may indicate that there is a threshold of aerobic and endurance work, which causes muscle stiffness to decrease following a soccer-specific stimulus. Previous research supports this theory, with studies from Morales Artacho et al. (2017) Sadedghi et al. (2018) and Andonian et al. (2016) all showing a decrease in muscle stiffness following endurance activities. Further research to assess an individual over a greater period of time would help to investigate what happens to the mechanical state of their muscle following consecutive bouts of decreasing muscle stiffness following activity. This would help to investigate whether the muscle becomes tolerant to the workload, or whether their physical threshold increases. If we refer to the phenomenon of the supercompensation curve, we could hypothesize that as a muscle tissue adapts to the stress of a Premier League academy football-match and compensates accordingly, that the response to the same stimulus may diminish as they become more tolerant to the stimulus (Mukhopadhyay, 2021).

That being said, one of the outcomes of study 4 (case study 1) highlighted a subsequent increase in muscle stiffness post-training in the days following Premier League academy match-play, when further training sessions were observed. This could potentially illustrate that there is a delayed response in muscle stiffness following match play and illustrate that when measuring muscle stiffness in Premier League academy football that ~75minutes post- training or match intervention may not be the most effective time point to measure. Future research to assess the time-course profile of a Premier League academy football match to evaluate whether there is an increase in muscle stiffness in the subsequent hours and days, would be useful to gain a more comprehensive understanding of the muscle mechanical response. If we hypothesize that the aerobic nature of a Premier League academy football match overrides the potential increase in muscle stiffness immediately post-match due to the aerobic demands, it would be beneficial to analyse a time-course profile post- match-play to see whether an increase in muscle stiffness does eventually occur. For example, if the acute response to the aerobic nature of the match-play gradually fades away and reveals an increase in muscle stiffness at a later time-point, it would still be beneficial in a football monitoring setting in forecasting that there could potentially be an onset of a degree of muscle damage. In a Premier League football environment, time is a significant asset, and if this technology is able to predict muscle damage ahead of time, even if it is not immediately post-exercise, it would still be of major value. Furthermore, the 2nd case study in Study 4 may support this argument, as there was a gradual increase in muscle stiffness in the pre-measurement scans across the first 3 days of the longitudinal analysis. This may also suggest a delayed response to the training stimulus, with an increase in stiffness occurring up to ~24hr post and emphasize that muscle stiffness may increase following consecutive bouts of activity. This reinforces that further research may need to be undertaken to identify the most effective time-point to measure muscle stiffness.

Despite both case studies in Study 4 showing the tendency for muscle stiffness to increase in some way across a series of training, it is important to deliberate that both case studies displayed alternating muscle stiffness responses to match and training session interventions.

The 1st case study revealed that despite a decrease in muscle stiffness post-match play, that for the 4 days following the match stimulus there was an increase in muscle stiffness immediately following the training sessions. Yet in the 2nd case study, despite the pre-measurements showing an increase day-to-day, each training session exhibited a decrease in muscle stiffness after each training session. During the 2nd case study, we did implement a CMJ alongside the USWE measurements to try and help interpret the impact of stiffness. Despite the relationship between passive muscle stiffness and some variables in the CMJ in Study 1, they did not seem to be any sensitivity to change when assessed together and indicated that muscle stiffness is not correlated with neuromuscular fatigue or force loss. The individual variation in muscle stiffness response may be expected, as different athletes will respond in different ways to the same stimulus due to factors such as muscle typology, pre-existing fatigue or injury, body composition, and physical capacity. Lievens and colleagues (2020) discussed that a one-size-fits-all training regime is present in most elite sports, despite the fact that athletes have a distinct muscle typology which substantially influences the time to recover from high-intensity exercise. For instance, fast-twitch muscle fibres can generate high power in a short amount of time but are easily fatigued, whereas slow-twitch muscle fibres are more fatigue resistant. Further research to begin to assess the type of athlete, whether this be through advancing methods such assessing muscle typology or through more traditional methods such as physical performance testing, and how different profiles of athletes and their muscle stiffness responds to a stimulus could transform load monitoring and recovery processes in elite football. By understanding the type of athletes that may experience a change in muscle stiffness, there could be scope to use USWE in a layered-screening approach where only a certain type of athlete or football in this case, who are sensitive to change in muscle stiffness, get regularly measured.

Although we were able to investigate 2 Premier League academy soccer players in a case series approach and examine 11 soccer players in a Premier League academy soccer match, it is difficult to infer any significant findings due to the small sample size, but this displays the challenges and complexities of collecting data within an elite sports team, especially in pre- and post- studies. The approach of the thesis was to perform a “real life” experiment with the additional value and significance of being able to collect data on Premier League academy athletes. Yet, as these Premier League academy footballers are full-time athletes, there was vast difficulty in controlling all external variables, as the data collected throughout this thesis was collected around day-to-day processes at a Premier League football club. As the data collection was performed in an applied setting and not in a laboratory setting, there was limitations of having to compromise on certain aspects of the methodology to collect data. That being said, that is why the Systematic Review was integral to this research project, to put in a place a framework to deliver the most effective and appropriate methodology we could have within a Premier League football club.

Practical Recommendations from Thesis

- When implementing USWE within an elite sport setting, make sure to review the methodological approach to ensure a consistent protocol can be continued. The systematic review provides practical applications for use of USWE in an applied setting, but further research may be required for specific muscle groups.

- When physically profiling players, attaining a measure of stiffness is important, due to its significance as a physical characteristic. A more holistic, lower-limb stiffness measure may be more effective to predict physical performance and general stiffness capability in a group setting. Collecting USWE on a more individual basis during processes such as recruitment, RTP or during a pre-season screening could be useful to evaluate a muscle stiffness profile on players, but tests such as the vertical hop test should be administered across a season to track changes in stiffness and the relationship to physical performance.

- USWE scans should be collected over a further battery of games using the same players, to start to build a profile of their muscular response to exercise. This will help to distinguish when players may need further attention if their muscular response shows a different response to normal. If Premier League academy footballers can be characterized by their physical profile, to differentiate typically what type of athletes may be sensitive to changes in muscle stiffness, the monitoring process could start to be individualised. For example, on a very basic level, if we knew players who accumulate more mechanical stress during a game through sprinting and COD, rather than metabolic fatigue through total volume and total distance, are more sensitive to changes in muscle stiffness, these could be the players targeted with the USWE and other players could use another monitoring test.
- If it can be expected that muscle stiffness will decrease immediately post- Premier League academy match play, USWE could be a beneficial way of monitoring players at a later time point to assess their stiffness and recovery process to investigate a truer reflection of the state of the muscular tissue. The early signs may suggest that the USWE could be implemented upon a MD + 2 screening, alongside other sport science and physio-led tests, to provide an insight into the muscular tissue to provide a more holistic recovery profile alongside other fatigue and recovery marker assessments.

Recommendations for Future Research

This thesis has completed a foundation of research for USWE within a Premier League football setting, for future researchers to build upon. A combination of continued research in elite sport settings, and more methodical and refined research within a university or laboratory setting would help to undress the performance questions from both sides. Despite the literature and research in USWE continuing to develop and advance, there is not yet concrete enough observations and outcomes to definitively use the data from USWE to help inform decisions in professional sport.

- Firstly, further research within Premier League football, collected from different clubs, would be useful to attain a normative set of data for each muscle group, to start to profile what level of muscle stiffness could be categorised as high, normal, or low. Collecting normative data from male academy football, as well as women's academy and first team level, would help to create benchmarks and a framework for profiling muscle stiffness. Until there is a greater amount of normative data for muscle stiffness measured via USWE in Premier League academy football, it will be difficult to make any informed decisions to influence practice.

- It would be extremely useful to repeat the studies completed in this thesis but with the measurements of further muscle groups to assess inter-muscle variability in response to soccer-specific stimulus. This could eventually lead to creating a muscle stiffness profile for each player and beginning to understand which muscles increase/decrease in specific individuals. This could help to begin a layered screening approach, where players who require further monitoring have a USWE scan performed on individualised focus areas such as past injury sites. Kawai and colleagues (2021b) stated that increased muscle stiffness can persist for several months after injury, thus athletes who have just returned from injury would be the targeted players to receive USWE scans to monitor their continued healing and re-conditioning.

- Similar to the 2nd case study in Study 4, future research would benefit from additional measures of other physical characteristics such as a CMJ to measure alongside USWE to help provide more context to the findings. Although there was no clear relationship with the CMJ, there would be several other useful markers to help interpret the impact of stiffness which we unable to measure during this thesis. Firstly, blood markers to help display the relationship between muscle stiffness and predicted muscle damage with assessments such as CK would be valuable. Also, in the context of this study, isometric contractions on the isokinetic dynamometer would be ideal to build upon work from Lacourpaille et al. (2017) to explore the force loss in comparison to muscle stiffness, but to also isolate the muscles such as the rectus femoris during the testing, rather than performing a more functional assessment of performance.

- Despite the quality of information that can be received from the USWE, it could be argued that it may not serve as the most practical within an elite setting, as it could be considered time-consuming and costly, especially to upskill or hire a skilled member of staff. Further research to assess the relationship between USWE and more practical technologies such as the MyotonPro, which could be deemed more useful in an applied setting, could be beneficial. Research from Feng and colleagues (2018) has demonstrated that the USWE and MyotonPRO measurements have significant correlations when measuring muscle stiffness which could offer valuable insight, as a MyotonPRO provides a much cheaper alternative to the USWE. Not only is the MyotonPRO less costly, but it is also a wireless device that can produce instantaneous feedback on mechanical properties of individual muscle sites, it makes the device both practical and attractive for applied sport scientists to collect data in an applied setting. Despite the advantages of exploiting the Myoton-Pro in an applied setting, the device cannot measure muscle stiffness when there is greater than 2cm of subcutaneous adipose tissue, meaning the measurements of participants with greater body fat percentages can be affected by overlying fascial, adipose, and skin tissue. This is a disadvantage of the MyotonPRO, as the ultrasound imaging of the USWE allows the examiner to specifically locate the designated ROI, with the intricacy of selecting the depth of the ultrasound scan and manoeuvring the ROI to avoid any unwanted muscle tissue. As USWE is more time-consuming, and requires a more skilled member of staff, there is a potential scope that the MyotonPro could perform preliminary assessments and regular monitoring, and measurements which are flagged could undergo a more thorough analysis on the USWE.

- From a more medical and physio-led perspective, further research on individual injury cases to monitor their return to play protocol could have interesting potential. The USWE could be extremely valuable in the rehabilitation setting, to measure the specific site of injury through a rehabilitation and reconditioning process to monitor healing and recovery. The ambition would be to assess an athlete's response to exercise and activity over the course of their rehabilitation, which may help to prescribe when a muscle may be safe or effective to load, and even assist in timelines of healing and injury.

Conclusions

This research has provided evidence that USWE could potentially be an important technology to measure muscle stiffness in a Premier League football setting. During this thesis, we have examined (1) practical recommendations for a suitable protocol to administer USWE in a Premier League academy football setting, (2) the relationship between muscle stiffness measured from USWE and athletic performance markers, (3) the muscle stiffness response to Premier League academy match-play, and (4) the influence of soccer-specific training on muscle stiffness. When observing the two ways in which USWE could be utilised within an elite sports setting, in identifying physical performance and being used as a readiness tool to assess the response to exercise, this thesis demonstrates the most effective use case for the USWE is in monitoring readiness and a response to exercise, and not assessing physical performance. Although there were limitations in this thesis due to collecting data within an Premier League academy environment, it has helped to provide an interesting insight into how USWE may be used within a professional sports team and provided a platform for future research.

Due to the fast-pace, and time-efficient nature of elite sports, it is recommended that USWE is implemented on an individual basis for special cases. As the USWE is so intricate and specific in the muscle site it is measuring, unless there is a general consensus of a muscle site that stakeholders are interested in, only measuring one muscle group may not provide enough context for monitoring and readiness purposes. Thus, scenarios such as rehabilitation and during individualised screenings may be the most optimal use case for the USWE to monitor high-quality, detailed information on specific regions of a muscle.

Advancing technologies such as the USWE, which are able to assess muscular tissue with instant feedback to provide insight on internal responses are inevitably going to become a fundamental part of the monitoring and readiness processes of Premier League football clubs. With the rising interest in USWE across elite sport, it is hypothesized that a vast amount of future research will be undertaken in this area, to build upon work from this thesis, to help develop USWE as a mainstay in all world-class monitoring and readiness processes within professional sport.

CHAPTER 9

APPENDICES

DECLARATION OF COVID-19 DISRUPTION TO PLANNED RESEARCH

Summary of Planned Research

The phase of research which was most heavily affected was the readiness and response to exercise phase, which was originally planned to be broken into two sub-categories: (1) efficacy and (2) effectiveness.

Efficacy Phase

The purpose of the efficacy phase was to examine how different soccer-specific training stimuli commonly performed within an elite academy football club training/competition programme affects muscle stiffness measured by USWE. This aspect of the research programme included 2 sub-phases of research. The first study in the efficacy phase aimed to assess how fundamental movements performed in elite football, executed in isolation, influenced muscle stiffness measured by USWE. Key components of training/match-play (jogging, sprinting, high speed running, accelerations, and decelerations) will be identified and then performed in isolation to help better understand the specific football movements that may result in changes in the physiological status of the muscle and in turn changes in muscle stiffness. An objective was to have a sample of 18-20 elite academy footballers (aged 16-18) measured pre- and post-different physical movements via the USWE. These movements were planned to be performed in isolation using specially constructed experimental protocols that mimic the relevant aspect of football movement.

The 2nd study of this phase was aimed to develop the understanding obtained in the first study by examining how actual training stimuli performed within an elite academy football club affects the response in muscle stiffness measured by shear-wave elastography. Between 18-20 elite academy footballers (aged 16-18) were to be scanned around a number of different training stimuli typically performed within a weekly micro-cycle at an elite premier league football academy. The players were scheduled to be scanned pre- and post- the following stimulus: MD+2 (2-Days Post Match recovery session focusing on low intensity technical work and linear running), MD-4 (4 days to the next game following a session focusing on smaller areas, and an increase in accelerations, decelerations and changes of direction), MD-3 (3 days to the next game following a session focusing on larger areas, and an increase in total distance, high speed running and sprint distance), MD-2 (Off-Pitch recovery session), MD-1 (1 day to the next game following a session focusing on smaller areas with a reactive stimulus, but lower in total duration and distance) and on MD (Match Day). The muscle stiffness response from each specific training stimulus would then be correlated against the external output (total distance covered, high speed running, sprint distance, accelerations, decelerations etc.) to examine whether there is a relationship between an increase in muscle stiffness and any of the isolated actions measured. The aim of this second study is therefore to establish how different training stimulus may influence the acute change of muscle stiffness, to help answer the question of what type of stimulus causes muscle stiffness, and to what severity.

Effectiveness Phase

The purpose of the effectiveness phase was to assess how muscle stiffness responded to multiple elite academy match-play stimulus, to try to provide insightful information on the muscular disruption caused by a match stimulus. The aim of this study was to measure muscle stiffness over a 5-day period across multiples football matches, to identify the effects of an elite academy match-stimulus. A sample of 18-20 players from an elite academy football club were scheduled to be scanned over selected time points to create a time-course profile of an elite academy football match. Scans were planned to take place at: MD-1, MD, MD+1, MD+2 and MD+3 in order to analyse when muscle stiffness may peak following a football match, and to evaluate whether there was any variation in muscle stiffness over a 5-day period. The muscle stiffness profile will be correlated with the external load (refer back to the efficacy phase), to identify whether an overload in one of the isolated soccer-specific movements may influence the muscle stiffness following the match-play.

Mitigation against COVID-19 Disruption

To mitigate against the disruption of COVID-19, the readiness and response to exercise phase of research was modified, but measures were taken to ensure the utmost quality was maintained. Due to the condensed time period to collect data, we still performed two sub-phases of research, but the content of these studies was altered. To continue to investigate the effectiveness of USWE to be a monitoring tool in in elite academy football, we aimed to explore the muscle stiffness response of a (1) elite academy football match, and a (2) series of training to show the effectiveness of USWE to provide insightful information in a "real-world" football monitoring programme. The first study in this phase of research aims to examine the effects of an elite academy football match on twelve academy players, with a USWE measurement ~75mins pre- and ~75mins post- to assess the acute response on muscle stiffness. Rather than perform a time-course profile of match-play, an intervention-based pre- and post-type study was performed to examine the acute effects of muscle stiffness following elite academy match-play. The second study in this phase of research was a case series approach to investigate how a series of soccer-specific stimuli (training/match interventions) effected muscle stiffness. The 1st case study followed an individual over a 5-day series of soccer-specific stimulus, with a USWE measurement ~75mins pre- and ~75mins post- each stimulus. In the 2nd case study, we repeated a similar longitudinal analysis, but included a countermovement jump, to help interpret the impact of stiffness. The 2nd part of this phase of research aimed to build upon the interpretation of the effects of muscle stiffness following match-play, to understand the impact of stiffness following multiple stimulus performed on consecutive days, similar to what is expected of elite academy footballers on a weekly basis. As a result, the thesis still shown a positive demonstration of the application of USWE to monitor readiness and the response to soccer-specific stimulus in elite academy footballers, despite the disruption from COVID-19, to satisfy the needs of the phase of research, alongside the methodological and physical performance phase outlined above.

GENERAL CONSENT FORM

USE OF DATA FOR RESEARCH (U18 PLAYERS)

As part of your involvement with Tottenham Hotspur Football Club, The Academy Medical and Sport Science department will collect data on you and your performances to help inform the training programmes set by the coaches and physical development staff.

At times this data will also be used by the club and selected partners for research projects. All data to be used in future research, will be made anonymous by removing personal identifying information, stored securely on password encrypted computers and be treated with strictest confidence.

1. I confirm that I have read and understand the information provided. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐

3. I understand that any personal information collected during the study will be anonymous and remain confidential. ☐

4. I agree to allow my data collected by the club to be used in research projects approved by the Academy Medical and Sport Science department. ☐

____________________	______________	____________
Name of Player	Date of Birth	Date

________________________________	_________________________
Parent/Guardian Signature *(if under 18 years old)*	Relationship to Player

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_____________________	______________	_______________
Name of Staff member	Date	Signature

GENERAL CONSENT FORM

USE OF DATA FOR RESEARCH (U23 & FIRST TEAM PLAYERS)

As part of your involvement with Tottenham Hotspur Football Club, The Academy Medical and Sport Science department will collect data on you and your performances to help inform the training programmes set by the coaches and physical development staff.

At times this data will also be used by the club and selected partners for research projects. All data to be used in future research, will be made anonymous by removing personal identifying information, stored securely on password encrypted computers and be treated with strictest confidence.

1. I confirm that I have read and understand the information provided. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐

3. I understand that any personal information collected during the study will be anonymous and remain confidential. ☐

4. I agree to allow my data collected by the club to be used in research projects approved by the Academy Medical and Sport Science department. ☐

______________________	______________	____________
Name of Player	Date of Birth	Date
______________________	______________	______________
Name of Staff member	Date	Signature

PARTICIPANT INFORMATION SHEET

THE APPLICATION OF ULTRASOUND SHEAR WAVE ELASTOGRAPHY TO ASSESS CHANGES IN MUSCLE STIFFNESS IN ELITE ACADEMY FOOTBALLERS.

PHD RESEARCH PROJECT

As the physicality and intensity of professional football continues to increase, there is a need to develop and research new technologies in order to monitor a footballer. Through training and competition in matches, there is a large physical stress placed upon a footballer's body, so our responsibility within the sports science department is to monitor and screen effectively in order to keep players injury-free and performing to their best.

A new, advancing and non-invasive technique being explored in multiple sports is the use of Ultrasound Shear-Wave Elastography, which has the potential to estimate muscle stiffness in a given area of the body which may help us to predict muscle damage and other structural muscle properties that we otherwise would not be able to evaluate (without the use of a muscle biopsy – which includes removal of a small portion the muscle, which is not ideal for footballers!)

Shear-Wave Elastography does not require any muscle contractions or physical work, the participant simply just has to lie on a medical bed and 'relax as much as possible'. The ultrasound machine used is similar to that of an Ultrasound machine used to look at the children of pregnant women, and simply requires a small amount of gel applied to the skin with an ultrasound probe held over the specified area with minimal pressure which then produces an image on the monitor of a view from inside your muscle.

If you were willing to participate in this study, you would be required to complete three testing sessions on your right quadricep muscle. Each testing session would take only ~5 minutes to complete. One would be a baseline test to compare against your other monitoring and profiling scores (jump testing, speed testing and strength testing etc.) and the other two testing sessions would occur 75 minutes before and after a game to assess the difference in muscle stiffness following a game.

During the testing sessions pre- and post- the game, you would be expected to have an Ultrasound scan 75mins- prior to the game before completing any warm-up, and following the game, you would be expected to have an Ultrasound scan 75-mins post the game before completing any recovery modalities (such as having an ice bath or active recovery in the swimming pool).

PRE- AND POST- STIMULUS RELIABILITY STUDY

INTRODUCTION

The study was performed to assess for the intra-class coefficient (ICC) and coefficient of variation (CV) of the USWE when performing 2 sets of measurements, 3 hours apart to replicate the study completed in Chapter 6, without the elite academy match-play intervention to ensure there was no natural fluctuation in muscle stiffness over that time period.

METHODS

8 non-athletes (age:27.2±1.2; height:182±7.1cm; mass:78.6kg±8.3kg), volunteered to take part in the reliability study. Please refer to Chapter 5 for a comprehensive overview of the *methods* for the USWE scan. The measurements were taken in a relaxed and passively stretched condition. The participants were asked to refrain from any physical activity during the time between scans.

RESULTS

Participant	Pre-Scan 1	Pre-Scan 2	Pre-Scan 3	**Pre-Average**	Post-Scan 1	Post-Scan 2	Post-Scan 3	**Post-Average**	**CV**	**ICC**
1	1.47	1.51	1.48	1.49	1.58	1.55	1.59	1.57	4.65%	-0.91
2	1.74	1.85	1.84	1.81	1.83	2.05	1.77	1.88	9.91%	-0.82
3	1.54	1.52	1.51	1.52	1.55	1.45	1.54	1.51	3.34%	-0.94
4	1.44	1.42	1.44	1.43	1.52	1.47	1.43	1.47	3.35%	-0.94
5	1.49	1.48	1.5	1.49	1.57	1.55	1.51	1.54	3.25%	-0.94
6	1.76	1.81	1.86	1.81	1.85	1.86	1.82	1.84	3.54%	-0.93
7	1.84	1.93	1.91	1.89	1.9	1.89	1.86	1.88	3.02%	-0.94
8	1.43	1.44	1.44	1.44	1.5	1.54	1.51	1.52	4.19%	-0.92
Average									**4.00%**	**-0.92**

Table 9.1: USWE measurements in a relaxed position during the reliability study

In a relaxed position, the average ICC was -0.92 showing excellent reliability, with a CV of 4% which is deemed as acceptable.

Participant	Pre-Scan 1	Pre-Scan 2	Pre-Scan 3	Pre-Average	Post-Scan 1	Post-Scan 2	Post-Scan 3	Post-Average	CV	ICC
1	2.79	2.8	2.87	2.82	2.83	2.72	2.75	2.77	4.92%	-0.91
2	2.59	2.53	2.58	2.57	2.77	2.79	2.87	2.81	12.68%	-0.77
3	2.57	2.59	2.81	2.66	2.58	2.59	2.62	2.60	8.34%	-0.85
4	2.16	2.14	2.15	2.15	2.09	2.09	2.16	2.11	3.02%	-0.94
5	2.77	2.81	2.95	2.84	2.75	2.8	2.83	2.79	6.44%	-0.88
6	2.31	2.35	2.43	2.36	2.25	2.25	2.21	2.24	7.37%	-0.86
7	2.26	2.33	2.37	2.32	2.26	2.29	2.24	2.26	4.52%	-0.91
8	2.55	2.54	2.53	2.54	2.77	2.72	2.59	2.69	9.37%	-0.83
Average									**7.83%**	**-0.87**

Table 9.2: USWE measurements in a passively stretched position during the reliability study

In a passively stretched position, the average ICC was -0.87 showing good reliability, with a CV of 7.83% which is deemed as acceptable.

DISCUSSION

The repeatability and reproducibility was categorised as excellent (ICC=0.92), with an acceptable within-subject variation (CV=4%) in a relaxed condition. The repeatability and reproducibility was categorised as good (ICC=0.87), with an acceptable within-subject variation (CV=7%) in a passively stretched condition. Therefore, the reliability shows good to excellent reproducibility, with acceptable within-subject variation, meaning this methodology is appropriate to use during Study 3 and Study 4 of the thesis.

CHAPTER 10

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