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EFFECTS OF REST POSITION ON ULTRASOUND-DERIVED MORPHOLOGICAL CHARACTERISTICS OF THE VASTUS LATERALIS AND LOWER-BODY FORCE PRODUCTION

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Learning Sciences and Educational Research in the College of Community Innovation and Education at the University of Central Florida Orlando, Florida

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ABSTRACT

INTRODUCTION: Ultrasound assessment of the lower body typically encompasses 10-15 minutes of supine rest prior to examination because of the potential influence of gravitational fluid shifts on tissue size and composition. However, examination of the vastus lateralis (VL) muscle requires individuals to lay in the lateral recumbent position, and this change in position may influence muscle morphological characteristics and their ability to predict muscle function. **PURPOSE:** The purpose of this investigation was to examine the effect of rest position on ultrasound-derived morphological characteristics of the VL and to determine whether or not rest position affects the relationships between muscle morphological characteristics of the VL and lower-body force and power production.

METHODS: Thirty-one resistance-trained males (age: 23.0 ± 2.1 years; height: 1.79 ± 0.08 m; body mass: 87.4 ± 11.7 kg) participated in this investigation. Muscle morphological characteristics, including cross-sectional area (CSA), muscle thickness (MT), pennation angle (PA), echo intensity (UnCorEI), subcutaneous adipose tissue thickness (SFT), and EI corrected for SFT (CorEI) of the VL were assessed in the dominant limb after 15 minutes of rest in 3 recumbent positions: supine (SUP), dominant lateral recumbent (DLR), non-dominant lateral recumbent (NDLR), as well as after 15 minutes of standing (ST) and immediately after laying down (IP). Following ultrasound assessments, participants completed unilateral performance assessments, including vertical jumps (UVJ), isometric/isokinetic testing, and a 1-repetition maximum (1-RM) leg press.

RESULTS: A repeated-measures analysis of variance revealed significantly different (p < 0.05) CSA, MT, PA, UnCorEI, and SFT in ST compared to recumbent positions after 15 minutes of rest (NDLR, DLR, and SUP). Additionally, significant differences were observed between recumbent positions for CSA, CorEI, and UnCorEI; however, no differences were observed for MT, PA, and SFT. Different magnitudes of relationships were observed between muscle morphological characteristics measured after rest in different positions and performance variables. However, muscle morphology after IP generally appears to be the best predictor of performance for most variables, although utilizing the NDLR and DLR positions may provide comparable, or potentially stronger results for variables such as IsokPF. The relationship between muscle morphology and various performance variables in ST were weaker compared to the recumbent positions examined, specifically for IsokPF, 1-RM leg press, and for all UVJ variables, except total work.

CONCLUSIONS: Muscle morphology differs depending on the rest position utilized prior to ultrasound analysis. These rest positions also affect the ability for muscle morphology to predict lower-body force production. Future research should consider evaluation of muscle morphology of the VL after IP in the prediction of muscle function; however, NDLR and DLR may also be used. SUP and ST provide significantly weaker relationships for some performance variables compared to the other recumbent positions.

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LIST OF ACRONYMS AND ABBREVIATIONS

1-RM	1-repetition maximum
3-RM	3-repetition maximum
ANOVA	Analysis of variance
AU	Arbitrary units
BIA	Bioelectrical impedance analysis
BIS	Bioelectrical impedance spectroscopy
CI	Confidence interval
CorEI	Echo intensity corrected for subcutaneous adipose tissue thickness
CSA	Cross-sectional area
CSCS	Certified Strength and Conditioning Specialist
DLR	Rest in the dominant lateral recumbent position for 15 minutes
EI	Echo intensity
FL	Fascicle length
ICC	Intraclass correlation
IMP	Impulse
IMP50	Impulse over 50 milliseconds
IMP100	Impulse over 100 milliseconds
IMP200	Impulse over 200 milliseconds
IP	Immediately post laying down in the non-dominant lateral
	recumbent position
IsokPF ($60^{\circ} \cdot s^{-1}$)	Isokinetic peak force at 60 degrees per second

IsokPF $(180^{\circ} \cdot s^{-1})$	Isokinetic peak force at 180 degrees per second
IsokPF $(240^{\circ} \cdot s^{-1})$	Isokinetic peak force at 240 degrees per second
IsokPT $(60^{\circ} \cdot s^{-1})$	Isokinetic peak torque at 60 degrees per second
IsokPT $(180^{\circ} \cdot s^{-1})$	Isokinetic peak torque at 180 degrees per second
IsokPT $(240^{\circ} \cdot s^{-1})$	Isokinetic peak torque at 240 degrees per second
LSD	Least significant difference
MD	Minimal difference
MHAQ	Medical history and activity questionnaire
MT	Muscle thickness
MVIC	Maximal voluntary isometric contraction
NDLR	Rest in the non-dominant lateral recumbent position for 15 minutes
NSCA	National Strength and Conditioning Association
PA	Pennation angle
PAR-Q+	Physical activity readiness questionnaire
PF	Peak force
PT	Peak torque
RFD	Rate of force development
RFD50	Rate of force development over 50 milliseconds
RFD100	Rate of force development over 100 milliseconds
RFD200	Rate of force development over 200 milliseconds
RM	Repetition maximum
RPD	Rate of power development

RTD	Rate of torque development
SEE	Standard error of the estimate
SEM	Standard error of measurement
SFT	Subcutaneous adipose tissue thickness
SPSS	Statistical Package for the Social Sciences
ST	Rest in the standing position for 15 minutes
SUP	Rest in the supine position for 15 minutes
T1	First visit to the laboratory (preliminary visit)
T2	Second visit to the laboratory (familiarization)
Т3	Third visit to the laboratory
UnCorEI	Uncorrected echo intensity
UVJ	Unilateral vertical jump
VL	Vastus lateralis

CHAPTER ONE: INTRODUCTION

The assessment of muscle morphology *in vivo* has been used to evaluate muscle function in response to various exercise and nutritional interventions, as well as in disease and other clinical conditions. Previously, the use of computerized tomography (CT), magnetic resonance imaging (MRI), and dual-energy x-ray absorptiometry (DEXA) have been considered the gold standards in the assessment of muscle size and composition. However, ultrasonography has gained significant attention due to its ability to provide valid and reliable measures of both muscle size and fiber orientation (Ahtiainen et al., 2010; Esformes, Narici, & Maganaris, 2002; Lixandrao et al., 2014; Noorkoiv, Nosaka, & Blazevich, 2010; Reeves, Maganaris, & Narici, 2004; Scott et al., 2012; Thomaes et al., 2012). Ultrasonography is portable, versatile, and does not produce ionizing radiation (Mourtzakis & Wischmeyer, 2014; Pillen & van Alfen, 2011), and thus represents a robust non-invasive method of skeletal muscle imaging.

Muscle force-producing capability is largely a result of the cross-bridge interaction of actin and myosin. Accordingly, a muscle that has a greater amount of contractile tissue and is larger in size characteristically has an increased ability to produce force (Finer, Simmons, & Spudich, 1994; Miller, Bedrin, Ades, Palmer, & Toth, 2015). As such, quantification of muscle size, including cross-sectional area (CSA) and muscle thickness (MT) is critical for predicting muscle strength and force production (Fukunaga et al., 2001; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Moreau, Simpson, Teefey, & Damiano, 2010). Conversely, the ability to produce force quickly, i.e., the rate of force development (RFD), appears to be related primarily to muscle fiber orientation, i.e., pennation angle (PA) and fascicle length (FL), and secondarily, muscle size (Maffiuletti et al., 2016; Zaras et al., 2016). Echo intensity (EI) has also been

previously investigated as a surrogate measure of muscle quality during ultrasonography and is thought to provide a measure of muscle composition by delineating between contractile and noncontractile tissue through the greyscale analysis of pixels within the image (Pillen & van Alfen, 2011). Given that measures of muscle size, fiber orientation, and quality directly impact strength and power performance, these parameters are often quantified in conjunction with functional strength and power assessments (Burkholder, Fingado, Baron, & Lieber, 1994).

Ultrasonography of the lower-body is typically completed while the subject is recumbent on an examination table, however the transition from an upright to recumbent position has been shown to induce rapid fluctuations in blood flow and resulting tissue volume (Berg, Tedner, & Tesch, 1993; Cerniglia, Delmonico, Lindle, Hurley, & Rogers, 2007; Maw, Mackenzie, & Taylor, 1995; Tan, Wilmshurst, Gleason, & Soeldner, 1973; Thoirs & English, 2009; Wagle et al., 2017). Specifically, a redistribution of blood out of the lower extremities and a decrease in hydrostatic pressure of the lower body result in a net absorption of fluid from the interstitial fluid into the capillaries, decreasing tissue volume (Fawcett & Wynn, 1960; Hagan, Diaz, & Horvath, 1978; Maw et al., 1995; Taylor, Halliwill, Brown, Hayano, & Eckberg, 1995; Thompson, Alper, & Thompson, 1928; Waterfield, 1931a, 1931b). Research has demonstrated that changes in body position result in alterations in muscle morphology of the lower body (Arroyo et al., 2018; Berg et al., 1993; Cerniglia et al., 2007; Lopez, Pinto, & Pinto, 2019; Shea, 2017; Thoirs & English, 2009; Tomko et al., 2018). Based off of these findings, ultrasonography is typically accomplished after a 10 to 15 minute period of rest in the supine position to allow for gravitational fluid shifts (Ahtiainen et al., 2010; Jajtner et al., 2015; Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014;

Varanoske et al., 2017a, 2017b; Wells et al., 2014). However, Wagle and colleagues (2017) observed stronger relationships between standing measures of muscle size and architecture with lower body strength and power than measures taken while recumbent, which was attributed to discrepancies between the position during examination and the position in which many sporting activities occur. As muscle morphological characteristics obtained via ultrasonography appear to differ depending on whether they are obtained while standing or while recumbent (Thoirs & English, 2009; Tomko et al., 2018; Wagle et al., 2017), the ability of these characteristics to predict muscle function during athletic activities may be compromised if ultrasound images are captured in the recumbent position.

Although there is research to support the use of standing measures of muscle morphology in the assessment of muscle function (Wagle et al., 2017), this positioning requires an additional level of difficulty on the subject as well as the technician. The ability to compare measures of muscle morphology obtained from standing ultrasounds to CT, MRI, and DEXA is also diminished because these techniques require participants to remain in the recumbent position during examination. Furthermore, ultrasound images captured while in the recumbent position may be altered by the rest position prior to assessment, affecting the ability of these images to predict muscle function. The VL is a muscle that is commonly examined in the evaluation of lower body strength and power, and it is located on the lateral side of the thigh. Typical ultrasound assessment of the VL reports that the subjects are instructed to lay in the supine position for fluid shifts to occur and then flip over onto their lateral side for assessment (Jajtner et al., 2015; Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Wagle et al., 2017; Wells et al., 2014). However, changes in hydrostatic pressure and blood distribution may also be induced with changes in recumbent positions (Bryan, 1974; Kallet, 2015), and a change in position (from rest in a supine position to examination in a lateral recumbent position) may alter muscle morphological characteristics, which may not reflect true changes in muscle function.

Measurements obtained during ultrasonography may further be influenced by compression of body tissues as a result of changes in body position. In the examination of bilateral asymmetries in muscle size and architecture via ultrasonography, previous investigations do not report a return to the supine position for the same duration prior to examination of the opposing muscle (Mangine, Fukuda, et al., 2014; Mangine, Hoffman, et al., 2014). Thus, the leg that was previously compressed against the examination table under the weight of the body in the lateral recumbent position is then examined without the potential for additional fluid shifts to occur. Compression of a tissue increases the interstitial hydrostatic pressure, which reduces filtration of fluid out of the capillaries, therefore minimizing changes in muscle size (Nehler et al., 1993). It remains unknown whether compression of a limb under the weight of the body would affect muscle morphological characteristics.

Therefore, it is possible that changes between recumbent positions affect muscle morphological characteristics of the lower body. Further, if differences in muscle morphology exist after rapid changes in body position, this may affect the ability for these characteristics to predict muscle function. Consequently, the purpose of this study was to examine the effect of rest position on ultrasound-derived morphological characteristics of the VL and to determine whether the rest position that is used prior to ultrasound assessment affects the relationships

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between muscle morphological characteristics of the VL and lower-body force and power production.

Purpose of the Study

The primary purpose of this study was to examine the effect of rest position on ultrasound-derived morphological characteristics of the VL. A secondary purpose of this investigation was to examine whether the rest position that is used prior to ultrasound assessment affects the relationships between muscle morphological characteristics of the VL and lower-body force and power production.

Research Questions

The research questions for this investigation were as follows:

- 1. Does rest in different positions prior to ultrasound analysis of the VL affect muscle morphological characteristics, including CSA, MT, UnCorEI, CorEI, PA, and SFT?
- 2. Does rest in different positions impact the relationships between VL muscle morphology and lower-body force and power production?

Hypotheses

The hypotheses for this investigation were as follows:

 Muscle morphological characteristics of the VL, including CSA, MT, UnCorEI, CorEI, PA, and SFT, will differ between rest positions. 2. The relationships between muscle morphological characteristics of the VL and lowerbody force and power production will differ between different rest positions, and therefore certain rest positions should be utilized prior to analysis of muscle morphology for specific purposes.

Delimitations

Inclusion Criteria

- 1. Participant is a male between the ages of 18 and 35 years old.
- 2. Participant was free of physical limitations as defined by medical history and activity questionnaire (MHAQ) and physical activity readiness questionnaire (PAR-Q+).
- 3. Participant was required to be resistance-trained, which was defined as performed resistance training at least 3 times per week for at least the previous year.
- 4. Participant was willing come into the laboratory for all visits and was willing to perform all requirements of the study.
- 5. Participant understood the study procedures and signed a form providing informed consent to participate in the study.

Exclusion Criteria

- 1. Participant did not provide consent to participate in the investigation.
- 2. Participant was unable to come into the laboratory for all visits.
- Participant was unable to perform physical exercise as determined by the MHAQ or PAR-Q+.

- Participant had previously taken performance-enhancing drugs (as determined by MHAQ).
- 5. Participant was regularly taking any type of prescription or over-the-counter medication or had any chronic illnesses requiring medical care.
- 6. Participant was unable to complete any of the exercise performance testing on the familiarization day.
- 7. Participant was an amputee.
- 8. Participant regularly smoked cigarettes.
- 9. Participant had a pacemaker.
- 10. The entire VL muscle could not be viewed at a depth of 5 cm on the ultrasound.

Limitations

- 1. Muscle morphological characteristics obtained via ultrasonography may be influenced by skill and speed of technician.
- 2. There was no inclusion criteria for body composition, so EI values obtained from participants with larger amounts of subcutaneous fat mass (SFT) may have inaccurately resulted in lower EI values due to non-systematic reflection of ultrasound waves with increasing amounts of adipose tissue (Pillen & van Alfen, 2011; H. J. Young, Jenkins, Zhao, & McCully, 2015).
- 3. Leg dominance was self-indicated by each respective participant.

Assumptions

- 1. Participants completed all performance assessments to their maximal capabilities.
- Participants did not engage in vigorous lower-body exercise for at least 72 hours prior to the testing session.
- 3. Participants did not engage in a new exercise training program or dietary regime throughout the duration of their enrollment in the study.
- 4. All equipment used in the study was properly calibrated and maintained.
- 5. Participants answered all surveys (MHAQ and PAR-Q+) honestly regarding health history, exercise habits, nutritional supplementation, etc.
- 6. Self-reported leg dominance reflects true leg dominance.
- 7. Participants visited the laboratory for the testing session well-rested.
- 8. A skilled, experienced, and reliable technician completed all ultrasound imaging and ensured proper pressure and placement of the ultrasound probe upon the muscle of interest.

CHAPTER TWO: REVIEW OF LITERATURE

The Use of Ultrasonography in the Assessment of Skeletal Muscle Morphology

The prevalence of ultrasonography as an imaging technique is often limited to its use in medical and clinical settings, specifically in the field of obstetrics, or during pregnancy and childbirth. However, due to the versatility of ultrasonography, in addition to its relatively low cost and quicker time of assessment in comparison to other imaging techniques such as MRI, CT, and DEXA, its use in research has grown exponentially, specifically throughout the past 20 years (Mourtzakis & Wischmeyer, 2014; Pillen & van Alfen, 2011). Furthermore, ultrasounds are a much safer alternative to these other imaging devices, as they do not emit radiation or other electromagnetic waves.

Because ultrasounds are non-invasive, relatively inexpensive, safe, and easy to use, they have been used widely in previous research to assess properties of various types of tissues in the human body (Bricker et al., 2000). Of particular interest to the field of exercise physiology is the examination of skeletal muscle due to the structure-function relationship of muscle tissue. Ultrasonography can provide insight into the function of skeletal muscle by examination of structural attributes of the muscle. For example, muscle morphological characteristics derived from ultrasound images, including CSA, MT, PA, FL, and EI can help researchers predict the functional capabilities of the muscle that is examined (Pillen & van Alfen, 2011). The following section details how ultrasonography can be used as a tool to assess skeletal muscle morphology.

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The Fundamentals of Ultrasonography

Ultrasonography is typically used in medical fields to produce digital images of internal body structures, including tendons, ligaments, muscles, blood vessels, and organs. Ultrasounds emit sound waves through a probe at frequencies above those that are audible to humans (Ihnatsenka & Boezaart, 2010). When the ultrasound probe is placed on the surface of the skin, these sound waves are sent into the body and reflect differently off each type of body tissue. When the sound waves encounter tissues with different densities, some of the waves are reflected back to the probe. These reflected 'echoes' create digital images on a screen based off of the degree of reflectance of the tissues examined as well as the total time it took for the echo to be received (Ihnatsenka & Boezaart, 2010).

Many different types of ultrasounds and imaging techniques are used in medical and research settings, the most common of which is the use of a brightness-mode (B-mode) ultrasound device. B-mode imaging produces a black-and-white, two-dimensional image of a cross-section of a tissue on screen, based off of the acoustic impedance of the tissue (Pillen & van Alfen, 2011). In non-pathological cases, skeletal muscle can be easily distinguished from other surrounding structures including bone, tendons, ligaments, and subcutaneous fat, due to the distinct appearance of these tissues on screen. Each type of tissue has a different echogenicity (also known as EI), which refers to the degree of reflectance of the ultrasound waves off of body tissues, therefore also affecting the image that is produced on screen. Tissues that are hyperechoic are those that reflect sound waves with a greater amplitude, resulting in a brighter image that is produced (Ihnatsenka & Boezaart, 2010). Tissues that are anechoic do not reflect sound waves and therefore result in a darker image. For example, during ultrasound imaging,

bones are characterized by an anechoic center, surrounded by a hyperechoic rim, which can be attributed to the inability of the ultrasound waves to penetrate past the outer surface of the bone. Blood vessels also appear anechoic due to the low reflectivity of fluid within the vessels. Subcutaneous fat has a relatively low EI; however, several sections of hyperechoic connective tissue often exist throughout this layer (Pillen & van Alfen, 2011). In healthy individuals, skeletal muscle also tends to have a low EI because of the low presence of fibrous tissue. However, skeletal muscle is naturally striated, and the appearance of these striations can be visualized in an ultrasound image due to the hyperechoic connective tissue surrounding muscle fascicles and intramuscular fascia. Additionally, although healthy skeletal muscle generally appears hypoechoic (low echogenicity), the degree of muscle echogenicity can be influenced by various factors. Specifically, an increase in fat or fibrous tissue content within a muscle will result in an ultrasound image with higher echogenicity (Pillen et al., 2009), a typical indicator of a muscle with "poor" quality. However, muscle quality is a subjective measurement and can differ depending on the individual goals and conditions (Fukumoto et al., 2012; Pillen et al., 2009; Pillen & van Alfen, 2011). Furthermore, skeletal muscle EI can be also be influenced by several other factors, including collagen infiltration (Arts, Pillen, Schelhaas, Overeem, & Zwarts, 2010), glycogen depletion (J. C. Hill & Millan, 2014), exercise (Jajtner et al., 2015), training status (Watanabe et al., 2013), age (Fukumoto et al., 2012; Watanabe et al., 2013; Wilhelm et al., 2014), and disease (Arts et al., 2010; Scholten, Pillen, Verrips, & Zwarts, 2002).

Ultrasound assessment of skeletal muscle can provide a practitioner or researcher with different information and attributes about the muscle of interest depending on the anatomical plane that the image is captured in, due to the non-uniform characteristics of muscles in different planes. For example, when ultrasound images are captured perpendicularly to the long axis of the muscle (often termed a "transverse scan"), a cross-sectional image of the muscle with a speckled appearance is produced, due to the reflection of perimysial connective tissue throughout the muscle (Pillen & van Alfen, 2011). These types of images are especially useful for quantifying aspects of muscle size, including the CSA of a muscle. In contrast, when ultrasound images are captured parallel to the long axis of the muscle (often termed a "longitudinal scan" or "sagittal scan"), the fascicular arrangement of the muscle becomes visible. These types of images are useful for quantifying aspects of muscle architecture, such as PA and FL. An important consideration to take into account is that differences in muscle fiber architecture on a macroscopic level will affect the muscle characteristics that can be viewed on an ultrasound image (Pillen & van Alfen, 2011).

Typical ultrasound imaging of skeletal muscle utilizes a linear probe with still imaging, in which a sonogram is captured while the probe remains still on the surface of the skin, and the size of the image is equal to the width of the transducer. However, this type of imaging often lacks topographical information if the entire muscle is unable to fit in the still image, which is commonly the case with larger muscles (Reeves et al., 2004). Because of this, panoramic ultrasound imaging has been used in recent years, in which the ultrasound probe is moved along the surface of the skin while multiple still images are captured. These still images are then compiled onto one another, creating one large comprehensive image. Panoramic imaging has been used widely in previous research to quantify muscle morphological characteristics, especially for larger muscles (Ahtiainen et al., 2010; Jajtner et al., 2015; Jajtner et al., 2014; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Noorkoiv

et al., 2010; Reeves et al., 2004; Varanoske et al., 2017a, 2017b; Wells et al., 2014). Panoramic imaging has also been shown to be a valid and reliable tool in comparison to still imaging (Ahtiainen et al., 2010; Jenkins et al., 2015; Varanoske et al., 2017b). However, this type of imaging places a greater emphasis on the technique of the practitioner and requires significantly more time, expertise, and cost than traditional still imaging does. Additionally, there is an increased possibility of error with panoramic imaging, due to the overlapping of images upon one another (Noorkoiv et al., 2010), which may limit the applicability of this type of imaging. Nevertheless, in order to examine some specific aspects of muscle morphological characteristics in larger muscles, panoramic imaging may be necessary.

Previous research has demonstrated that ultrasonography is a valid and reliable tool to assess skeletal muscle morphology in comparison to other imaging techniques such as magnetic MRI, CT, and DEXA, especially for quantification of muscle size and quality (Ahtiainen et al., 2010; Esformes et al., 2002; Lixandrao et al., 2014; Noorkoiv et al., 2010; Reeves et al., 2004; Scott et al., 2012; Thomaes et al., 2012). MRI has often been considered the gold standard in the assessment of muscle CSA and volume, as it discriminates between different body tissues by creating drastic levels of contrast, and it can capture both superficial and deep tissue in a single image, making the assessment of each tissue relatively simple (Ahtiainen et al., 2010; Pillen & van Alfen, 2011; Reeves et al., 2004). Additionally, in contrast to CT, MRI and ultrasonography do not emit ionizing radiation, and therefore may be preferred in most cases for the quantification of muscle morphology (Pillen & van Alfen, 2011). Furthermore, MRI and ultrasonography appear to be more sensitive at detecting changes in neuromuscular function than CT, making CT of limited use when MRI and ultrasonography are available. However, both MRI and CT devices are not easily accessible for use in research due to their high cost, limited availability, and lack of portability. In addition, ultrasonography is capable of visualizing muscle architecture, allowing for quantification of measures such as FL and PA, making it an attractive tool for both researchers and clinicians.

Relationship between Muscle Morphology and Strength and Power Production

The non-invasive assessment of skeletal muscle morphological characteristics obtained via ultrasonography can provide a researcher or practitioner with important information about the functionality of a muscle because of the relationship that muscle structure has with its function (Burkholder et al., 1994). Specifically, measures of muscle fiber arrangement, muscle size, and muscle quality acquired through ultrasonography can help predict how a specific muscle functions and its ability to produce force. This section will detail the measurements of muscle CSA, MT, EI, PA, and SFT and how each of these measurements can be used to evaluate skeletal muscle function.

Measures of Muscle Size: Muscle Thickness (MT) and Cross-Sectional Area (CSA)

Measures of muscle size typically reported in the ultrasound literature include CSA, defined as the area of a two-dimensional cross-section of a muscle, and MT, defined as the perpendicular distance between the superficial and deep aponeurosis of a muscle (Ahtiainen et al., 2010; Esformes et al., 2002; Lixandrao et al., 2014; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Noorkoiv et al., 2010; Reeves et al., 2004; Scanlon et al., 2014; Scott et al., 2012; Thomaes et al., 2012; Varanoske et al., 2017b; Wells et al., 2014). If measurements of muscle CSA are desired and the muscle of interest is larger than the width of the ultrasound probe, panoramic imaging is required to capture the entire cross-section of a muscle in a single ultrasound image. On the other hand, quantifying MT usually only requires still ultrasound images and is, therefore, easier, less technical, and more time-efficient to complete. However, a recent investigation by Varanoske et al. (2017b) observed significant differences in both CSA and MT of the VL muscle measured from still ultrasound images and panoramic ultrasound images and demonstrated that values obtained from both types of images were significantly correlated (p < 0.001). Additionally, MT measurements were significantly correlated with CSA measurements from both types of images (p < 0.001), indicating that both MT and CSA may be used as measures of muscle size (Varanoske et al., 2017b).

Quantification of muscle size is a critical component of the ability to predict muscle strength and force production, and previous research has shown that measures of muscle size assessed via ultrasonography, including CSA and MT, are positively associated with maximal force production (Fukunaga et al., 2001; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Moreau et al., 2010). As muscle force-producing capability is largely a result of the cross-bridge interaction of actin and myosin, a muscle that is larger in size with greater myofilaments characteristically has an increased ability to produce force. In accordance with this notion, previous research has also demonstrated that resistance training significantly increases muscle size, and CSA in particular, through the addition of contractile protein as a result of increased muscle myofibrillar protein synthesis (Glass, 2003a, 2003b; Scanlon et al., 2014; Seynnes, de Boer, & Narici, 2007; Wells et al., 2014; J. C. Young, Chen, & Holloszy, 1983). However, an investigation by Wells and colleagues (2014) suggested that increases in lower-body muscle

strength following a resistance training program may be better elucidated by changes in VL MT rather than changes in CSA. Furthermore, research has shown that MT of the VL accounts for over 90% of the variance in maximal knee extensor torque after controlling for age and gross motor function in children and adolescents (Moreau et al., 2010). Therefore, although ultrasound measures of CSA and MT both quantify muscle size and appear to be related (Varanoske et al., 2017b), evaluating both may be necessary for a comprehensive understanding of muscle function and force production (Wells et al., 2014).

Measures of Muscle Architecture: Pennation Angle (PA) and Fascicle Length (FL)

Measures of muscle architecture that can be obtained via ultrasonography include PA, defined as the angle between the muscle fascicles and the deep aponeurosis of the muscle, and FL, defined as the length of an individual fascicle along the distance of the muscle. A unique inverse relationship typically exists between PA and FL within a muscle, as muscles with greater PA are usually associated with shorter FL (Blazevich, Gill, & Zhou, 2006). Pennate muscles within the body exhibit these characteristics, having shorter FL and greater PA, allowing for a greater amount of contractile protein to be arranged in parallel within a given volume, resulting in an increased ability of the given muscle to produce force. On the other hand, parallel muscles typically contain fibers with longer FL and lower PA, resulting in a decreased ability to produce force. However, muscles with a greater number of fibers arranged in series (as in parallel muscles) often allow for a greater range of motion at the joint and a greater velocity of muscle contraction, offering them a unique advantage over pennate muscles (Aagaard et al., 2001).

Inconclusive research has shown that muscle architectural characteristics may change with resistance training, which have been associated with changes in muscle strength and power. Although some investigations have reported no change in FL after exercise training (Blazevich, Gill, Deans, & Zhou, 2007; Ema, Wakahara, Miyamoto, Kanehisa, & Kawakami, 2013; Erskine, Jones, Williams, Stewart, & Degens, 2010; Nimphius, McGuigan, & Newton, 2012), most research has demonstrated that FL increases after either resistance training, isokinetic training, or marathon training (Alegre, Jimenez, Gonzalo-Orden, Martin-Acero, & Aguado, 2006; Baroni et al., 2013; Blazevich, Cannavan, Coleman, & Horne, 2007; Franchi, Atherton, Maganaris, & Narici, 2016; Franchi et al., 2014; McMahon, Morse, Burden, Winwood, & Onambele, 2014; Murach, Greever, & Luden, 2015; Reeves, Maganaris, Longo, & Narici, 2009; Seynnes et al., 2007). Furthermore, although some studies have observed that exercise training does not change PA (Alegre et al., 2006; Rutherford & Jones, 1992), others have reported that PA significantly increases after resistance training (Aagaard et al., 2001; Ema et al., 2013; Kawakami, Abe, Kuno, & Fukunaga, 1995). It has been suggested that the discrepancies in these findings may be related to the type of contraction the muscle endures (Franchi et al., 2014). Specifically, Franchi and colleagues (2014) proposed that changes in FL are promoted by eccentric training, whereas changes in PA are promoted by concentric training. Pre-clinical studies have previously reported that muscle lengthening actions, including passive and intermittent stretch, cause skeletal muscle to respond by adding new sarcomeres in series (i.e., increasing FL) (Goldspink, 1985; Holly, Barnett, Ashmore, Taylor, & Mole, 1980; Williams, Catanese, Lucey, & Goldspink, 1988). This phenomenon has also been observed after eccentric exercise and overload (Butterfield, Leonard, & Herzog, 2005; Goldspink, 1999; Lynn & Morgan, 1994; Proske & Morgan, 2001), which is

thought to be a protective mechanism acting after eccentric muscle damage (Morgan & Talbot, 2002). On the other hand, concentric exercise tends to promote increases in PA through the addition of sarcomeres in parallel as a result of increased muscle myofibrillar protein synthesis (Atherton & Smith, 2012; Glass, 2003a, 2003b), increasing the thickness of each fascicle (Franchi et al., 2014). Therefore, an exercise-induced change in PA may be partially attributed to the degree of muscle hypertrophy experienced (Ema et al., 2013). Previous research has demonstrated that a significant relationship between MT and PA exists (Ema et al., 2013; Kawakami et al., 1995), and that this relationship was still present after exercise training eliciting hypertrophy (i.e., an increase in MT was also associated with an increase in PA) (Azizi & Brainerd, 2007; Ema et al., 2013). Furthermore, it has been suggested that an increase in FL does not necessarily accompany an increase in muscle hypertrophy (Fukutani & Kurihara, 2015). However, research has shown that in muscles with very large PA ($<45^{\circ}$), contractile forces may not be transmitted to the tendon as effectively, which may be accompanied by a decrease in specific tension (Kawakami, 2005). Taken together, it is possible that the type of contraction that a muscle experiences affects the changes in muscle architecture with training.

In addition to force production, another vital aspect of sporting performance is the ability to produce force quickly, i.e., the RFD. Mirkov and colleagues (2004) suggested that the ability to produce strength is largely correlated with RFD and that the magnitude of this relationship increases especially during the later phases of muscle contraction. Therefore, it is sensible to propose that the main factors influencing maximal strength probably also influence the RFD (Aagaard et al., 2001; Andersen & Aagaard, 2006; Maffiuletti et al., 2016). As greater PA typically allow for greater physiological CSA for a given muscle size, this may be associated with a greater ability of the muscle to produce force (Maffiuletti et al., 2016). In contrast to the later phases of muscle contraction, research has demonstrated that the rise in force measured early in a contraction is largely a result of the requirement of the muscle to take up the series elastic component (Edman & Josephson, 2007). Muscles with longer fascicles typically contain a greater amount of series elastic material, and therefore may display a slower rise in force because of the time it takes to take up the series elastic slack prior to true muscle contraction (Blazevich, Cannavan, Horne, Coleman, & Aagaard, 2009; Maffiuletti et al., 2016). It is generally well-known that muscles capable of high rates of force development, including the VL, soleus, and gastrocnemius possess shorter FL and accompanying high PA (Lieber & Ward, 2011). Therefore, it is apparent that muscle architecture may affect both maximal strength as well as rate of force production.

Measures of Muscle Quality: Echo Intensity (EI)

In additional to muscle size and architecture, EI can provide useful information about muscle composition and functionality. EI, previously introduced as the degree of reflectance of the ultrasound waves off of body tissues, is quantified by averaging the grayscale value of each individual pixel within a region of interest using an image analysis software (Jenkins et al., 2015; Pillen et al., 2009; Pillen & van Alfen, 2011; Scanlon et al., 2014). Higher EI values within a muscle are representative of a tissue with a higher degree of reflectance of sound waves and correspond with a brighter overall image (Pillen & van Alfen, 2011). Muscles with greater EI values typically contain greater amounts of non-contractile tissue, including intramuscular fibrous tissue, connective tissue, and/or fat, as these tissues increase the number of reflections

within the muscle (Pillen & van Alfen, 2011; Watanabe et al., 2013). Therefore, in healthy populations, a muscle with lower EI is characteristic of a greater proportion of contractile tissue and lower amounts of non-contractile tissue, thus signifying a muscle with a greater efficiency at producing force, potentially indicating higher muscle quality.

Previous research has demonstrated that muscle EI values differ depending on various factors, some of which include age, gender, and training status. Specifically, EI values are positively correlated with age (Li et al., 2012; Scanlon et al., 2014; Watanabe et al., 2013), which may reflect the increased infiltration of intramuscular fat, fibrous tissue, and connective tissue that usually accompanies aging. Additionally, research has shown that EI differs between genders, with females typically possessing greater muscle EI (Arts et al., 2010; Caresio, Molinari, Emanuel, & Minetto, 2015; H. J. Young et al., 2015), which may be due to differences in body composition or the physiological consequences of hormone concentration differences between genders (H. J. Young et al., 2015). EI values have also been shown to be negatively correlated with strength and power as well as anaerobic sporting performance (Cadore et al., 2012; Fukumoto et al., 2012; Jajtner et al., 2015; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Watanabe et al., 2013; H. J. Young et al., 2015). Additionally, there is evidence to suggest that chronic resistance training can also influence EI values (Scanlon et al., 2014; Strasser, Draskovits, Praschak, Quittan, & Graf, 2013; Watanabe et al., 2013; Wilhelm et al., 2014), potentially reflecting an increased proportion of contractile tissue within the muscle, and therefore increased fluid retention. As water reflects ultrasound waves poorly, a greater fluid content of the muscle typically results in lower echogenicity (Pillen & van Alfen, 2011).

Muscle glycogen content and muscle damage may also affect EI values (J. C. Hill & Millan, 2014; Jajtner et al., 2015; Nosaka & Sakamoto, 2001). Previous research conducted in trained cyclists indicated that lower muscle EI values in the rectus femoris muscle were associated with increased intramuscular glycogen content quantified via muscle biopsy (J. C. Hill & Millan, 2014). Maximizing intramuscular glycogen content is beneficial for endurance performance, as decreased muscle glycogen is associated with fatigue, hypoglycemia, decreased muscle contractility, and decreased calcium release (Bangsbo, Graham, Kiens, & Saltin, 1992; Chin & Allen, 1997; Hargreaves, Meredith, & Jennings, 1992). It is generally well-known that a greater intramuscular glycogen content is associated with increased water retention (MacKay & Bergman, 1932; Olsson & Saltin, 1970). In accordance with this, EI values measured after a bout of exhaustive exercise have been shown to increase (J. C. Hill & Millan, 2014; Jajtner et al., 2015; Nosaka & Sakamoto, 2001), potentially reflecting glycogen depletion. Additionally, the increase in EI observed after exercise has been shown to be associated with increased muscle damage and edema (Fujikake, Hart, & Nosaka, 2009; Jajtner et al., 2015; Longo, Jacobson, Fessell, & Mautner, 2016; Nosaka & Clarkson, 1996; Nosaka & Newton, 2002; Nosaka, Newton, & Sacco, 2002; Radaelli, Bottaro, Wilhelm, Wagner, & Pinto, 2012). Fujukake et al. (2009) suggested that the increase in EI following muscle damage in mice, as induced via an injection of bupivacaine hydrochloride, was characterized by interstitial edema and associated with a significant increase in muscle size and weight. Specifically, these researchers suggested that a large difference in acoustic impedance between the muscle fibers and increased volume of interstitial spaces induced by edema may have resulted in an increase in EI (Fujikake et al., 2009), however other researchers have suggested that infiltration of inflammatory cells and

cytokines may be responsible for the increases in echogenicity (Nosaka & Clarkson, 1996; Nosaka & Sakamoto, 2001). Furthermore, research has shown that exercise-induced muscle damage may cause EI values to remain elevated for 72 hours or more, indicating that it may be an adequate marker of muscle damage and edema (Jajtner et al., 2015; Nosaka & Newton, 2002; Nosaka et al., 2002; Nosaka & Sakamoto, 2001; Radaelli et al., 2012).

Measures of Adiposity: Subcutaneous Adipose Tissue Thickness (SFT)

In addition to muscle morphological characteristics, ultrasonography has also been used as a tool to assess SFT (Pineau, Filliard, & Bocquet, 2009; Pineau et al., 2010; Selkow, Pietrosimone, & Saliba, 2011; H. J. Young et al., 2015). Subcutaneous adipose tissue is the layer of fat that lies between the dermis and the muscle (Selkow et al., 2011), and accurate assessment of adiposity can provide vital information about body fat percentage and composition in the evaluation of disease risk and health status. The use of ultrasonography in the assessment of SFT has been proven to be valid and reliable in comparison to MRI, CT, bioelectrical impedance analysis (BIA), arm circumference analysis, and skinfold thickness (Fanelli & Kuczmarski, 1984; Fukumoto et al., 2012; Jenkins et al., 2015; Selkow et al., 2011). However, previous research has suggested that the SFT adjacent to the muscle of interest may affect the EI of that tissue (Pillen & van Alfen, 2011; H. J. Young et al., 2015). Previous research with MRI and CT has demonstrated that an increase in total body adiposity is associated with an increased accumulation of intramuscular fat (Goodpaster et al., 2001; A. S. Ryan & Nicklas, 1999; Sinha et al., 2002). Based off of these findings, it may be expected that an increase in SFT would be related to an increase in EI values. However, some previous research has found no significant
correlations between EI and SFT (Fukumoto et al., 2012; Melvin et al., 2014; Scholten et al., 2002; Trexler, Smith-Ryan, Roelofs, & Hirsch, 2015; Varanoske et al., 2017b; Wu, Darras, & Rutkove, 2010). Some of these authors have suggested that a measure of total body adiposity may not be representative of the intramuscular fat contained within individual muscles, and therefore a limb-specific measure of body fat may be more related to limb-specific intramuscular fat (E. D. Ryan et al., 2016). Others have suggested that the SFT above the muscle may affect the EI values obtained from the ultrasound images (Pillen & van Alfen, 2011; H. J. Young et al., 2015). As ultrasonography is based on the ability of sound waves to penetrate different body tissues and reflect off of them, tissues with greater thicknesses may affect the ultrasound beam in a way that does not allow the image to accurately affect true muscle composition (Pillen & van Alfen, 2011). In support of this theory, Young and colleagues (2015) reported a negative correlation between SFT and EI values, proposing that an underestimation of EI may occur when SFT increases due to the non-systematic reflection of ultrasound waves. These researchers suggested that SFT should, therefore, be accounted for when assessing muscle EI, and thus they developed an equation with a correction factor for SFT in the assessment of EI. To further validate this correction factor, Ryan and colleagues (2016) examined the relationship between EI and body composition prior to and after accounting for SFT. The uncorrected EI values (UnCorEI) suggested that muscle quality improved with increasing adiposity (r = -0.329 - -0.224; p = 0.038 - 0.165), whereas the corrected echo intensity values (CorEI) suggested that muscle quality significantly decreased with increasing adiposity (r = 0.711 - 0.798; p < 0.001) (E. D. Ryan et al., 2016). Therefore, it is evident that adjusting EI values for SFT may be necessary for the assessment of skeletal muscle morphology.

Specific Considerations during Ultrasound Assessment of Skeletal Muscle

Due to its highly-technical nature, ultrasonography is a procedure that requires stringent guidelines on the part of the technician, as well as the subject, in order for accurate measurements of muscle morphology to be made. For example, ensuring that the technician is applying consistent pressure to the probe on the surface of the skin is essential for precise measurement of muscle morphological characteristics (Ihnatsenka & Boezaart, 2010). Changes in the pressure applied to the probe on the skin may compress the underlying tissue, therefore altering muscle and SFT, as well as EI. Additionally, ensuring that other characteristics of probe manipulation, including probe tilt and rotation, are consistent, is imperative in the accurate measurement of muscle morphology (Ihnatsenka & Boezaart, 2010). Previous research has shown that tilting the probe as little as 2% can produce significant changes in EI of muscle (Dankel et al., 2018). Additionally, changes in the rotation, or clockwise/counterclockwise shift of the probe, will affect the orientation of muscle fibers and therefore muscle morphological characteristics (Ihnatsenka & Boezaart, 2010). Furthermore, when performing panoramic ultrasonography, additional consideration must be taken to ensure that the probe is moved along the skin while maintaining a constant pressure and sliding speed throughout the entire sweep (Ihnatsenka & Boezaart, 2010). Because technical abilities and methodologies during ultrasonography may differ between examiners, values obtained from one technician may vary from those of another, with no real change in the properties of the muscle. Therefore, it is important to use the same technician when performing ultrasound imaging in the quantification of muscle morphology, particularly in research settings.

In addition to the skills of the technician that can affect ultrasound images, different ultrasounds have different adjustable features (i.e., gain, frequency, depth) that result in drastic changes to the image presented on screen (Ihnatsenka & Boezaart, 2010). For example, altering the gain, or brightness, of the image will have a direct effect on the EI values generated by an image analysis software. Additionally, altering the depth of the image will change how much of the tissue of interest can be viewed in a single image, affecting the overall quality of the image. Specifically, increasing the depth will result in a decreased overall image quality due to more of the surrounding musculature being included in the image. This will affect the pixilation within the image, also influencing EI. Ultrasound probes can also emit sound waves of different frequencies, which will affect the ability of the waves penetrate tissues. Higher-frequency probes are preferred when examining superficial tissues at a maximum of 4 cm from the surface of the skin, whereas lower-frequency probes are useful when viewing much deeper tissues, 10 or more centimeters from the surface of the skin. Higher-frequency probes, therefore, provide better image resolution than low-frequency probes; however, low-frequency probes permit a greater image depth (Ihnatsenka & Boezaart, 2010). Additionally, different researchers use different methodologies for image capture (i.e., panoramic vs. still imaging) and examine tissues in different planes. Research has shown that the type of image and the plane of movement in which the image is captured can influence muscle morphological characteristics and the reliability of ultrasound procedures (Caresio et al., 2015; Jenkins et al., 2015; Varanoske et al., 2017b). Therefore, direct comparisons of muscle morphological characteristics in different research studies may not be practical due to differences in ultrasound settings.

Because valid measurements of ultrasonography are also highly dependent on the subject, it is important to standardize certain subject requirements prior to ultrasound assessment. For example, subjects should not participate in exercise that utilizes the muscle of interest for at least 72 hours prior to testing, as exercise has been shown to result in significant changes in muscle morphology, including CSA, MT, PA, and EI (Jajtner et al., 2015; Nosaka & Newton, 2002; Nosaka et al., 2002; Nosaka & Sakamoto, 2001; Radaelli et al., 2012). Additionally, it is essential to ensure that the muscle-specific joint angles are standardized and are kept consistent across subjects because research has demonstrated that changes in joint angle alone can affect muscle morphology (Hacker, Peters, & Garkova, 2016; Maganaris, 2001; Myers et al., 2013; Narici et al., 1996). For example, Hacker and colleagues (2016) revealed that a change in hip angle of 20° (from supine to 20° of bed elevation) resulted in a significant increase in rectus femoris CSA. Rectus femoris CSA also continued to significantly increase with every 20° increase in bed elevation (Hacker et al., 2016). In addition, previous research has demonstrated that changes in body position (from a standing to supine position) induces significant changes in blood flow and hydrostatic pressure throughout the body (Maw et al., 1995). This postural change prompts gravitational fluid shifts, which have been shown to result in alterations in muscle size and architecture (Cerniglia et al., 2007; Thoirs & English, 2009; Wagle et al., 2017). Therefore, changes in the proximity of exercise, joint position, and body posture alone may result in changes in muscle structure, without concomitant changes in muscle function. Thus, these represent just a few of the specific requirements that must be considered prior to ultrasound assessment of skeletal muscle. Due to the significance of posture on measures muscle

morphology, the remainder of this review will focus on physiological responses to postural changes.

Cardiovascular Responses to Changes in Posture

Blood pressure measured at different anatomical locations within the human body is impacted by various factors; one of the most influential being gravity. For a standard adult male 180 cm tall resting in the supine anatomical position, the mean arterial pressure (representing the average blood pressure within the arteries during one cardiac cycle) is about 95 mmHg at the level of both the feet and the head; on the venous side, the pressure is approximately 3 mmHg at the level of both the feet and the head (Martin-Du Pan, Benoit, & Girardier, 2004). Thus, in the recumbent position, pressures and blood volumes within similar vessels tend to be equal throughout all parts of the body because gravitational forces act similarly in the same horizontal plane (Martin-Du Pan et al., 2004). However, when transitioning from a recumbent to erect position suddenly, the pressures within similar vessels differ drastically due to the influence of gravity on changes in blood flow and hydrostatic pressure throughout the body (Martin-Du Pan et al., 2004). Theoretically, in a microgravity environment, changes in body posture do not result in changes in blood flow, other than what is caused by muscular contraction. However, on upon transitioning from a supine to upright position on Earth, the force of gravity causes a change in the fluid distribution throughout the body. Within seconds of standing, blood accumulates in the lower extremities, specifically in the venous system due to the high compliance of veins (Smith, Porth, & Erickson, 1994). This causes the venous volume and pressure of the lower body increase drastically, which reduces venous return back to the heart (Martin-Du Pan et al., 2004).

The decreased right ventricular filling therefore reduces stretching of the heart, thereby resulting in a decrease in stroke volume by the Frank-Starling mechanism and a decrease in parasympathetic stimulation (Frey, Tomaselli, & Hoffler, 1994; Smith et al., 1984; Sprangers, Wesseling, Imholz, Imholz, & Wieling, 1991). This causes a reduction in cardiac output and mean arterial pressure within the arterial system, the latter of which is sensed by the arterial baroreceptors to increase activation of the sympathetic nervous system (Smith et al., 1984). This sympathetic stimulation acts to increase heart rate and contractility, increase systemic vascular resistance, and decrease venous compliance to maintain cardiac output and mean arterial pressure (Borst et al., 1982; Ewing, Campbell, Murray, Neilson, & Clarke, 1978). Additionally, operation of other important compensatory mechanisms, including neurogenic vasoconstriction of veins, the respiratory pump, the muscle pump, and the release of neurohormones help to maintain moderate increases in the capillary and venous pressures in the feet (Smith et al., 1984). Without these compensatory mechanisms, orthostatic hypotension, edema of the feet, and syncope would be inevitable.

Hydrostatic pressure, defined as the pressure that is exerted by a fluid due to the effects of gravity, increases in proportion to depth from the surface of the fluid (i.e., reference point) due to the weight of the fluid exerting a force above it. The effects of gravity and distance from the reference point on pressure is demonstrated in the equation for positional hydrostatic pressure (Equation 1) (Martin-Du Pan et al., 2004),

Positional Hydrostatic Factor =
$$p \times g \times h$$
 (1)

where p is the blood density, g is the acceleration due to gravity, and h is the distance from the reference point. Since blood density and the acceleration due to gravity are stable, the hydrostatic

factor is solely influenced by the distance from the reference point. In this equation, the positional hydrostatic factor is negative for distances above the reference point and is positive for distances below the reference point. In the human body, the reference point referred to is the heart; therefore, during standing, the pressure is decreased in any vessel above the level of the heart and is increased in any vessel below the level of the heart (Martin-Du Pan et al., 2004). These changes in pressures are even more pronounced in vessels further from the reference point, as demonstrated by the feet having the greatest pressure during standing.

Upon return to a recumbent position after a duration of standing, the hydrostatic pressures within similar vessels in different parts of the body begin to equilibrate, and blood redistributes throughout the body. Because gravitational forces act similarly in the same horizontal plane, venous volume and pressure decline, increasing venous return to the heart. This results in an increased right ventricular filling and stroke volume, increasing cardiac output and mean arterial pressure. The increase in mean arterial pressure is sensed by the arterial baroreceptors, which decrease sympathetic stimulation of the cardiovascular system, allowing heart rate and contractility to decline.

Although the magnitude of disparity in hydrostatic pressures between different parts of the body are very pronounced during standing, postural changes within the horizontal plane may also induce variations in hydrostatic pressure and blood flow, but to a lesser extent. As hydrostatic pressure in the body is based on the vertical height from the heart (Martin-Du Pan et al., 2004), transitioning from a supine to lateral recumbent position alters the positioning of body parts relative to the heart and therefore may alter blood flow. In the supine position, the posterior side of the body is vertically positioned at a height below the heart, whereas the anterior side of the body is vertically positioned at a height above or equal to the heart, which may result in a greater hydrostatic pressure and increased accumulation of blood in the posterior vessels compared to the anterior vessels (Bryan, 1974; Kallet, 2015). When transitioning from a supine to a lateral recumbent position, a similar change in hydrostatic pressures may occur. For example, in the left lateral recumbent position, the right side of the body will be vertically positioned at a height above the heart, which may result in a lower hydrostatic pressure and decreased accumulation of blood in the vessels in the right side of the body compared to those in the left side. Although there is conflicting research on hemodynamic changes induced by recumbent postural changes (Atkins, Watt, Milan, Davies, & Crawford, 1981; Ueland & Hansen, 1969; Whitman, Howaniak, & Verga, 1982), there is a possibility for these recumbent postural changes to affect blood distribution.

Effects of Changes in Posture on Blood Volume, Distribution, and Composition

Immediately after transitioning from a recumbent to upright position, the distensibility of the venous system allows for an accumulation of blood in the lower extremities. This blood accumulation begins rapidly, and within 2-3 minutes, about 10% of total blood volume is displaced to the lower body, although the total amount of redistributed blood is dependent on net lower body vascular compliance (Smith et al., 1984). Because a substantial amount of blood has been displaced to the lower body, cerebral blood flow, splanchnic blood flow, and blood flow to the upper extremities decreases significantly (Sjostrand, 1953). The accumulation of blood in the lower sody, increasing hydrostatic pressure within these capillaries. At the level of the capillary,

Starling's law applies: when hydrostatic pressure is greater than plasma oncotic pressure, net filtration of fluid occurs; when plasma oncotic pressure is greater than hydrostatic pressure, net absorption occurs (Thompson et al., 1928; Waterfield, 1931b). Although upright rest results in vasoconstriction of blood vessels in the lower body to maintain cardiac output, which tends to decrease capillary pressure, the increase in hydrostatic pressure within these vessels, specifically within the non-constricted capillaries, overrides the decrease in capillary pressure (Stick, Hiedl, & Witzleb, 1993). Therefore, with an increase in hydrostatic pressure, the rate of capillary filtration of fluid into the interstitial space increases, resulting in a decrease in plasma volume (Fawcett & Wynn, 1960; Hagan et al., 1978; Taylor et al., 1995; Thompson et al., 1928; Waterfield, 1931b). Previous research has demonstrated that there is a decrease in blood volume after transitioning from a recumbent to upright position, with losses originating from the plasma (Hagan et al., 1978; Lundvall, Bjerkhoel, Quittenbaum, & Lindgren, 1996; Taylor et al., 1995; Thompson et al., 1928; Waterfield, 1931b). Specifically, Hagan et al. (1978) discovered an average loss blood volume loss of 9.5% and a corresponding plasma volume loss of 16.2% after transitioning from a supine to standing position. Additionally, Thompson et al. (1928) discovered a net plasma volume loss of 11% after standing for 20-30 minutes. Similar findings have also been reported when transitioning from a seated to standing position, as Maw et al. (1995) reported a 6% loss in blood volume 30 minutes after transitioning from a seated to standing position, which was accounted for by a significant decrease in plasma volume and a significant increase in interstitial fluid volume. Additionally, these researchers discovered elevated concentrations of plasma protein, which is in line with previous research (Fawcett & Wynn, 1960; Hagan et al., 1978; Thompson et al., 1928; Waterfield, 1931b). Decreases in plasma

volume after transitioning to a standing position have also been associated with significant increases in hemoconcentration (Eisenberg, 1963; Eisenberg & Wolf, 1965; Fawcett & Wynn, 1960; Hagan et al., 1978; Tan et al., 1973; Waterfield, 1931b) and increases in concentrations of other plasma constituents, including hormones, ions, and metabolites (Husdan, Rapoport, & Locke, 1973; Stoker, Wynn, & Robertson, 1966; Tan et al., 1973). Due to the pressure increases that occur specifically within the venous system, changes in blood composition observed while maintaining an upright posture appear to be more pronounced in venous blood than arterial blood (Thompson et al., 1928). However, transitioning from the supine to sitting position, or from the sitting to upright position, appears to result in smaller magnitudes of change in blood volume, plasma volume, and blood composition than transitioning from the supine to standing position (Tan et al., 1973).

In contrast to the physiological responses observed when transitioning from a recumbent to upright position, the opposite has been observed when transitioning from an upright to recumbent position. Due to the gravitational forces acting on the body in the same horizontal plane, blood no longer accumulates in the lower body and is instead redirected back to the thorax and head (Baccelli et al., 1995; Hildebrandt et al., 1994; Yadollahi, Singh, & Bradley, 2015). Additionally, vessels begin to dilate, and the hydrostatic pressure within vessels of the lower extremity declines, allowing for net absorption of fluid from the interstitial space into the capillaries (Maw et al., 1995). Previous research has demonstrated that blood volume and plasma volume increase when transitioning from a standing to supine position, reflecting the fluid absorption of fluid from the interstitial space into the capillaries (Eisenberg, 1963; Fawcett & Wynn, 1960; Hagan et al., 1978; Hinghofer-Szalkay & Moser, 1986; Maw et al., 1995; Tan et al., 1973). Hagan and colleagues (1978) reported a 6.4% increase in blood volume and 11.7% increase in plasma volume after movement from the upright position to the supine position, which was associated with significant hemodilution, decreased hematocrit and hemoglobin levels, and significantly decreased concentrations of plasma proteins. Similar results have been reported when transitioning from a seated to supine position (Maw et al., 1995; Tan et al., 1973). Maw et al. (1995) reported an increase in blood volume and plasma volume 30 minutes after transitioning from a seated to supine position, which was accompanied by a decrease in interstitial fluid volume, although these values did not reach statistical significance. These findings align with those of Maxfield et al. (1941) who reported a lower magnitude of change in blood composition when transitioning from the supine to sitting position, or from the sitting to upright position, compared to transitioning from the supine to standing position.

Postural changes within in the recumbent position affect hydrostatic pressures within opposing sides of the body (Bryan, 1974; Kallet, 2015), which therefore may induce changes in blood distribution. Previous research has suggested that, in the recumbent position, pressures and blood volumes within similar vessels tend to be equal throughout all parts of the body because gravitational forces act similarly in the same horizontal plane (Martin-Du Pan et al., 2004). This is based on the assumption that the hydrostatic pressure is measured in vessels at the same vertical position relative to the heart; thus it does not account for vessels in different vertical positions. In the supine position, the accumulation of blood towards the posterior side of the body may increase capillary hydrostatic pressure enough to cause a fluid shift out of the capillaries and into the interstitial space of the posterior tissues. Likewise, in the left lateral recumbent position, the accumulation of blood towards the left side of the body may increase capillary hydrostatic pressure enough to cause a fluid shift out of the capillaries and into the interstitial space of the tissues on the left side of the body. Therefore, based on the changes in hydrostatic pressures during postural changes within the recumbent position, blood distribution throughout the body may increase fluid shifts into specific tissues. Future research is necessary to determine whether or not changes in total blood volume, plasma volume, or blood constituents occur with postural transitions in the recumbent position, although this seems unlikely because the postural changes are occurring within the same vertical plane.

Effects of Changes in Body Posture on Intracellular and Extracellular Water

When postural shifts are made, changes in cardiovascular function, hemodynamics, and blood composition occur within a relatively short period. The transition from a supine to upright position elicits rapid decreases in plasma volume due to net filtration of fluid out of capillaries, specifically within the lower body (Fawcett & Wynn, 1960; Hagan et al., 1978; Taylor et al., 1995; Thompson et al., 1928; Waterfield, 1931b). Despite the gravitational effect of fluid shifts upon the modification of body posture, previous research has suggested that the transition from a supine to standing position does not affect total body water (Gibson, Beam, Alencar, Zuhl, & Mermier, 2015; Maw et al., 1995), but rather the distribution of fluid throughout the body, as most of the blood volume is shifted to the lower body. Cerniglia and colleagues (2007) suggested that moving from a standing to recumbent position and the resultant decrease in hydrostatic pressure within the lower body may allow intracellular water to move out of muscle cells of the lower body, into the interstitial space, and finally into the vascular system. This, in theory, implies that there is movement of fluid from the intracellular fluid compartment to the

extracellular fluid compartment upon attaining the supine position. However, previous research has demonstrated that 30 minutes after transitioning from a seated to standing position resulted in a significant increase in interstitial fluid volume and significant decreases in blood volume and plasma volume, with no significant changes in intracellular or extracellular fluid volumes (Maw et al., 1995). These findings propose that postural manipulations result in a loss of plasma filtrate from the intravascular space, which is eluted only into the extracellular interstitial space and not into the extravascular intracellular compartment (Maw et al., 1995). Similar increases in interstitial fluid volume have also been observed in other investigations upon postural changes from the supine to standing position (Eichler et al., 2000; Husmann, Barton, Amann-Vesti, & Franzeck, 2006). Additionally, transitioning from a standing to a supine 5% head-down tilt position resulted in significantly decreased interstitial fluid pressure and volume of the leg; however, no significant changes were observed in the areas of Type I and Type II muscle fibers (Hargens, 1983). Scharfetter and colleagues (1997) suggested that a fluid shift from the extracellular compartment to the intracellular compartment would only occur as a result of changes in osmolality between the extracellular and intracellular fluid. Because of the ability of sodium to transfer easily between the plasma and interstitial fluid, an increase in interstitial fluid volume with postural changes likely does not change the osmolality of the plasma or interstitial fluid. This has been confirmed in previous research demonstrating no change in plasma osmolality or plasma sodium concentrations after postural changes, despite significant changes in plasma volume (Hagan et al., 1978; Lippi et al., 2015; Shirreffs & Maughan, 1994).

Confounding research has observed significant changes in whole body and segmental extracellular and intracellular fluid volume with positional changes as measured by BIA and

bioelectrical impedance spectroscopy (BIS) (Fenech & Jaffrin, 2004; Gibson et al., 2015; Scharfetter et al., 1997; Zhu, Schneditz, Wang, & Levin, 1998). Zhu and colleagues (1998) suggested that upon transitioning positions, regional fluid shifts are likely to affect the measured extracellular fluid volume as assessed using a whole-body technique because of the changes in bioimpedance with blood accumulation in the lower body, therefore increasing extracellular fluid volume measurements upon transitioning from a supine to standing position. In the same investigation, total body extracellular fluid volume was also measured as the sum of segmental extracellular fluid volumes; these researchers observed no changes in the sum of segmental extracellular fluid volumes with changes in posture, although leg extracellular fluid volume significantly decreased upon changing from a standing to supine body position, reflecting fluid redistribution to the head and thorax (Zhu et al., 1998). In agreement with these findings, Scharfetter and colleagues (1997) discovered significant decreases in segmental leg extracellular fluid volume upon transitioning from a standing to supine body position, reflecting fluid redistribution to the head and thorax, however, this was also accompanied by a decrease in total body extracellular fluid volume and increase in intracellular volume. These researchers suggested that the method of measuring whole body impedance that was utilized was not very sensitive to fluid changes in the trunk, head, and neck, and therefore fluid shifts in the legs and arms completely determined the changes in total body impedance, therefore resulting in spurious changes in compartment fluid volume with changes in position (Scharfetter et al., 1997). Similarly, Shea et al. (2017) observed significant decreases in extracellular water and increases in intracellular water in both older and younger individuals after transitioning from a standing to supine position. Likewise, Gibson and colleagues (2015) observed significant increases in

extracellular water after transitioning from a supine to standing position. Additionally, these researchers found no significant change in intracellular water when maintaining an upright position, indicating that the change in extracellular water may be due to fluid moving out of the torso into the lower body, rather than fluid shifts between the extracellular and intracellular compartments. However, when transitioning from a standing to supine position, extracellular fluid volume decreased, and intracellular fluid volume increased, which may be attributed to the change in impedance and resistance of the body when large fluid shifts occur (Gibson et al., 2015). Furthermore, it appears that the time course for increases in extracellular fluid and decreases in intracellular fluid when changing from a supine to standing position may not mirror each other (Gibson et al., 2015; Scharfetter et al., 1997). Therefore, although some researchers suggest that fluid shifts between the intracellular and extracellular compartments may occur with postural changes (Cerniglia et al., 2007; Kose, Hur, Taskin, Bicak, & Duman, 2014), changes in osmolality between the intracellular and extracellular compartments is the underlying stimulus of fluid transfer between compartments, which does not appear to occur with positional changes.

Time Course of Postural Fluid Shifts

Previous research has demonstrated that switching from a supine to upright position, and vice versa, elicits changes in fluid distribution and composition throughout the body (Fawcett & Wynn, 1960; Hagan et al., 1978; Taylor et al., 1995; Thompson et al., 1928; Waterfield, 1931a, 1931b). Hematological variations upon attaining a new posture seem to follow an asymptotic behavior, with initial changes in blood constituents occurring rapidly, followed by a decrease in the rate of change (Hagan et al., 1978; Husdan et al., 1973; Stoker et al., 1966; Tan et al., 1973).

Tan and colleagues (1973) observed significant decreases in serum cholesterol, triglycerides, and hematocrit after 5 minutes of transitioning from an upright to recumbent position, and blood concentrations of these elements continued to fall until they reached a maximum decrease of about 10-12% after 20-30 minutes of recumbency. Upon return to the upright position, these changes were reversed at a similar rate. Additionally, these researchers noted that the transition from an upright to sitting position resulted in a smaller magnitude of change in blood variables than the transition from an upright to recumbent position; however, the changes followed the same asymptotic pattern that transitioning from an upright to supine position did (Tan et al., 1973). Similarly, Thompson et al. (1928) suggested that the maximum fluid loss occurs after 20-30 minutes of transitioning from the recumbent to standing positions. In alignment with these findings, Hagan et al. (1978) observed significant changes in plasma volume, hemoglobin, hematocrit, and plasma protein concentration 20 minutes after transitioning from a standing to supine position, with a stability appearing to occur 20 minutes after recumbency, as no statistically significant differences in blood constituents were observed between the 20th and 35th minutes of recumbency. However, these researchers noted that maximal fluid shifts may not yet have been accomplished even after 35 minutes of recumbency, as changes in blood variables continued to progress even after 35 minutes, albeit not significantly. In a follow-up test of 2 subjects, these researchers examined the effects of 1 hour of recumbency on fluid shifts and reported that maximal hemoconcentration occurred between 40 and 60 minutes after transitioning to the supine position. Therefore, although fluid shifts may be approaching stability 20 minutes after changing positions, complete stabilization of fluid shifts may require an hour to complete (Hagan et al., 1978). Additionally, Maw and colleagues (1995) observed a significant

5.1% decrease in plasma volume 15 minutes after transitioning from a seated to standing posture, which increased only to 6.0% after 30 minutes of standing; however, there was no significant difference in plasma volume between the 15th and 30th minute of standing. Although these authors report that it is unknown whether or not plasma volume would have continued to decrease after 30 minutes of standing, other authors have also suggested that complete stability may take at least 40 minutes to complete (Waterfield, 1931b; Youmans, Wells, Donley, Miller, & Frank, 1934).

Maw and colleagues (1995) suggested that the extent of plasma fluid shifts into the interstitial space after transitioning from a supine to standing position may be limited by the increases in both interstitial hydrostatic pressure (Aratow, Fortney, Watenpaugh, Crenshaw, & Hargens, 1993; Husmann et al., 2006) and plasma oncotic pressure (Hinghofer-Szalkay & Moser, 1986), due to fluid efflux out the plasma into the interstitial space. As the increased capillary hydrostatic pressure and plasma oncotic pressure begins to equilibrate with the increase in interstitial hydrostatic pressure after standing, further movement of fluid into the interstitium may be prevented. Therefore, this equilibration period may require at least 20 minutes to complete for complete stability to be attained (Hagan et al., 1978; Maw et al., 1995; Tan et al., 1973; Thompson et al., 1928; Waterfield, 1931b), which will result in a corresponding increase in interstitial fluid volume and pressure (Husmann et al., 2006; Maw et al., 1995).

Previous research utilizing BIA and BIS to monitor fluid shifts between extracellular and intracellular fluid with changes in position has demonstrated that extracellular water may increase, and intracellular water may decrease, when transitioning from a supine to upright position (Fenech & Jaffrin, 2004; Gibson et al., 2015; Scharfetter et al., 1997; Zhu et al., 1998).

However, researchers have suggested that these findings may be a result of fluid redistribution from the head and thorax to the lower body, which results in changes in resistance and impedance between different body parts, therefore resulting in changes in artificial changes in intracellular and extracellular fluid volumes, rather than a true movement of fluid between the extracellular and intracellular spaces because of the lack of osmotic change with changes in posture (Scharfetter et al., 1997). Despite these findings, data from these investigations show that the time course for fluid shifts to occur is similar to those directly assessing blood volume and blood constituents (Hagan et al., 1978; Maw et al., 1995; Tan et al., 1973; Thompson et al., 1928). Specifically, when transitioning from a supine to standing position, a rapid increase in extracellular fluid volume and decrease in intracellular fluid volume occurs, followed by a more progressive increase and decrease in extracellular and intracellular fluid volume, respectively (Scharfetter et al., 1997; Zhu et al., 1998). The initial rapid phase implies a redirection of venous blood from the lower body to the abdomen and head, whereas during the second phase, a reabsorption of interstitial fluid occurs (Berg et al., 1993). Gibson et al. (2015) observed significant changes in extracellular and intracellular fluid volume within only 5 minutes of transitioning from an upright to recumbent position. However, even after 30 minutes of transitioning to a new position, extracellular fluid volume was still unstable (Gibson et al., 2015). These findings align with those of other researchers (Scharfetter et al., 1997; Zhu et al., 1998), who observed that a steady state in extracellular and/or intracellular fluid volume could not be reached within 30 minutes of changing positions. Taken together, these results may indicate that fluid shifts occur within the body rapidly after transitioning to a new position, although complete redistribution of fluid may take at least 30 minutes to accomplish.

Effects of Changes in Posture on Measurements of Muscle Size and Composition

Previous research has shown that the rapid changes in blood flow and composition that occur upon moving from a supine to upright position are associated with a decrease in plasma volume and increase in interstitial fluid volume (Maw et al., 1995). Although interstitial fluid is part of the extracellular compartment, due to its anatomical extravascular location surrounding cells, an increase in interstitial fluid volume would likely result in an increase in tissue size. In accordance with this hypothesis, Waterfield (1931a) observed that 40 minutes after transitioning from a recumbent to upright position resulted in an increase in leg volume of 60-120 mL, and the greatest swelling occurred within 20-25 minutes. Similarly, Yadollahi and colleagues (2015) suggested that, upon attaining a recumbent position, rapid changes in fluid distribution and leg volume (as assessed by BIA) occurred, followed by shower changes over time. However, these researchers observed that longer periods of time may be required for complete fluid shifts to occur, as they observed that 80% of the fluid would be redistributed out of the leg after 2 hours in the supine position (Yadollahi et al., 2015). Furthermore, Berg and colleagues (1993) discovered significant decreases in calf (5.5%) and thigh (1.9%) CSA 2 hours after transitioning from a standing to supine position (as assessed by CT), which was associated with the changes in limb fluid volume in the calf (10.9%) and thigh (2.5%) (as assessed by BIA). The change in calf CSA was about 66% complete, and the change in thigh CSA was about 99% complete after 1 hour in the recumbent position. This was explained by a significant fluid loss in the calf from 60 and 120 minutes in the supine position, whereas the thigh showed no change between 60 and 120 minutes. Additionally, a significant increase in radiological density was observed in the calf after 60 minutes in the supine position, whereas a small but non-significant increase was observed in

the thigh. Radiological density refers to the ability of electromagnetic radiation to pass through certain materials, which results in varying levels of opaqueness on a radiograph. Air has a very low radiological density, appearing black on a radiograph, whereas metal has a very high radiological density, appearing white on a radiograph. Body tissues have varying levels of radiological densities, with bone appearing the whitest, followed by soft tissue, and finally fat, that appears dark on a radiograph. Therefore, radiological density of skeletal muscle is reduced in individuals with greater amounts of intramuscular fat and is increased in individuals with lower amounts of intramuscular fat (Termote, Baert, Crolla, Palmers, & Bulcke, 1980). In the investigation by Berg et al. (1993), a significant increase in radiological density of the calf after supine rest appeared to be associated with the decrease in muscle CSA, indicating that the loss of fluid with a low radiological density during supine rest had an effect on both muscle size and apparent composition. Although the change was not significant for the thigh, a small increase in radiological density was observed after 2 hours of recumbency; however, no significant differences in radiological density were observed in either the calf or the thigh between 60 and 120 minutes of supine rest. These researchers stated that the potential discrepancy in the magnitude of change between the calf and the thigh was due to a greater hydrostatic pressure in the calf than the thigh when in the erect position, therefore leading to a greater fluid accumulation in the calf. Interestingly, subcutaneous fat CSA in the thigh and calf also significantly decreased during recumbency, which is in alignment with previous research following simulated weightlessness (Hargens, 1983). Although fat has a relatively low water content, research has shown that fluid movement through adipose tissue may be equal to or greater than that in skeletal muscle (Lundvall & Lanne, 1989; Oberg & Rosell, 1967). Therefore,

rest in the supine position may not only affect muscle tissue size, but also adipose fat thickness and apparent measures of muscle composition.

In another investigation utilizing CT to assess changes in muscle size after changing positions, Cerniglia and colleagues (2007) attempted to compare thigh CSA after 5, 10, and 15 minutes of transitioning from a standing to recumbent position. They proposed that an equilibration period of 60 minutes prior to each CT scan, as supported by Berg et al. (1993), may tax important resources and potentially introduce error into the measurement of CSA, and therefore wanted to examine the changes in muscle size with shorter rest durations. Additionally, these researchers compared the time courses of changes in low-density muscle, which was characterized by a lower radiological density and therefore higher amounts of intramuscular fat and non-contractile tissue, with those of normal-density muscle, which was characterized by a higher radiological density and therefore lower amounts of intramuscular fat and non-contractile tissue. Cerniglia et al. (2007) observed a significant decrease (1.6%) in normal-density muscle when CT scans were taken after 5 minutes of supine rest compared to 15 minutes; however, no differences were observed between 5 and 10 minutes, 10 and 15 minutes, or between any time periods for low-density muscle. These researchers hypothesized that, because fat tissue is relatively anhydrous in comparison to skeletal muscle, low-density muscle, characterized by greater amounts of intramuscular fat, is likely less affected by posture changes than normaldensity muscle. Additionally, in contrast to the findings of Berg et al. (1993), Cerniglia and colleagues (2007) discovered no significant changes in SFT for any time interval. A potential explanation for the discrepancies in these findings are that the methods used to evaluate SFT in both studies differed, as Berg et al. (1993) used a manual technique to define the borders of the

areas of interest, whereas Cerniglia et al. (2007) used an automated technique based off of radiological attenuation. Another potential explanation is that fluid flux through adipose tissue may take longer than 15 minutes to occur due to the low water content of adipose tissue, despite Berg et al. (1993) reporting that fluid flux through adipose tissue may be comparable to that of skeletal muscle (Lundvall & Lanne, 1989; Oberg & Rosell, 1967). Therefore, Cerniglia et al. (2007) suggested that the potential measurement error associated with fluid shifts after transitioning from a standing to supine position in the measurement of muscle size via CT may be minimized when scans are completed within 10 minutes, as longer durations of rest result in significant changes in muscle size.

Effects of Changes in Posture on Measurements of Muscle Size and Composition Assessed via Ultrasonography

Based off of the recommendations of Berg et al. (1993) and Cerniglia et al. (2007) using CT to assess changes in muscle size with changes in body position, most previous research utilizing ultrasonography to assess muscle morphology of the lower body has encompassed a 10 to 15 minute period of supine rest prior to ultrasound imaging in order for fluid shifts to occur (Ahtiainen et al., 2010; Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014). However, because ultrasonography utilizes different technology and has different methodological considerations than CT, fluid shifts and the effects that they have on muscle morphology may appear differently on ultrasound images.

Time Course of Postural Fluid Shifts Assessed via Ultrasonography

In an investigation similar to that of Cerniglia et al. (2007), Lopez et al. (2019) examined changes in total quadriceps femoris MT and rectus femoris CSA immediately, 5 minutes, 10 minutes, and 15 minutes after transitioning from a standing to supine position. These researchers observed no change in any measures of muscle size between any time points. Similarly, Tomko et al. (2018) observed no change in the CSA of the rectus femoris 5 minutes after transitioning from a supine to seated position or 5 minutes after transitioning from a seated to supine position. In contrast to these findings, Arroyo and colleagues (2018) observed a significant decrease in VL CSA between 0 and 10 minutes and 0 and 15 minutes after transitioning from a standing to recumbent position, although these changes in CSA did not exceed the technician's standard error of measurement (SEM). However, no changes in VL MT were observed between any time points (Arroyo et al., 2018). Likewise, an investigation by Shea (2017) observed significant decrease and 30 minutes of supine rest, with no significant differences in CSA between other time points.

The discrepancy between the findings of Lopez et al. (2019), Tomko et al. (2018), Arroyo et al. (2018), and Shea (2017) may be a result of a multitude of factors. Specifically, the investigations of Lopez et al. (2019) and Tomko et al. (2018) examined the CSA of the rectus femoris, whereas the investigations of Arroyo et al. (2018) and Shea (2017) examined the CSA of the VL. Differences in muscle size, structure, origin and insertion, and function may result in different physiological responses upon postural changes. Although speculative, the larger size of the VL in comparison to the rectus femoris may partially explain the different responses of these muscles to changes in posture. The VL contains a greater amount of contractile tissue than the

rectus femoris because of its size, which therefore may provide a greater potential for fluid shifts to occur. It is also possible that the differences in muscle architecture between the VL and rectus femoris results in different responses to positional changes. The VL is a unipennate, uniarticular muscle that assists in knee extension, whereas the rectus femoris is a bipennate, biarticular muscle that assists in hip flexion and knee extension. The rectus femoris has two distinct origins, one at the anterior inferior iliac spine and the other at the ilium above the acetabulum, which converge into an aponeurosis in the center of the muscle and create its bipennate structure. It may be possible that the bipennate structure of the rectus femoris and its aponeurosis are less sensitive to fluid shifts than the VL.

Additionally, Lopez et al. (2019) suggested that the discrepancies in findings may be a result of the populations investigated, as Arroyo et al. (2018) observed significant changes in CSA in healthy, young adults (24.3 ± 3.4 y), whereas Lopez et al. (2019) did not observe changes in CSA in older adults (men: 68.1 ± 4.6 y, women: 66.8 ± 4.1 y). With aging, it is expected that sarcopenia may result in a decrease in contractile tissue within skeletal muscle and an increase in the proportion of intramuscular fat and fibrous tissue. Although speculative, the loss of muscle mass in older adults may lead to a decline in fluid storage within the muscle (Hooper, Bunn, Jimoh, & Fairweather-Tait, 2014), therefore potentially affecting the ability for fluid shifts to occur with changes in posture (Lopez et al., 2019). This conjecture aligns with the findings of Cerniglia et al. (2007), as they observed no change in the CSA of low-density muscle after 15 minutes of supine rest, which was characterized by lower contractile tissue and greater non-contractile tissue. The findings of Shea (2017) also seem to align with this hypothesis, where significant changes in CSA of the VL were observed only between 20 and 30 minutes after

recumbency, in contrast to those of Arroyo et al. (2018), who observed significant changes after 10 minutes. The subjects recruited in the investigation by Shea (2017) engaged in low amounts of physical activity, which has been shown to be related to a lower intracellular to extracellular fluid ratio (Riley et al., 1990; Wang et al., 2004). Therefore, in an untrained population, fluid shifts from the intracellular compartments to extracellular compartments may be dependent on fluid efflux capacity. However, it is unlikely that fluid shifts from the intracellular space to the extracellular space occur with postural changes because changes in plasma osmolality are not induced with changes in position (Lippi et al., 2015; Scharfetter et al., 1997; Shirreffs & Maughan, 1994), although significant changes in thigh extracellular and intracellular water were observed in the investigation of Shea (2017), as assessed via BIS. In contrast, the findings of Tomko et al. (2018) do not seem to support the population difference hypothesis because no difference in rectus femoris CSA was observed after changes in position in physically-active, young males and females. However, an important point of consideration is that CSA was only measured 5 minutes after changing position. Although fluid shifts have been shown to occur rapidly upon changes in posture (Hagan et al., 1978; Husdan et al., 1973; Stoker et al., 1966; Tan et al., 1973), Arroyo et al. (2018) did not report significant changes in VL CSA until after 10 minutes recumbency in young individuals. Therefore, a longer time frame may be necessary to observe changes in muscle CSA, specifically within the rectus femoris. Furthermore, the subjects in the Tomko et al. (2018) investigation transitioned from a seated to supine position, whereas the subjects in the investigations by Arroyo et al. (2018), Lopez et al. (2019), and Shea (2017) transitioned from a standing to supine position. Previous research has demonstrated that the transition from the supine to sitting position, or from the sitting to upright position, appears to

result in smaller fluid shifts than transitioning from the supine to standing position (Maxfield et al., 1941; Tan et al., 1973). Consequently, the lack of significant changes in rectus femoris CSA in the study by Tomko and colleagues (2018) may be partially explained by the time frame as well as type of postural change induced.

In addition to examining the time course of changes in muscle size measured via ultrasonography after supine rest, researchers have also examined changes in EI after postural transitions (Arroyo et al., 2018; Lopez et al., 2019; Shea, 2017; Tomko et al., 2018). Lopez et al. (2019) observed a significant increase in EI of the rectus femoris, vastus intermedius, and total quadriceps femoris between 0 to 5 minutes and 10 to 15 minutes of supine rest, however, no changes in the EI of the vastus medialis or VL was detected. Similarly, Arroyo et al. (2018) did not find significant changes in EI in the VL after 15 minutes of supine rest. Contradicting findings by Tomko et al. (2018) demonstrated no significant changes in rectus femoris EI after 5 minutes of supine rest, although EI was significantly increased after 5 minutes in the seated position. An important point of consideration in these investigations is that they lack correcting for possible alterations in SFT with postural changes. Previous research has suggested that an attenuation of ultrasound waves may occur in tissues that are examined at a greater depth (Pillen & van Alfen, 2011; H. J. Young et al., 2015). This attenuation can decrease the acoustic reflectivity of the ultrasound waves, therefore artificially decreasing EI values in deeper tissues (Pillen & van Alfen, 2011). With a postural change from the standing to supine position, research has demonstrated that SFT may decrease over time due to fluid shifts out of the subcutaneous layer (Berg et al., 1993; Hargens, 1983). Therefore, with all other things being equal, a decrease in only SFT should result in a decreased EI. However, with a postural change from the standing

to supine position, fluid shifts from the muscle also accompany fluid shifts from the subcutaneous layer, which results in a lower water content of the muscle, hypothetically increasing EI values. Although speculative, the EI values obtained after a postural change from the standing to supine position may, therefore, reflect the combination of the decreased thickness of subcutaneous layer and content of muscle, which both act to increase EI. Consequently, a maintenance of EI values with changes in position (Arroyo et al., 2018; Lopez et al., 2019) may indicate that fluid shifts from the muscle have the same effect on EI as fluid shifts from the subcutaneous tissue, or may indicate that fluid shifts with postural changes do not affect EI values. On the other hand, an increase in EI values during supine rest (Lopez et al., 2019) may indicate that greater fluid shifts are occurring in the muscle, relative to the subcutaneous layer. Nevertheless, the findings of Tomko et al. (2018), who observed significant increases in EI values 5 minutes after transitioning from a supine to sitting position, cannot be explained by this hypothesis, and therefore further research in this area is warranted.

In an investigation examining postural fluid shifts on both UnCorEI and CorEI, Shea (2017) observed significant increases in UnCorEI of the VL after transitioning from a standing to recumbent position, which was followed by a subsequent decline back to original values. These researchers discovered that, when EI values were corrected for SFT, older individuals (69.3 ± 8.3 y) had significantly elevated CorEI values over the first 20 minutes of supine rest, with values peaking 10 minutes after recumbency, whereas the CorEI values in younger individuals (21.4 ± 2.5 y) did not change over time. This is counterintuitive, considering that previous research has demonstrated that gravitational fluid shifts occur to a lesser extent in older individuals compared to younger individuals (Fu et al., 1999). However, these researchers attributed the group

differences to changes in SFT, as fat thickness was significantly decreased in the older group from 15 minutes to 30 of recumbency, whereas no change was observed in the younger individuals (Shea, 2017).

In the investigations mentioned above, the association between changes in muscle EI and muscle size with postural shifts does not appear to exhibit a linear relationship (Arroyo et al., 2018; Lopez et al., 2019; Shea, 2017; Tomko et al., 2018). This contradicts previous research demonstrating that fluid shifts and increases in muscle size reported in the case of muscle damage or glycogen depletion is typically associated with changes in echogenicity (J. C. Hill & Millan, 2014; Jajtner et al., 2015; Nosaka & Clarkson, 1996; Nosaka & Sakamoto, 2001; Radaelli et al., 2012). From these findings, is apparent that changes in muscle EI may not directly reflect absolute changes in muscle fluid shifts, but may be more sensitive to the rate of change in fluid within the muscle (Lopez et al., 2019), SFT (Shea, 2017), intramuscular adipose tissue content (Strasser et al., 2013), or another unknown factor.

Effects of Position on Muscle Morphology Assessed via Ultrasonography

Most previous research utilizing ultrasonography to assess skeletal muscle morphology of the lower body has been completed while the subject is recumbent on an examination table because of the ability to directly compare variables obtained from ultrasound images to those from CT, MRI, and DEXA. Ultrasounds performed in this position have demonstrated high reliability and validity in comparison to CT, MRI, and DEXA for quantification of muscle size (Ahtiainen et al., 2010; Esformes et al., 2002; Lixandrao et al., 2014; Noorkoiv et al., 2010; Reeves et al., 2004; Scott et al., 2012; Thomaes et al., 2012). Additionally, this positioning warrants few physical requirements for subject, as there is little need for balance, strength, and coordination while recumbent. The high stability of the subject may also help to provide improved reliability of variables obtained from ultrasound images. Furthermore, this positioning is easy for technician because the subject is usually placed on a table at waist-height in front of the technician, allowing for simultaneous viewing of the ultrasound monitor as the image is being captured. However, previous research has demonstrated that changes in body position (from a standing to supine position) may induce large variations in muscle morphology (Arroyo et al., 2018; Berg et al., 1993; Cerniglia et al., 2007; Lopez et al., 2019), which may change the ability for muscle morphological characteristics to predict muscle function. Ultrasonography provides greater positional versatility than CT, MRI, and DEXA; therefore, practitioners and researchers have the capability of examining muscle morphology via ultrasonography in different positions with ease.

Thoirs & English (2009) were one of the firsts to compare muscle size in different positions via ultrasonography. These researchers compared the reliability of measures of MT of 9 bilateral measurement sites in the body when participants were standing to when they were supine. They observed significantly smaller MT values in the supine position compared to standing in 7 of the 9 sites assessed, with no differences observed in the lateral forearm and subscapular measurements upon changes in position. An interesting finding in this investigation was that the two measurement sites that did not exhibit changes in MT after positional changes were located in the upper body, indicating that gravitational fluid shifts may have a greater effect on tissue size in the lower body due to increased hydrostatic pressure, which is consistent with previous research (Berg et al., 1993). In addition, these researchers indicated that, in contrast to previous research (Berg et al., 1993; Cerniglia et al., 2007), the time spent in recumbency did not have an effect on MT measurements, as there were no significant differences between those taken immediately after laying down and 1 hour later. However, it was noted that the total assessment protocol required approximately 15 minutes to perform, and therefore, fluid shifts may have already occurred within the muscle by the time the first round of assessments in the supine position were complete. This aligns with previous research showing that the greatest fluid shifts occur within the first few minutes of recumbency (Berg et al., 1993; Cerniglia et al., 2007; Hagan et al., 1978; Husdan et al., 1973; Stoker et al., 1966; Tan et al., 1973). Nevertheless, measures of MT in both the standing and supine positions exhibited high test-retest reliability; however, this also may be a factor of the longer total assessment time and the stabilization of fluid shifts that were allowed to occur (Thoirs & English, 2009).

In another investigation examining the influence of position on muscle morphology, Tomko et al. (2018) observed a significantly greater CSA of the rectus femoris when subjects were in a seated position compared to a supine position. These findings align with those of Thoirs & English (2009) who found significantly greater anterior thigh musculature thickness while standing compared to laying down. Although postural fluid shifts may account for much of the change in muscle size during positional changes, a simple change in joint position may also have resulted in significant changes in rectus femoris muscle morphology (Hacker et al., 2016; Maganaris, 2001; Myers et al., 2013; Narici et al., 1996). Because the rectus femoris is a biarticular muscle that crosses both the hip and knee joint and aids in knee extension and hip flexion, a change in either hip or knee angle may result in changes in muscle shortening, and therefore, muscle size. For example, Hacker and colleagues (2016) demonstrated that when the knee is kept at full extension, rectus femoris CSA significantly increases for every 20° decrease in hip angle, which can be attributed to a shortening of the muscle. More specifically, the rectus femoris is maximally shortened when the hip is flexed and the knee is extended, and is maximally lengthened when the hip is extended and the knee is flexed. However, Tomko et al. (2018) reported that the rectus femoris is shortened in the sitting position when both the knee and hip are flexed and is lengthened in the supine position when the knee and hip are extended. This assumption was based on an investigation reporting that the rectus femoris is shortened while in a seated position compared to a recumbent position (Maffiuletti & Lepers, 2003); however, in this study, knee flexion was fixed at 90° during both the sitting and recumbent positions. In this case, the transition from a sitting to supine position undoubtedly lengthens the rectus femoris, as the knee angle is kept consistent and the hip is extended (Maffiuletti & Lepers, 2003). In contrast, the investigation by Tomko et al. (2018) utilized a fixed knee flexion of 90° during the sitting position, which was extended to 180° during the supine position. In this study, the transition from a sitting to supine position results in both knee and hip extension, therefore resulting in a simultaneous shortening and lengthening of the rectus femoris, potentially resulting in negligible changes in overall muscle length (Tomko et al., 2018). Consequently, while it is possible that changes in joint position resulted in changes in CSA of the rectus femoris, the overall shortening of the muscle in the sitting position compared to the supine position may be insignificant, as an increase in hip flexion is also coupled with an increase in knee flexion. A more plausible explanation for the differences in muscle size observed in this investigation may be a result of the change in position and shape of muscles during changes in joint angle and posture (Thoirs & English, 2009). Although all measurements of the muscle were obtained at the

same marked sites, it is possible that these sites marked on the skin were not consistent with the same locations on the underlying muscle following positional changes, therefore potentially affecting muscle size and EI (Thoirs & English, 2009). It was noted that the shape of the rectus femoris appeared flatter in the supine than seated images, indicating that changes in joint position can result in changes in underlying muscle location and shape (Tomko et al., 2018). However, the differences in muscle size may also be attributed to postural fluid shifts, which have been shown to occur with transitioning from a supine to seated position (Maw et al., 1995).

In addition to the differences in CSA between different positions, Tomko et al. (2018) observed a significantly lower EI in the seated position compared to the standing position. The authors suggest that this difference may be attributed to the alterations in the shape and curvature of the thigh upon positional changes (Tomko et al., 2018), where the researchers noted that the rectus femoris appeared flatter in the supine position compared to the seated position (Tomko et al., 2018). This caused the deep aponeurosis of the muscle to be located more proximally to the ultrasound probe, which may have reduced the attenuation of sound waves that occurs with deeper tissues (Pillen & van Alfen, 2011). Previous research has suggested that, when ultrasound settings are kept constant, tissues that are examined at a greater depth experience a greater attenuation of sound waves, and thus lower EI (Pillen & van Alfen, 2011; H. J. Young et al., 2015). Tomko et al. (2018) observed significantly greater EI values in the supine compared to seated positions, which may be due to a reduced attenuation in the supine position. Nevertheless, the potential for postural fluid shifts to affect EI values along with CSA cannot be discounted. Furthermore, these researchers indicated that the test-retest reliability of CSA and EI measured in

both positions and after all time points was high and did not depend on the position or time at which the images were taken (Tomko et al., 2018).

Effects of Position on the Relationship between Muscle Morphology Assessed via Ultrasonography and Performance

There has been a recent research interest in the use of ultrasonography as a way to evaluate skeletal muscle morphology in an attempt to predict athletic performance and muscle function. Most of these studies have examined muscle morphology while subjects remain in the recumbent position (Jajtner et al., 2015; Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014), however this positioning induces a discrepancy between the position in which the muscles are assessed and the position in which many sporting activities occur (Wagle et al., 2017). Previous research has shown that muscle morphological characteristics obtained via ultrasonography differ depending on position (Thoirs & English, 2009; Tomko et al., 2018), however these changes in characteristics do not reflect true changes in muscle function. Therefore, the ability of characteristics to predict muscle function during athletic activities may be compromised.

An investigation by Wagle et al. (2017) compared the relationships between muscle morphology of the VL assessed in both the recumbent and standing positions to lower-body force production. These researchers observed significantly greater measures of muscle size and architecture, including MT, CSA, and PA during the standing position in comparison to the recumbent position, which is in alignment with previous research (Thoirs & English, 2009; Tomko et al., 2018). However, the magnitude of change in muscle size was greater for MT than for CSA, indicating that changes in the size of the muscle belly may be greater than those throughout the entire muscle area. This was reported as a potential result of muscle gearing, where muscle fibers shorten in the longitudinal direction and expand in the transverse direction, causing them to rotate to a greater PA, thus creating a bulging effect in the center of the muscle (Azizi & Brainerd, 2007; Wakeling & Randhawa, 2014). Muscle gearing is typically reported in the case of muscle contraction, when a change in the length of the muscle is induced (Azizi & Brainerd, 2007); however, it is apparent that changes in position can create a similar musclebulging effect due to the influence of gravity on muscle shape (Thoirs & English, 2009; Tomko et al., 2018). In addition, standing measures of muscle size exhibited overall stronger relationships with performance than the lying measurements. Specifically, standing CSA and MT yielded stronger relationships with isometric squat peak force (PF), RFD, and impulse (IMP) than lying measurements. Standing CSA was also more strongly associated with 1-repetition maximum (1-RM) squat than lying CSA; however, MT values in the different positions yielded similar correlations. Furthermore, muscle PA was also significantly greater in the standing compared to recumbent position, and standing measures of PA exhibited overall stronger relationships with performance than the lying measurements except for 1-RM squat. These findings may indicate that the strengths of the relationships between standing and recumbent muscle morphology and force production may be dependent on the type of test administered. Measures of standing muscle size exhibited stronger relationships with all isometric variables than lying measurements; however, this positional relationship difference subsided during dynamic 1-RM squats. Dynamic activities involve changes in joint angle, muscle length, and

force-producing capabilities throughout the entire movement, whereas isometric activities involve force production at a constant joint angle and muscle length. Due to the influence of joint angle and muscle length on muscle size (Hacker et al., 2016; Maganaris, 2001; Myers et al., 2013; Narici et al., 1996), the ability of muscle morphology to predict performance may be related to the position in which the muscle is analyzed. This may explain why stronger relationships were observed between standing muscle characteristics and isometric squat variables, as the isometric squat was performed in the upright position with no change in joint angle.

A Proposition for Further Exploration

Although there is research to support the use of standing measures of muscle morphology in the assessment of muscle function (Wagle et al., 2017), this positioning requires an additional level of difficulty on the subject as well as the technician, which may be unappealing for its use in research settings. Additionally, some limitations to the investigation of Wagle et al. (2017) should not be discounted. For example, all strength assessments in this investigation involved the use of both limbs; however, muscle morphology was assessed only in the right VL muscle, without consideration of leg dominance on performance measures. Additionally, the duration of time in each position was not reported, and this has been demonstrated to affect muscle morphology of the VL (Arroyo et al., 2018; Shea, 2017). Although the authors report that joint angle was standardized for both the standing and recumbent positions, the standing position did not involve resting the leg against a device or plinth, and therefore some degree of muscle contraction may have been required to retain the joint angle during the assessment (Wagle et al., 2017). Also, despite the report of the interpretation of the magnitude of correlation coefficients between muscle morphology and performance, over 60% of the correlations did not reach statistical significance; thus the results should be reported with caution.

Traditional ultrasonography in the assessment of muscle morphology of the lower body typically reports that subject are required to lay on an examination table for a period of 10 to 15 minutes in the supine position (Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014) to allow for fluid shifts to occur when transitioning to a new position (Berg et al., 1993; Cerniglia et al., 2007). However, ultrasound assessment of certain muscles requires subjects to lay in other recumbent positions during examination, which induces a discrepancy between the rest position and the position in which the muscle is examined. For example, the VL is a muscle that is commonly examined during ultrasonography in the evaluation of lower body strength and power due to its extensive involvement in knee extension, its larger size in comparison to the other quadriceps muscles, and its superficial location. However, due to the anatomical position of this muscle on the lateral side of the body, ultrasound assessment of the VL requires the subject to lay on their lateral side and not in the supine position. Previous research has shown that differences in hydrostatic pressure and blood distribution as a result of changing from a standing to recumbent position result in fluid shifts to tissues of the lower body, thereby increasing muscle size and altering muscle composition (Berg et al., 1993; Cerniglia et al., 2007; Maw et al., 1995). Because changes in hydrostatic pressure and blood distribution may also be induced with changes in recumbent positions (Bryan, 1974; Kallet, 2015), a change in
position (from rest in a supine position to examination in a lateral recumbent position) may alter muscle morphological characteristics, which may not reflect true changes in muscle function.

Furthermore, the use of ultrasonography in the examination of bilateral asymmetries in muscle size and composition is an important component in the evaluation of athletic performance (Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Sanders, Boos, Shipley, & Peacock, 2018). However, if the examination of the VL muscle on the opposing limb to detect for bilateral asymmetries is desired, the subject must flip over to the opposite lateral recumbent side. Previous reports of bilateral differences in muscle morphology of the VL do not report a return to the supine position prior to examination of the opposing muscle (J. C. Hill & Millan, 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Sanders et al., 2018). Therefore, the leg that was previously compressed against the examination table is examined without the potential for fluid shifts to occur in another plane. Previous research has suggested that muscle compression may reduce blood flow, swelling, and muscle size during and after exercise (J. Hill, Howatson, van Someren, Leeder, & Pedlar, 2014; Kraemer et al., 2010; Kraemer et al., 2000; Sperlich, Born, Kaskinoro, Kalliokoski, & Laaksonen, 2013). Additionally, muscle compression has been shown to reduce venous pooling in the lower body when standing (Mills, Scurr, & Wood, 2011; Partsch, Flour, Smith, & International Compression, 2008; Redolfi, Arnulf, Pottier, Bradley, & Similowski, 2011). Investigations involving ultrasonography assessments of the lower body have previously reported that subjects should avoid wearing tight shorts to reduce compression of the thigh musculature (Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014). Compression of a tissue increases the interstitial hydrostatic pressure, which reduces filtration of fluid out of the capillaries, therefore minimizing changes in muscle size (Nehler et al., 1993).

Although these assumptions are primarily concerned with the use of compression garments, it remains unknown whether a similar effect would occur following compression of a limb under the weight of the body. Therefore, it is possible that changes in recumbent positions affect muscle morphological characteristics of the lower body. Further, if differences in muscle morphology exist after rapid changes in body position, this may affect the ability for these characteristics to predict muscle function.

CHAPTER THREE: RESEARCH DESIGN AND METHODOLOGY

Experimental Design

Participants reported to the Human Performance Laboratory at the University of Central Florida on three separate occasions. During visit one (T1), participants completed a written form of consent, an MHAQ, and a PAR-Q+ to establish eligibility. During visit 2 (T2), participants underwent a familiarization session with all physical performance assessments to minimize any learning effect of the assessments on outcome variables. At least 72 hours after T2, participants visited the laboratory for their final visit (T3), which consisted of hydration status assessment, anthropometric testing, body composition assessment, ultrasound assessments, and physical performance testing. A depiction of all visits to the laboratory and associated assessments is presented in Figure 1.



Figure 1: Study Design

Timeline of study procedures. PAR-Q+: Physical activity readiness questionnaire; MHAQ: Medical history and activity questionnaire; RM: Repetition maximum; MVIC: Maximal voluntary isometric contraction

Participants

Thirty-five recreationally-active males between the ages of 18 and 35 years old were recruited for this study. Participants were instructed to maintain normal dietary and exercise habits throughout the duration of enrollment in the study. Following an explanation of all procedures, risks, and benefits, each participant provided their written informed consent to participate (T1). All participants were required to be free of any physical limitations (as determined by the MHAQ and PAR-Q+) and were deemed as resistance-trained, having participated in resistance training at least three times per week for at least the previous year. Furthermore, participants were required to be non-smokers and be free from previous use of any performance-enhancing drugs. All performance assessments and ultrasounds were performed on the dominant leg, which was designated by each participant. This investigation was approved by the University of Central Florida Institutional Review Board for human subjects, and all procedures were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments.

Procedures

Familiarization (T2)

During T2, participants were familiarized with all of the physical performance assessments used in this investigation to minimize any learning effect of the assessments on dependent outcome variables. Prior to all physical performance assessments, participants were required to complete a standardized dynamic warm-up including: pedaling on a cycle ergometer for 5 minutes at a self-selected pace, 10 body-weight squats, 10 body-weight walking lunges, 10 dynamic walking hamstring stretches, 10 dynamic walking quadriceps stretches, 10 squat jumps, 10 arm circles, and 10 arm swings.

Unilateral Vertical Jump Assessment (UVJ)

During T2, participants became familiar with performing maximal unilateral vertical jumps (UVJ). Participants were instructed to stand on a force plate (AccuPower, AMTI Watertown, MA, USA, 1000 Hz) with their hands placed on their hips throughout the duration of each UVJ. Participants were instructed to stand on one leg and perform a maximal countermovement jump. Participants were instructed on proper landing mechanics (e.g., no tucking) as not to affect flight time. To decrease the risk of injury, participants were instructed that they could land on two legs. A series of maximal UVJ were completed until the participant felt comfortable with the exercise.

Isometric and Isokinetic Knee Extension Assessments

Participants were seated in an isokinetic dynamometer (S4, Biodex Medical System, Inc., New York, NY, USA), strapped into the chair at the waist, shoulders, and across the thigh to complete a series of isometric and isokinetic strength assessments. Chair and dynamometer settings were adjusted for each participant to properly align the axis of rotation with the lateral condyle of the femur. All participants were tested on their dominant leg, which was secured to the dynamometer arm just above the medial and lateral malleoli. The range of motion was assessed for each participant, and isokinetic dynamometer settings for each participant were consistent throughout testing. Participants first completed isometric and isokinetic warm-up sets at 50% of their perceived maximum. The isometric warm-up sets were performed while the knee remained positioned at angle of 110°, considering full extension is 180°. The isometric warm-up sets consisted of three consecutive repetitions of leg extension, which incorporated 10 seconds of contraction, with a 10 second rest in between each repetition. Participants then completed an isokinetic warm-up, consisting of three sets of three isokinetic contractions of the knee extensor muscles at different angular velocities ($60^{\circ} \cdot s^{-1}$, $180^{\circ} \cdot s^{-1}$, and $240^{\circ} \cdot s^{-1}$). Each isokinetic set consisted of concentric knee extension and passive knee flexion. Sixty-seconds of rest were provided between each set, and 3 minutes of rest were provided after the last set.

Participants were then instructed to perform two maximal voluntary isometric contractions (MVIC) at a knee angle of 110°, which was held for 6 seconds. Additionally, participants then performed three sets of three isokinetic contractions at different angular velocities $(60^{\circ} \cdot s^{-1}, 180^{\circ} \cdot s^{-1})$, and $240^{\circ} \cdot s^{-1}$). Participants were required to achieve an acceptable range of motion (~90°-170°) from to knee flexion to extension for each contraction. Between MVIC and isokinetic testing sets, 3 minutes of rest were provided to each participant.

Unilateral Leg Press Assessment

Participants were seated in a unilateral leg press machine (Uni/Bi-Lateral Leg Press, PowerLift, Jefferson, IA) and were familiarized with the unilateral leg press assessment. Participants were provided with proper instruction and technique for optimal exercise form and were instructed to complete unilateral leg presses with the dominant leg only. The seat position on the leg press apparatus was kept consistent for each participant for all testing days. Due to the unfamiliarity of a 1-RM unilateral leg press, participants underwent a 3-RM protocol on T2, which was then used to predict their 1-RM for T3. Each participant performed three warm-up sets before attempting a 3-RM lift. Following each warm-up set, additional weight was added to the leg-press based upon the subject's perceived difficulty. Repetition ranges for each of the three warm-up sets were 8-10 repetitions, 6-8 repetitions, and 4-6 repetitions, followed by 1, 2, and 3 minute rest periods, respectively. Following the warm-up sets, additional weight was added to the leg-press, and a 3-RM was attempted. Two to four subsequent trials were performed to determine each participant's 3-RM. Trials not meeting the range of motion criteria for each exercise were discarded. Each participant's 1-RM was then predicted using Equation 2 (Brzycki, 1993) for use during T3.

$$1 - RM = Weight / [1.0278 - (0.0278 \cdot Number of Repetitions)]$$
(2)

Testing Day (T3)

After a period of at least 72 hours, participants returned to the laboratory for their testing visit (T3), which consisted of a hydration status assessment, anthropometric and body composition testing, ultrasound assessments, and physical performance testing. Participants were instructed to wear loose-fitting shorts during T3 to avoid compression of the upper leg musculature. All participants were required to have refrained from vigorous lower-body exercise for 72 hours prior to the testing visit and from consuming alcohol and caffeine for at least 24 hours prior to the testing visit. Participants were required to arrive for T3 in a hydrated state and having been fasted for a period of 4 hours. A standardized snack (total energy: 260 kcal; carbohydrates: 48 g; protein: 3.1 ± 0.7 g; fat: 6 g) was provided to all participants after the ultrasound assessments and before physical performance assessments were completed.

Hydration Status Assessment

Hydration status was assessed upon arrival at T3 to ensure that each participant was in a state of euhydration prior to testing. Each participant was asked to provide a urine sample in a sterile container. Urine samples were analyzed for hydration status via refractometry by placing a drop of urine on a refractometer (Human Urine Refractometer, MISCO Refractometer, Cleveland, OH, USA) and digitally inspecting its osmolarity. Participants were considered euhydrated if the osmolarity of the urine was less than or equal to 1.020. If the participant was not properly hydrated at the time of assessment, they were asked to drink water and provide another urine sample until properly hydrated. Participants could not continue with the assessments until properly hydrated.

Anthropometric and Body Composition Assessments

After the participant was confirmed to be in a state of euhydration, participants were asked to remove their footwear, socks, and jewelry. Body mass (±0.1 kg) and height (±0.1 cm) were assessed using a Health-O-Meter Professional scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Body composition (percent body fat, fat-free mass) was assessed via multi-frequency BIA (InBody770, InBody, Cerritos, CA), as previously described (Arroyo et al., 2017). Participants were asked to stand on the platform with their heels placed on the circular rear sole electrode and the forefoot on the front sole electrode. Participants then picked up the handles of the BIA device, ensuring that the surface of the hand electrode was placed in contact with each of the five fingers. The BIA device then sent a small electrical current through the

electrodes, which was transferred into the body in order to calculate the proportion of lean tissue to fat mass contained within the body.

Ultrasound Assessments

Ultrasound Technical Procedures

The ultrasound imaging techniques utilized in this investigation to assess the VL muscle have been previously described (Jajtner et al., 2015; Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014). However, due to the primary research question of this investigation, the rest position that was utilized prior to ultrasound image capture was altered to examine the effects of the rest position on ultrasound characteristics. All anatomical locations of interest were identified using standardized landmarks for the VL muscle in the participants' selfreported dominant limb. The landmarks for the VL were identified along the longitudinal distance over the femur at 50% of the distance from the greater trochanter to the lateral border of the patella (Scanlon et al., 2014; Wells et al., 2014). To ensure proper probe placement and consistent image capture location, a semi-permanent marker was used to draw a dotted line transversely and longitudinally along the surface of the skin at the aforementioned location. The anatomical measurements for the VL were taken prior to anthropometric measurements on T3 to minimize the effect of time required to mark the leg during ultrasound assessment. All measures of muscle morphology were obtained using a B-mode, 12-MHz linear probe (General Electric LOGIQ P5, Wauwatosa, WI, USA), coated with transmission gel (AquasonicVR 100, Parker Laboratories, Fairfield, NJ, USA) to provide acoustic contact without depressing the dermal

layer of the skin (Scanlon et al., 2014; Wells et al., 2014). Ultrasound settings remained fixed for examination of each participant to minimize instrumentation bias, to optimize spatial resolution, and to ensure consistency (Scanlon et al., 2014; Wells et al., 2014). Image gain was set at 50 dB, dynamic range was set at 72, and image depth was set at 5 cm. Ultrasound images were captured in the transverse and sagittal planes, utilizing panoramic and still imaging (Figures 2 and 3, respectively). For each round of assessment, three panoramic images were captured in the transverse plane, perpendicular to the long axis of the muscle. Extended-field-of-view ultrasonography (LogiqView[™]) was used to capture panoramic images, which utilized a sweep of the probe along the VL from the anterior portion of the muscle to the posterior portion of the muscle in order to capture the entire area of the muscle in a single image. Additionally, three still images were captured in the sagittal plane, parallel to the long axis of the muscle (Varanoske et al., 2017b). All ultrasound assessments were performed by the same examiner and were captured from the same anatomical locations.



Figure 2: Panoramic Ultrasound Image Assessment

A sample participant laying down for panoramic ultrasound image assessments. Panoramic images were captured in the transverse plane, perpendicular to the force-generating axis of the muscle, using extended-field-of-view ultrasonography (LogiqViewTM). The yellow box represents the probe head orientation, and the solid yellow line represents the direction of probe manipulation along the leg during image capture.



Figure 3: Still Ultrasound Image Assessment

A sample participant laying down for still ultrasound image assessments. Still images were captured in the sagittal plane, parallel to the force-generating axis of the muscle, using a still image. The yellow box represents the probe head orientation and image capture location.

Each participant underwent five rounds of non-invasive ultrasound assessment of the VL in the dominant leg. In the first assessment, participants were positioned on an examination table on the non-dominant side in the non-dominant lateral recumbent position. Participants were required to have their legs stacked together, and a foam pad was placed between their ankles. The legs were positioned to allow a 10° bend in the knees, as measured by a goniometer. Ultrasound images of the VL were captured immediately after the participant was positioned (IP). IP assessments took, on average, 113.9 ± 12.6 seconds to complete. In the next assessment, participants were instructed to remain in the non-dominant lateral recumbent (NDLR) position for a period of 15 minutes. After the 15-minute duration had elapsed, additional ultrasound images were captured. Following the first two rounds of ultrasound assessments, each participant was asked to stand for a period of 15 minutes. After the 15-minute duration elapsed, participants were asked to lay supine (SUP) on an examination table for a period of 15 minutes. After the 15minute duration elapsed, participants were instructed to quickly flip over onto their nondominant side for another round of ultrasound assessments. Following the third round of assessments, the participant was asked to stand for another period of 15 minutes. After the 15minute duration elapsed, participants were asked to lay on an examination table in the dominant lateral recumbent (DLR) position for a period of 15 minutes. After this 15-minute duration elapsed, participants were instructed to quickly flip over onto their non-dominant side for another round of ultrasound assessments. Following the fourth round of assessments, participants were asked to stand for a period of 15 minutes. After the 15-minute duration elapsed, participants were asked to stand on an elevated platform to obtain standing ultrasound images. Participants were instructed to bear weight only on their non-dominant limb, while the shin of the dominant limb

rested against a higher platform to allow for a 10° bend in the knee. Participants were instructed to completely relax the dominant leg against the higher limb to avoid muscle contraction of the VL. Ultrasound images were captured while participants remained in the standing position (ST), and were identical to those used during the recumbent positions. The order of all assessments except ST were randomized for each participant. Ultrasound rest positions are depicted in Figures 4a-d.



Figure 4: Ultrasound Rest Positions Utilized

An example participant in different rest positions prior to ultrasound analysis of the vastus lateralis (VL) muscle. A: Participant laying in the non-dominant lateral recumbent (NDLR) position with the dominant limb exposed. This was the position that all ultrasound images were captured in (except for standing), however, the rest position utilized beforehand differed. The participant utilized this rest position for immediately post (IP) analysis and rest for 15 minutes in the NDLR position. B: Participant laying in the supine (SUP) position. After 15 minutes in this position, the participant was instructed to flip over to the NDLR position and an ultrasound image was captured immediately following. C: Participant laying in the dominant lateral recumbent (DLR) position and an ultrasound image was captured immediately following. D: Participant was instructed to flip over to the NDLR position and an ultrasound image was captured immediately following. D: Participant in the standing (ST) position. The ultrasound images were captured while the participant remained standing. Participants were instructed to bear weight on the non-dominant leg while resting the dominant leg against a platform to allow for a bend in the knee.

Ultrasound Image Analysis

All ultrasound images were analyzed offline by an experienced researcher using an image analysis software (ImageJ, National Institutes of Health, USA, version 1.45s) to quantify muscle morphological characteristics. A known distance shown in each ultrasound image was used to calibrate the image analysis software.

Cross-Sectional Area (CSA) Quantification

CSA of the VL was quantified using panoramic images captured in the transverse plane. The outline of the VL was located in each image and was traced using the polygon function tool in ImageJ, ensuring to include as much lean tissue as possible without including any surrounding bone or fascia (Wells et al., 2014). The total area of each traced polygon was then calculated and reported in centimeters². The average CSA of the three images taken in each rest position was then used for further analysis. A sample image for CSA analysis is presented in Figure 5.

Inter-day reliability for the quantification of the CSA of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at least 24 hours between examinations. The intraclass correlation coefficient using model "3,1" (ICC_{3,1}), SEM, minimal difference (MD), and coefficient of variation (CV) for CSA between ultrasound images taken on two separate days were determined to be: ICC_{3,1} = 0.997; SEM = 0.423 cm^2 ; MD = 1.173 cm^2 ; CV = 1.027%.



Figure 5: Cross-Sectional Area (CSA) Quantification

A sample panoramic ultrasound image of the vastus lateralis (VL) captured in the transverse plane used for CSA analysis. The outline of the VL muscle was located in the image and traced using the polygon function tool in ImageJ, which included as much lean mass as possible without including any surrounding bone, muscle, or fascia. The CSA value is highlighted in red and recorded in centimeters² (cm²).

Uncorrected Echo Intensity (UnCorEI) Quantification

UnCorEI was quantified within the region of interest previously demarcated for CSA determination. UnCorEI of the traced polygon was determined using the standard histogram function in ImageJ. Quantification of the grayscale of each individual pixel in the region of interest was expressed as a value between 0 and 255 arbitrary units (AU) (0: black; 255: white) (Pillen & van Alfen, 2011; Scanlon et al., 2014; Wells et al., 2014). The grayscale of each individual pixel was then projected on a histogram plot, and UnCorEI was quantified as the mean grayscale of the entire region of interest (Pillen & van Alfen, 2011; Scanlon et al., 2014). The average UnCorEI of the three images taken in each rest position was then used for further analysis. A sample image for UnCorEI analysis is presented in Figure 6.

Inter-day reliability for the quantification of UnCorEI of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at least 24 hours between examinations. Reliability values were determined to be: $ICC_{3,1} = 0.935$; SEM = 3.679 AU; MD = 10.199 AU; CV = 5.509%.



Figure 6: Uncorrected Echo Intensity (UnCorEI) Quantification

A sample panoramic ultrasound image of the vastus lateralis (VL) captured in the transverse plane used for UnCorEI analysis. The same region of interest used for cross-sectional area (CSA) analysis is again used for UnCorEI analysis. The UnCorEI value is highlighted in red and recorded in arbitrary units (AU).

Subcutaneous Adipose Tissue Thickness (SFT) Quantification

In order to examine the potential influence of SFT (SFT) on EI, SFT superficial to the VL was assessed in the images previously used for CSA and UnCorEI quantification. SFT is defined as the perpendicular distance between the inferior border of the epithelium and the superior border of the superficial aponeurosis (H. J. Young et al., 2015). Quantification of SFT was determined as the average SFT adjacent to the lateral, mid-line, and medial portions of the VL using the line tool in ImageJ and is reported in centimeters (E. D. Ryan et al., 2016; H. J. Young et al., 2015). The average SFT of the three images taken in each rest position was then used for further analysis. A sample image for SFT analysis is presented in Figure 7.

Inter-day reliability for the quantification of SFT of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at least 24 hours between examinations. Reliability values were determined to be: $ICC_{3,1} = 0.999$; SEM = 0.022 cm; MD = 0.061 cm; CV = 3.044%.



Figure 7: Subcutaneous Adipose Tissue Thickness (SFT) Quantification

A sample panoramic ultrasound image of the vastus lateralis (VL) captured in the transverse plane used for SFT analysis. SFT is defined as the distance between the inferior border of the epithelium and the superior border of the superficial aponeurosis. SFT was determined as the average of the lateral, mid-line and medial SFT values, highlighted as the yellow lines in the above image. One SFT value is highlighted in red and recorded in centimeters (cm).

Corrected Echo Intensity (CorEI) Quantification

In order to examine the effect of SFT on EI, the UnCorEI values for each panoramic image were then corrected for SFT (averaged from the SFT at the medial, mid-line, and lateral portions of the muscle) using Equation 3 previously established by Young et al. (2015):

$$Corrected EI = Uncorrected EI + (SFT \times 40.5278)$$
(3)

The average corrected EI (CorEI) values of the three images taken in each rest position was then used for further analysis.

Inter-day reliability for the quantification of CorEI of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at least 24 hours between examinations. Reliability values were determined to be: $ICC_{3,1} = 0.980$; SEM = 4.308 AU; MD = 11.942 AU; CV = 4.747%.

Muscle Thickness (MT) Quantification

MT was assessed using still images captured in the sagittal plane. MT was measured as the perpendicular distance from the superficial aponeurosis to the deep aponeurosis (Mangine et al., 2015). MT was quantified using the straight-line tool in ImageJ at 50% of the horizontal distance of the image length and was reported in centimeters (Figure 3). The average MT of the three images taken in each rest position was then used for further analysis. A sample image for MT analysis is presented in Figure 8.

Inter-day reliability for the quantification of MT of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at

least 24 hours between examinations. Reliability values were determined to be: $ICC_{3,1} = 0.995$; SEM = 0.029 cm; MD = 0.081 cm; CV = 1.071%.



Figure 8: Muscle Thickness (MT) Quantification

A sample still image of the vastus lateralis (VL) captured in the sagittal plane with MT measurement highlighted in yellow. MT is defined as the distance between the inferior border of the superficial aponeurosis and the superior border of the deep aponeurosis. MT was quantified using the line tool at the midpoint of the horizontal distance between the left and right sides of the vastus lateralis (VL). The MT value is highlighted in red and reported in centimeters (cm).

Pennation Angle (PA) Quantification

PA was assessed using the same images that were used for MT quantification. PA is defined as the angle of the intersection of the fascicles with the deep aponeurosis. PA was quantified using the angle tool in ImageJ and is reported in degrees (°). The PA of three fascicles was measured in each image, and the average of the three were used for that image. The average PA of the three images taken in each rest position was then used for further analysis. A sample image for PA analysis is presented in Figure 9.

Inter-day reliability for the quantification of PA of the VL using ultrasonography following rest in the SUP position were completed on a separate sample of participants, with at least 24 hours between examinations. Reliability values were determined to be: $ICC_{3,1} = 0.998$; $SEM = 0.272^\circ$; $MD = 0.754^\circ$; CV = 2.103%.



Figure 9: Pennation Angle (PA) Quantification

A sample still image of the vastus lateralis (VL) captured in the sagittal plane with PA measurement highlighted in yellow. PA was quantified using the angle tool at the intersection of the fascicles with the deep aponeurosis. The PA value is highlighted in red and reported in degrees (°).

Physical Performance Assessments

Prior to all physical performance assessments, participants were required to complete a standardized dynamic warm-up including: pedaling on a cycle ergometer for 5 minutes at a self-selected pace, 10 body-weight squats, 10 body-weight walking lunges, 10 dynamic walking hamstring stretches, 10 dynamic walking quadriceps stretches, 10 squat jumps, 10 arm circles, and 10 arm swings. Each participant then performed the same physical performance assessments that they completed on the familiarization day, with minor adjustments. All physical performance assessments were administered to each participant by the same researcher. Instructions for each assessment were provided to the participants through reciting a script. Verbal encouragement was given during each physical performance assessment. All assessments were supervised by a Certified Strength and Conditioning Specialist (CSCS) through the National Strength and Conditioning Association (NSCA).

Unilateral Vertical Jump (UVJ) Assessment

Participants were instructed to stand on the force plate on their dominant leg, with their hands placed on their hips. Participants performed a total of three maximal UVJ, with 3 minutes of rest between each jump. Participants were instructed that they could land on two feet if they preferred. Flight time was calculated as the time interval from toe-off to landing, and UVJ height was calculated using flight time. Furthermore, PF was measured, and peak power, the rate of power development (RPD), total work, and peak velocity were calculated for each UVJ. The greatest values for each variable from the three UVJ were then used for further analysis. An example participant completing UVJ assessment is presented in Figure 10.



Figure 10: Unilateral Vertical Jump (UVJ) Assessment

An example participant completing UVJ assessment. Three total countermovement jumps were completed on the dominant leg, while the participants' hands remained on their hips throughout the duration of the UVJ. Each UVJ was separated by three minutes of rest.

Isometric and Isokinetic Knee Extension Assessments

Following the UVJ assessment, participants underwent the same unilateral isometric and isokinetic testing protocol that was completed during the familiarization day. An example participant completing isometric and isokinetic assessments is presented in Figure 11.



Figure 11: Isometric and Isokinetic Knee Extension Assessments

An example participant completing unilateral isometric and isokinetic knee extension assessments. Two maximal voluntary isometric contractions (MVIC) were performed, with three minutes of rest between each MVIC. Three maximal voluntary isokinetic contractions were performed at different angular velocities $(60^{\circ} \cdot s^{-1}, 180^{\circ} \cdot s^{-1})$, with three minutes of rest between each set.

For each test, torque signals were sampled at 1 kHz with a data acquisition system (MP150 BIOPAC Systems, Inc., Santa Barbara, CA, USA), recorded on a personal computer, and processed offline. For each MVIC, a torque-time curve was created. Due to the influence of dynamometer arm length on torque, a correction was applied to the torque values to independently examine the effects of muscle morphology in different rest positions on isometric and isokinetic force. The torque values obtained from the isokinetic dynamometer were divided by the dynamometer arm length setting of the Biodex for each participant to account for the influence of moment arm on torque (Equation 4).

$$Torque = Moment Arm \times Force \tag{4}$$

Therefore, muscle force production was examined after accounting for dynamometer arm setting length. For each MVIC, the onset of torque was determined when the torque signal crossed the value equal to 10% above the baseline. PF, RFD over 50 ms (RFD50), 100 ms (RFD100), 200 ms (RFD200), and impulse over 50 ms (IMP50), 100 ms (IMP100), and 200 ms (IMP200) were recorded for each MVIC. PF was identified as the greatest force achieved on the force-time curve for each repetition. RFD was defined as the greatest rate of change of force development over time between sampled data points. IMP was defined as the average force generated over time. For each set of isokinetic kicks, PF was recorded: isokinetic PF at $60^{\circ} \cdot s^{-1}$ [IsokPF ($60^{\circ} \cdot s^{-1}$)], isokinetic PF at $180^{\circ} \cdot s^{-1}$ [IsokPF ($180^{\circ} \cdot s^{-1}$)], and isokinetic PF at $240^{\circ} \cdot s^{-1}$ [IsokPF ($240^{\circ} \cdot s^{-1}$)]. The greatest values for each variable was used for further analysis.

In support of the correction for moment arm in the examination of muscle morphology and its relationship with force production, Biodex dynamometer arm length was found to be a significant predictor of performance on all isometric and isokinetic torque variables [peak torque (PT), rate of torque development (RTD) over 50 ms, (RTD50), 100 ms (RTD100), 200 ms (RTD200), and impulse over 50 ms (IMP50), 100 ms (IMP100), and 200 ms (IMP200)] except RTD100 (Table 1). Therefore, torque values obtained via isokinetic dynamometry may not reflect true muscle force-producing capabilities unless the dynamometer length is accounted for.

Biodex Variable	r	<i>p</i> -value
MVIC PT	0.400	0.026*
MVIC RTD50	0.471	0.008*
MVIC RTD100	0.344	0.058
MVIC RTD200	0.364	0.044*
MVIC IMP50	0.588	0.001*
MVIC IMP100	0.492	0.005*
MVIC IMP200	0.472	0.007*
IsokPT $(60^{\circ} \cdot s^{-1})$	0.496	0.005*
IsokPT (180°·s ⁻¹)	0.429	0.016*
IsokPT (240°·s ⁻¹)	0.504	0.004*

 Table 1: Associations between Biodex Dynamometer Arm Length Setting and Unilateral

 Isometric and Isokinetic Knee Extension Performance Variables

r: Pearson's correlation coefficient; R^2 : Shared variance; SEE: Standard error of the estimate; MVIC: Maximal voluntary isometric contraction; PT: Peak torque; RTD50: Rate of torque development over 50 ms; RTD100: Rate of torque development over 100 ms; RTD200: Rate of torque development over 200 ms; IMP50: Impulse over 50 ms; IMP100: Impulse over 100 ms; IMP200: Impulse over 200 ms; IsokPT ($60^{\circ} \cdot s^{-1}$): Isokinetic peak torque at 60° per second; IsokPT ($180^{\circ} \cdot s^{-1}$): Isokinetic peak torque at 180° per second; IsokPT ($240^{\circ} \cdot s^{-1}$): Isokinetic peak torque at 240° per second.

*Statistically significant correlation ($p \le 0.05$)

Unilateral Leg Press Assessment

To determine the maximal strength of each individual, participants then completed a unilateral leg press assessment according to guidelines published by the NSCA. Each participant performed three warm-up sets before attempting a 1-RM lift. The external loads used during the warm-up sets and the 1-RM attempts were determined based off of a percentage of the estimated 1-RM from the familiarization day. Following each warm-up set, additional weight was added to the leg-press based upon the subject's perceived difficulty. Repetition ranges for each of the three warm-up sets were 8-10 repetitions, 4-6 repetitions, and 2-3 repetitions, followed by 1, 2, and 3 minute rest periods, respectively. Following the warm-up sets, additional weight was added to the leg-press, and a 1-RM was attempted. Two to four subsequent trials were performed to determine a 1-RM. Trials not meeting the range of motion criteria for each exercise were discarded. An example participant completing a 1-RM unilateral leg press is presented in Figure 12.



Figure 12: 1-Repetition Maximum (1-RM) Unilateral Leg Press Assessment An example participant completing a 1-RM unilateral leg press assessment. Following a warmup, two to four trials were used to determine 1-RM. Trials not meeting the range of motion criteria were discarded. Each attempt was separated by three minutes of rest.
Statistical Analyses

Intra-examiner precision between three consecutive panoramic and still images captured from each subject was analyzed using the SEM for CSA, UnCorEI, CorEI, MT, PA, and SFT (Varanoske et al., 2017b). The SEM indicates how precise a measurement is compared to its true value and it is not sensitive to within- or between-subject variability (Vincent & Weir, 2012). The CV and ICC_{3,1} for each muscle morphological characteristic was also calculated (Weir, 2005). Lower CVs and higher ICCs indicate greater reproducibility between measurements. Additionally, the MD was calculated for each muscle morphological characteristic, which refers to the minimum difference between values that reflects a true change. Prior to statistical procedures, all data was assessed for normality and sphericity. If the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied. To analyze within-subject differences in ultrasound-derived morphological characteristics of the VL (CSA, UnCorEI, CorEI, MT, PA, SFT), a repeated-measures analysis of variance (ANOVA) was used. In the event of a significant interaction, least significant differences (LSD) post-hoc tests were used for pairwise comparisons. Rest position effects were further analyzed using partial eta squared (η_p^2). Interpretations of η_p^2 were evaluated in accordance with Cohen (1988) at the following levels: small effect (0.01-0.058), medium effect (0.059-0.137), and large effect (>0.138). Comparisons between rest positions were further analyzed using 95% confidence intervals (CI) and Cohen's d. Magnitudes of the standardized effects were interpreted using thresholds of < 0.2, 0.2-0.6, 0.6-1.2, 1.2-2.0, 2.0-4.0. These values corresponded to trivial, small, moderate, large, and very large effect sizes (ES), respectively.

Associations between muscle morphological characteristics (CSA, UnCorEI, CorEI, MT, PA, and SFT) and physical performance variables were examined using Pearson's *r*. Additionally, stepwise linear regression was used to assess the shared variance (R^2) between muscle morphological characteristics and physical performance variables. Entry into the model occurred if the significance of the F value was *p* < 0.05, and the independent variable with the highest correlation to the dependent variable was included into the regression equation. Correlation magnitudes were quantified using the following descriptors: 0.00–0.10: *trivial*; 0.11-0.30: *small*; 0.31–0.50: *moderate*; 0.51–0.70: *large*; 0.71–0.90: *very large*; 0.91–1.00: *almost perfect* (Hopkins, Marshall, Batterham, & Hanin, 2009). For all analyses, a criterion alpha level of $\alpha \leq 0.05$ was used to determine statistical significance, and statistical software (Statistical Package for the Social Sciences [SPSS] V.23.0, Chicago, IL, USA) was used. All data are reported as mean ± standard deviation.

Differences between two dependent correlation coefficients with one variable in common were tested using the Williams modification of the Hotelling test (Kenny, 1987). This method was used to determine if one correlation was significantly different than another correlation with one common variable, using the following equation (Equation 5):

$$t(n-3) = \frac{(r_{12}-r_{23})\sqrt{(n-1)(1+r_{12})}}{\sqrt{2K\frac{(n-1)}{(n-3)} + \frac{(r_{23}+r_{13})^2}{4}(1-r_{12})^3}}$$
(5)

where

$$K = 1 - r_{12}^2 - r_{13}^2 - r_{23}^2 + 2r_{12}r_{13}r_{23}$$

The two correlation coefficients to be compared (i.e., r_{12} and r_{13}), along with the correlation coefficient between the two unshared variables (i.e., r_{23}), and the sample size were inputted into a published spreadsheet ("Comparing Pairs of Correlations," University of Cambridge, accessible at <u>http://imaging.mrc-</u>

<u>cbu.cam.ac.uk/statswiki/FAQ/WilliamsSPSS?action=AttachFile&do=view&target=Williams-</u> <u>test.xlsx</u>. The *p*-value associated with a two-tailed test of significance was then computed. Results were considered significant at an alpha-level of $\alpha \le 0.05$.

CHAPTER FOUR: RESULTS

Participants

One participant withdrew from the investigation after the familiarization day due to reasons unrelated to the study. Two participants were removed from the final data analysis due to issues related to ultrasound image analysis. Therefore, a total of 31 participants were included in the final analysis.

Anthropometric Measurements

The anthropometrics (age, height, body mass, body fat percentage) of participants included in the final analysis are presented in Table 2.

N	Age (yrs)	Height (m)	Body Mass (kg)	Body Fat Percentage (%)
31	23.0 ± 2.1	1.79 ± 0.08	87.4 ± 11.7	18.0 ± 5.2

Table 2: Anthropometric Measurements of Participants Included in the Final Data Analysis

Values are presented as mean \pm standard deviation.

Ultrasound Assessments

The majority of ultrasound morphological variables exhibited normality, and therefore, comparisons of mean differences in muscle morphological characteristics after rest in different positions was assessed using parametric analysis.

Reliability and precision values for all muscle morphological characteristics are presented in Table 3. These results indicate high reliability and precision between images for each variable after rest in all positions; however, PA consistently provided the lowest reliability and precision values, regardless of rest position.

Rest Position	Variable	ICC _{3,1}	CV	SEM	MD
	CSA	0.996	1.054	0.404	1.120
	UnCorEI	0.976	2.316	1.059	2.934
ID	CorEI	0.994	1.162	1.197	3.317
IP	MT	0.990	1.643	0.045	0.123
	PA	0.811	11.216	2.270	6.293
	SFT	0.996	2.826	0.018	0.049
	CSA	0.995	1.186	0.486	1.346
	UnCorEI	0.967	2.186	1.206	3.342
	CorEI	0.992	1.594	1.376	3.815
NDLK	MT	0.988	1.735	0.049	0.135
	PA	0.861	8.838	1.752	4.859
	SFT	0.997	2.235	0.015	0.043
SUP	CSA	0.995	1.300	0.473	1.310
	UnCorEI	0.957	2.947	1.444	4.004
	CorEI	0.988	2.072	4.512	1.628
	MT	0.989	1.787	0.044	0.123
	PA	0.857	9.358	1.755	4.865
	SFT	0.998	2.111	0.014	0.038
	CSA	0.972	1.515	1.081	2.996
	UnCorEI	0.940	3.216	1.552	4.301
ם וח	CorEI	0.989	2.157	1.525	4.227
DLK	MT	0.991	1.739	0.044	0.123
	PA	0.911	8.570	1.511	4.187
	SFT	0.998	2.303	0.015	0.041
	CSA	0.995	1.205	0.494	1.369
	UnCorEI	0.959	2.784	1.309	3.629
SТ	CorEI	0.994	1.666	1.210	3.354
51	MT	0.970	2.302	0.063	0.174
	PA	0.801	8.782	2.192	6.076
	SFT	0.995	3.431	0.023	0.065
	CSA	0.991	1.252	0.588	1.628
	UnCorEI	0.960	2.690	1.314	3.642
Averego	CorEI	0.991	1.730	1.964	3.268
Avelage	MT	0.986	1.841	0.049	0.136
	PA	0.848	9.353	1.896	5.256
	SFT	0.997	2.581	0.017	0.047

Table 3: Reliability and Precision Values for Ultrasound-Derived Morphological Characteristics of the Vastus Lateralis after Rest in Different Positions

CSA: Cross-sectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; NDLR: Assessments taken 15 minutes after laying down in the non-dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after standing up; ICC: Intraclass correlation coefficient; CV: Coefficient of variation; SEM: Standard error of measurement; MD: Minimal difference.

Values for ultrasound-derived muscle morphological characteristics between different rest positions are presented in Table 4. A significant main effect for rest position was observed for CSA ($F_{2.941, 88.238} = 7.206, p < 0.001, \eta_p^2 = 0.194$). CSA was significantly greater after rest in ST compared to NDLR (p < 0.001; d = 0.12; 95% CI = 0.442 to 1.147), SUP (p < 0.001; d =0.12; 95% CI = 0.392 to 1.156), and DLR (p = 0.009; d = 0.10; 95% CI = 0.171 to 1.107), although a trend towards a significant difference was observed between ST and IP (p = 0.070; d= 0.06; 95% CI = -0.036 to 0.861). Additionally, CSA was significantly greater after rest in IP compared to NDLR (p = 0.010; d = 0.06; 95% CI = 0.099 to 0.665) and SUP (p = 0.007; d =0.06; 95% CI = 0.106 to 0.617), but was not significantly different from DLR (p = 0.167; d =0.04; 95% CI = -0.100 to 0.554). No other differences in CSA existed between rest positions (p >0.05).

A significant main effect for rest position was observed for UnCorEI (F_{2.311,69.345} = 18.196, p < 0.001, $\eta_p^2 = 0.378$). UnCorEI was significantly lower after rest in ST compared to all other positions: IP (p < 0.001; d = 0.50; 95% CI = -4.455 to -2.057), NDLR (p < 0.001; d = 0.47; 95% CI = -4.176 to -1.814), SUP (p < 0.001; d = 0.37; 95% CI = -3.577 to -1.263), and DLR (p = 0.001; d = 0.30; 95% CI = -2.896 to -0.805). Additionally, UnCorEI was significantly greater after rest in IP compared to SUP (p = 0.017; d = 0.12; 95% CI = 0.163 to 1.509) and DLR (p < 0.001; d = 0.22; 95% CI = 0.789 to 2.021), but was not significantly different from NDLR (p = 0.359; d = 0.04; 95% CI = -0.310 to 0.831). UnCorEI was significantly greater after rest in NDLR compared to DLR (p = 0.001; d = 0.18; 95% CI = 0.517 to 1.772). A trend towards a significant difference was observed after rest in NDLR compared to SUP (p = 0.092; d = 0.09;

95% CI = -0.100 to 1.250). Additionally, a trend towards a significant difference was observed after rest in DLR compared to SUP (p = 0.083; d = 0.09; 95% CI = -1.218 to 0.079).

A significant main effect for rest position was observed for CorEI ($F_{2.522, 69.345} = 5.046, p$ = 0.005, $\eta_p^2 = 0.144$). CorEI was significantly lower after rest in ST compared to IP (p = 0.019; d= 0.11; 95% CI = -3.141 to -0.306), and NDLR (p = 0.037; d = 0.09; 95% CI = -2.590 to -0.085), but was not significantly different from SUP (p = 0.983; d = 0.00; 95% CI = -1.258 to 1.231) or DLR (p = 0.649; d = 0.02; 95% CI = -1.544 to 0.976). Additionally, CorEI at was significantly greater after rest in IP compared to SUP (p = 0.001; d = 0.12; 95% CI = 0.721 to 2.700) and DLR (p = 0.001; d = 0.10; 95% CI = 0.670 to 2.209), but was not significantly different from NDLR (p = 0.182; d = 0.03; 95% CI = -0.190 to 0.963). CorEI was significantly greater after rest in NDLR compared to SUP (p = 0.008; d = 0.09; 95% CI = 0.377 to 2.271) and DLR (p = 0.004; d= 0.07; 95% CI = 0.357 to 1.750). No significant differences in CorEI were observed rest in DLR compared to SUP (p = 0.510; d = 0.02; 95% CI = -0.557 to 1.099).

A significant main effect for rest position was observed for MT (F_{1.891, 56.723} = 85.671, p < 0.001, $\eta_p^2 = 0.741$). MT was significantly greater after rest in ST compared to all other positions: IP (p < 0.001; d = 0.99; 95% CI = 0.321 to 0.465), NDLR (p < 0.001; d = 0.97; 95% CI = 0.311 to 0.461), SUP (p < 0.001; d = 1.02; 95% CI = 0.322 to 0.475), and DLR (p < 0.001; d = 0.98; 95% CI = 0.322 to 0.481). No other differences in MT existed between rest positions (p > 0.05).

A significant main effect for rest position was observed for PA (F_{2.577, 77.322} = 35.621, p < 0.001, $\eta_p^2 = 0.543$). PA was significantly greater after rest in ST compared to all other positions: IP (p < 0.001; d = 1.17; 95% CI = 3.953 to 6.974), NDLR (p < 0.001; d = 1.22; 95% CI = 3.862 to 6.991), SUP (p < 0.001; d = 1.32; 95% CI = 4.357 to 7.300), and DLR (p < 0.001; d = 1.33; 95% CI = 4.491 to 7.917). No other differences in PA existed between rest positions (p > 0.05).

A significant main effect for rest position was observed for SFT ($F_{1.978, 59.335} = 12.660, p < 0.001, \eta_p^2 = 0.297$). SFT was significantly greater after rest in ST than in all other positions: IP (p < 0.001; d = 0.13; 95% CI = 0.018 to 0.057), NDLR (p < 0.001; d = 0.14; 95% CI = 0.021 to 0.061), SUP (p < 0.001; d = 0.12; 95% CI = 0.019 to 0.053), and DLR (p < 0.001; d = 0.13; 95% CI = 0.13; 95% CI = 0.019 to 0.053), and DLR (p < 0.001; d = 0.13; 95% CI = 0.020 to 0.057). No other differences in SFT existed between rest positions (p > 0.05).

Rest Position	CSA (cm ²)	UnCorEI (AU)	CorEI (AU)	MT (cm)	PA (°)	SFT (cm)
IP	34.91 ± 6.48	$49.93 \pm 6.76^*$	$74.58 \pm 14.87*$	$2.077 \pm 0.445*$	$17.45 \pm 4.89^*$	$0.608 \pm 0.294*$
NDLR	34.52 ± 6.58*↓	$49.67 \pm 6.52^*$	74.19 ± 15.19*	$2.083 \pm 0.443*$	$17.49 \pm 4.48*$	$0.605 \pm 0.289*$
SUP	34.54 ± 6.38*↓	49.09 ± 6.85*∔	72.87 ± 14.69↓‡	$2.071 \pm 0.428*$	$17.09 \pm 4.41*$	$0.610 \pm 0.298*$
DLR	$34.68 \pm 6.42*$	48.52 ± 6.21*∔‡	73.14 ± 14.49↓‡	$2.068 \pm 0.463*$	16.71 ± 4.92*	$0.607 \pm 0.293*$
ST	35.32 ± 6.72	46.67 ± 6.39	72.86 ± 15.67	2.470 ± 0.360	22.92 ± 4.57	0.646 ± 0.318

Table 4: Values for Ultrasound-Derived Muscle Morphological Characteristics of the Vastus Lateralis after Rest in Different Positions

Values are presented as mean ± standard deviation. CSA: Cross-sectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; NDLR: Assessments taken 15 minutes after laying down in the non-dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the supine position; DLR: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after standing up.

*Significantly different from ST (p < 0.05).

 \downarrow Significantly different from IP (p < 0.05).

 \pm Significantly different from NDLR ($p \le 0.05$).

Association between Muscle Morphology and Physical Performance

All physical performance data exhibited normality except for UVJ RPD and leg press 1-RM. However, the associations between muscle morphological characteristics after rest in different positions and physical performance was assessed using parametric analysis due to the majority of the variables exhibiting normality.

Unilateral Vertical Jump (UVJ) Performance

Associations between UVJ performance and ultrasound morphological characteristics are presented in Table 5. CSA after rest in all positions was a significant predictor of UVJ PF, UVJ peak power, and UVJ total work. CSA was the best predictor of UVJ PF after rest in DLR, and the best predictor of both UVJ peak power and UVJ total work after rest in IP. Nevertheless, CSA was also significantly correlated with UVJ PF after rest in all other rest positions (r = 0.551 - 0.571, p = 0.001), as well as with peak power (r = 0.510 - 0.531, p = 0.002 - 0.003) and total work (r = 0.385 - 0.418, p = 0.019 - 0.032) after rest in all other positions. The correlation between CSA and PF after rest in DLR (r = 0.592, p < 0.001) was significantly greater than that after rest in SUP (r = 0.551, p = 0.001) (t = -2.126, p = 0.042). No other statistically significant differences were observed between rest positions for CSA and UVJ peak power, or CSA and UVJ total work correlation coefficients (p < 0.05). Additionally, no other statistically significant differences were observed between rest positions for CSA and PF correlation coefficients. CSA did not significantly predict UVJ height, peak velocity, or RPD after rest in any position.

CorEI after rest in all positions was a significant predictor of UVJ height and peak velocity. CorEI was the best predictor of UVJ height after rest in NDLR and the best predictor of peak velocity after rest in IP. Nevertheless, CorEI was also significantly correlated with UVJ height after rest in all other rest positions (r = -0.555 - -0.497, p = 0.001 - 0.004), and with UVJ peak velocity after rest in all other positions (r = -0.479 - -0.419, p = 0.006 - 0.019). Neither the correlation between CorEI and UVJ height in IP, nor the correlation between CorEI and peak velocity in IP were significantly different from any other rest position (p < 0.05). CorEI did not significantly predict PF, peak power, total work, or RPD after rest in any position. Additionally, UnCorEI was not a significant predictor of any UVJ performance variable.

MT was a significant predictor of PF and peak power. MT was the best predictor of PF after rest in IP, and the best predictor of peak power after rest in ST. Nevertheless, MT was significantly correlated with PF after rest in all other positions (r = 0.394 - 0.423, p = 0.018 - 0.028) and with peak power after rest in all positions, except SUP (IP: r = 0.385, p = 0.032; NDLR: r = 0.391, p = 0.030; DLR: r = 0.373, p = 0.039; SUP: r = 0.345, p = 0.058). Neither the correlation between MT and PF after rest in IP, nor the correlation between MT and peak power after rest in IP, nor the correlation between MT and peak power after rest in ST were significantly different from any other rest position (p < 0.05). MT was not a significant predictor of UVJ height, peak velocity, total work, or RPD after rest in any position.

PA was a significant predictor of UVJ height, UVJ PF, UVJ peak power, UVJ peak velocity, and UVJ RPD. PA was the best predictor of UVJ height, peak power, peak velocity, and RPD after rest in IP. PA was also significantly correlated with RPD after rest in all other positions (r = 0.363 - 0.568, p = 0.001 - 0.045). The correlation between PA and RPD after rest in IP (r = 0.646, p < 0.001) was significantly greater than that after rest in ST (r = 0.363, p = 0.045) (t = 2.244, p = 0.033). Although PA was also the best predictor of peak power after rest in IP, it was also significantly correlated with peak power after rest in NDLR and DRL, but not

SUP or ST (NDLR: r = 0.428, p = 0.016; DLR: r = 0.415, p = 0.020; SUP: r = 0.317, p = 0.082; ST: r = 0.106, p = 0.571). The correlation between PA and peak power after rest in IP (r = 0.475, p = 0.007) was significantly greater than that after rest in ST (r = 0.106, p = 0.571) (t = 2.652, p = 0.013). No other statistically significant differences were observed between rest positions for PA and peak power correlation coefficients (p > 0.05).

PA was the best predictor of PF after rest in DLR. Nevertheless, PA was significantly correlated with PF after rest in all positions, except ST (IP: r = 0.414, p = 0.021; NDLR: r = 0.417, p = 0.019; SUP: r = 0.419, p = 0.019; ST: r = 0.091, p = 0.625). The correlation between PA and PF after rest in in NDLR, IP, DLR, and SUP was significantly greater than that after rest in ST (IP: t = 2.221, p = 0.035; NDLR: t = 2.045, p = 0.050; DLR: t = 2.221, p = 0.035; SUP: t = 2.193, p = 0.037). No other statistically significant differences were observed between rest positions for PA and peak UVJ PF correlation coefficients (p > 0.05).

PA was not significantly correlated with UVJ height (r = 0.107 - 0.271, p = 0.140 - 0.565) or UVJ peak velocity (r = 0.108 - 0.255, p = 0.167 - 0.563), except for after rest in IP (UVJ height: r = 0.363, p = 0.045; UVJ peak velocity: r = 0.360; p = 0.047). The correlation between PA and UVJ height was significantly greater after rest in IP than that after rest in SUP (t = -2.250, p = 0.032) and DLR (t = 2.206, p = 0.035), but no statistically significant differences were observed between IP and NDLR, or IP and ST (p > 0.05). The correlation between PA and peak velocity was significantly greater after rest in SUP (t = -2.208, p = 0.036), but no significant differences were observed between IP and NDLR, or ST (p > 0.036), but no significant differences were observed between IP and NDLR, predict total work after rest in any position.

SFT did not significantly predict PF, peak power, total work, or RPD after rest in any position. However, SFT was a significant predictor of UVJ height and peak velocity. SFT was the best predictor of both UVJ height and peak velocity after rest in IP. Nevertheless, SFT was also significantly correlated with UVJ height and peak velocity after rest in all other positions (UVJ height: NDLR: r = -0.561, p = 0.001; DLR: r = -0.562, p = 0.001; SUP: r = -0.540, p =0.002; ST: r = -0.555, p = 0.001; peak velocity: NDLR: r = -0.497, p = 0.004; DLR: r = -0.496, p =0.005; SUP: r = -0.479, p = 0.006; ST: r = -0.484, p = 0.006). Neither the correlation between SFT and UVJ height or SFT and peak velocity were significantly different from any other position (p < 0.05).

Morphological Variable	UVJ Variable	Best Position Predictor	r	R ²	SEE	<i>p</i> -value	Other Potential Positions
	Height	None	-	-	-	-	-
	PF	DLR↓ ^d	0.592	0.350	191.44	< 0.001	IP↓, NDLR↓, SUP↓, ST↓
CEA	Peak Power	IP∔	0.537	0.288	403.65	0.002	NDLR↓, SUP↓, DLR↓, ST↓
CSA	Peak Velocity	None	-	-	-	-	-
	Total Work	IP*	0.425	0.181	94.06	0.017	NDLR*, SUP*, DLR*, ST*
	RPD	None	-	-	-	-	-
	Height	None	-	-	-	-	-
	PF	None	-	-	-	-	-
U-C-FI	Peak Power	None	-	-	-	-	-
UnCorEl	Peak Velocity	None	-	-	-	-	-
	Total Work	None	-	-	-	-	-
	RPD	None	-	-	-	-	-
	Height	NDLR↓	-0.556	0.309	2.61	0.001	IP↓, SUP↓, DLR↓, ST*
	PF	None	-	-	-	-	-
ConFI	Peak Power	None	-	-	-	-	-
CorEl	Peak Velocity	IP*	-0.484	0.235	0.15	0.006	NDLR*, SUP*, DLR*, ST*
	Total Work	None	-	-	-	-	-
	RPD	None	-	-	-	-	-
	Height	None	-	-	-	-	-
	PF	IP*	0.449	0.202	212.12	0.011	NDLR*, SUP*, DLR*, ST*
МТ	Peak Power	ST*	0.433	0.187	431.25	0.015	IP*, NDLR*, DLR*
INI I	Peak Velocity	None	-	-	-	-	-
	Total Work	None	-	-	-	-	-
	RPD	None	-	-	-	-	-
	Height	IP*cd	0.363	0.132	2.93	0.045	None
	PF	DLR* ^e	0.453	0.205	211.67	0.010	IP* ^e , NDLR* ^e , SUP* ^e
DA	Peak Power	IP* ^e	0.475	0.226	420.85	0.007	NDLR*, DLR*
ГA	Peak Velocity	IP^{*d}	0.360	0.129	0.16	0.047	None
	Total Work	None	-	-	-	-	-
	RPD	IP↓ ^e	0.646	0.418	2295.56	< 0.001	NDLR↓, SUP↓, DLR↓, ST*
	Height	IP∔	-0.565	0.319	2.60	0.001	NDLR \downarrow , SUP \downarrow , DLR \downarrow , ST \downarrow
	PF	-	-	-	-	-	-
SET	Peak Power	-	-	-	-	-	-
SFT	Peak Velocity	IP*	-0.503	0.253	0.15	0.004	NDLR*, SUP*, DLR*, ST*
	Total Work	-	-	-	-	-	-
	RPD	-	-	-	-	-	-

Table 5: Associations between Ultrasound-Derived Muscle Morphological Characteristics after Rest in Different Positions and Unilateral Vertical Jump (UVJ) Outcome Measures Associations between ultrasound-derived morphological characteristics and UVJ outcome measures based on the rest position having the greatest shared variance with the outcome variable. The morphological variable is presented first, followed by the rest position that best predicts the dependent variable. "Other Potential Positions" denotes rest positions also having a significant association with the dependent variable. "None" indicates that the specific measure of morphology was not a significant predictor of jump performance after rest in any position. *r*: Pearson's correlation coefficient; R^2 : Shared variance; SEE: Standard error of the estimate; CSA: Cross-sectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; PF: Peak force; RPD: Rate of power development; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the supine position; DLR: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after laying up.

Statistically significant ($p \le 0.05$) correlation magnitudes were quantified using the following descriptors (Hopkins et al., 2009):

*Moderate

.↓Large

Differences between correlation coefficients were examined using the Williams modification of the Hotelling test (Kenny, 1987):

^aSignificantly stronger than IP ($p \le 0.05$)

^bSignificantly stronger than NDLR ($p \le 0.05$)

^cSignificantly stronger than DLR ($p \le 0.05$)

^dSignificantly stronger than SUP ($p \le 0.05$)

^eSignificantly stronger than ST ($p \le 0.05$)

Unilateral Isometric and Isokinetic Performance

No significant associations existed between uncorrected isometric and isokinetic performance values and muscle morphological characteristics after rest in any position. Associations between muscle morphological characteristics and isometric and isokinetic performance values after adjusting for dynamometer arm length with their best rest position predictor, along with their practical interpretation, are presented in Tables 6 and 7, respectively.

UnCorEI was a significant predictor of MVIC IMP50. UnCorEI was the best predictor of MVIC IMP50 after rest in IP. Nevertheless, UnCorEI was significantly correlated with MVIC IMP50 after rest in SUP and ST, but not after rest in NDLR or DLR (NDLR: r = 0.276, p = 0.132; DLR: r = 0.309, p = 0.091; SUP: r = 0.356, p = 0.049; ST: r = 0.359, p = 0.047). The correlation between UnCorEI and MVIC IMP50 was significantly greater after rest in IP than that after rest in ST (t = 2.373, p = 0.025). No other statistically significant differences were observed between rest positions for UnCorEI and MVIC IMP50 correlation coefficients (p > 0.05).

MT was a significant predictor of IsokPF ($180^{\circ} \cdot s^{-1}$) and IsokPF ($240^{\circ} \cdot s^{-1}$), but was not a significant predictor of any of the isometric variables. MT was the best predictor of IsokPF ($180^{\circ} \cdot s^{-1}$) and IsokPF ($240^{\circ} \cdot s^{-1}$) after rest in DLR. Nevertheless, MT was significantly correlated with IsokPF ($180^{\circ} \cdot s^{-1}$) after rest in all other positions (IP: r = 0.394, p = 0.028; NDLR: r = 0.427, p = 0.017; SUP: r = 0.401, p = 0.025; ST: r = 0.421, p = 0.018), and with IsokPF ($240^{\circ} \cdot s^{-1}$) after rest in SUP and ST (IP: r = 0.362, p = 0.045; NDLR: r = 0.373, p = 0.039; SUP: r = 0.349, p = 0.054; ST: r = 0.331, p = 0.069). No other statistically

significant differences were observed between rest positions for MT and IsokPF ($180^{\circ} \cdot s^{-1}$) or MT and IsokPF ($240^{\circ} \cdot s^{-1}$) correlation coefficients (p < 0.05).

PA was a significant predictor of IsokPF ($60^{\circ} \cdot s^{-1}$), IsokPF ($180^{\circ} \cdot s^{-1}$), and IsokPF ($240^{\circ} \cdot s^{-1}$), but was not a significant predictor of any of the isometric variables. PA was the best predictor of IsokPF ($60^{\circ} \cdot s^{-1}$) after rest in DLR, but was not significantly correlated with IsokPF ($60^{\circ} \cdot s^{-1}$) after rest in any other position (IP: r = 0.261, p = 0.156; NDLR: r = 0.201, p = 0.279; SUP: r = 0.102, p = 0.587; ST: r = 0.210, p = 0.257). The correlation between PA and IsokPF ($60^{\circ} \cdot s^{-1}$) after rest in DLR was significantly greater than that after rest in SUP (t = -2.489, p = 0.019). No other statistically significant differences were observed between rest positions for PA and IsokPF ($60^{\circ} \cdot s^{-1}$) correlation coefficients (p > 0.05).

PA was the best predictor of IsokPF ($180^{\circ} \cdot s^{-1}$) after rest in ST. Nevertheless, PA was significantly correlated with IsokPF ($180^{\circ} \cdot s^{-1}$) after rest in all other positions, except for SUP (IP: r = 0.435, p = 0.014; NDLR: r = 0.370, p = 0.040; SUP: r = 0.311, p = 0.088; DLR: r = 0.370, p = 0.040). No other statistically significant differences were observed between rest positions for PA and IsokPF ($180^{\circ} \cdot s^{-1}$) correlation coefficients (p < 0.05).

PA was the best predictor of IsokPF ($240^{\circ} \cdot s^{-1}$) after rest in DLR, but was not significantly correlated with IsokPF ($240^{\circ} \cdot s^{-1}$) after rest in any other position (IP: r = 0.347, p = 0.056; NDLR: r = 0.340, p = 0.061; SUP: r = 0.339, p = 0.062; ST: r = 0.321, p = 0.079). No other statistically significant differences were observed between rest positions for PA and IsokPF ($240^{\circ} \cdot s^{-1}$) correlation coefficients (p < 0.05).

CSA, CorEI, and SFT were not significant predictors of any isometric or isokinetic variables after rest in any position.

Morphological Variable	Isometric Variable	Best Position Predictor	r	R^2	SEE	<i>p</i> -value	Other Potential Positions
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
CSA	RFD100	None	-	-	-	-	-
	RFD200	None	-	-	-	-	-
	IMP50	None	-	-	-	-	-
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
	RFD100	None	-	-	-	-	-
UnCorEI	RFD200	None	-	-	-	-	-
	IMP50	IP* ^b	0.366	0.134	2.28	0.043	SUP*, ST*
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
	RFD100	None	-	-	-	-	-
CorEI	RFD200	None	-	-	-	-	-
	IMP50	None	-	-	-	-	-
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
	RFD100	None	-	-	-	-	-
MT	RFD200	None	-	-	-	-	-
	IMP50	None	-	-	-	-	-
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
	RFD100	None	-	-	-	-	-
PA	RFD200	None	-	-	-	-	-
	IMP50	None	_	-	_	-	-
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-

Table 6: Associations between Muscle Morphological Characteristics after Rest in Different Positions and Isometric Variables after Correcting for Biodex Dynamometer Arm Length.

Morphological Variable	Isometric Variable	Best Position Predictor	r	R ²	SEE	<i>p</i> -value	Other Potential Positions
	PF	None	-	-	-	-	-
	RFD50	None	-	-	-	-	-
	RFD100	None	-	-	-	-	-
SFT	RFD200	None	-	-	-	-	-
	IMP50	None	-	-	-	-	-
	IMP100	None	-	-	-	-	-
	IMP200	None	-	-	-	-	-

Associations between ultrasound-derived morphological characteristics and isometric and isokinetic outcome measures based on the rest position having the greatest shared variance with the dependent variable after correcting for dynamometer arm length. The morphological variable is presented first, followed by the rest position that best predicts the dependent variable. "None" indicates that the specific measure of morphology was not a significant predictor of isometric or isokinetic performance after rest in any position. "Other Potential Positions" denotes rest positions also having a significant association with the dependent variable. r: Pearson's correlation coefficient; R^2 : Shared variance; SEE: Standard error of the estimate; CSA: Crosssectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; PF: Peak force; RFD50: Rate of force development over 50 ms; RFD100: Rate of force development over 100 ms; RFD200: Rate of force development over 200 ms; IMP50: Impulse over 50 ms; IMP100: Impulse over 100 ms; IMP200: Impulse over 200 ms; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; NDLR: Assessments taken 15 minutes after laying down in the non-dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the supine position; DLR: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after standing up. Statistically significant ($p \le 0.05$) correlation magnitudes were quantified using the following descriptors (Hopkins et al., 2009): *Moderate

Large

Differences between correlation coefficients were examined using the Williams modification of the Hotelling test (Kenny, 1987):

^aSignificantly stronger than IP ($p \le 0.05$)

^bSignificantly stronger than NDLR ($p \le 0.05$)

^cSignificantly stronger than DLR ($p \le 0.05$)

^dSignificantly stronger than SUP ($p \le 0.05$)

^eSignificantly stronger than ST ($p \le 0.05$)

Table 7: Associations between Muscle Morphological Characteristics after Rest in Different Positions and Isokinetic Variables after Correcting for Biodex Dynamometer Arm Length.

Morphological Variable	Isokinetic Variable	Best Position Predictor	r	R^2	SEE	<i>p</i> -value	Other Potential Positions
	IsokPF (60°⋅s ⁻¹)	None	-	-	-	-	-
CSA	IsokPF (180°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (240°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (60°·s ⁻¹)	None	-	-	-	-	-
UnCorEI	IsokPF (180°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (240°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (60°·s ⁻¹)	None	-	-	-	-	-
CorEI	IsokPF (180°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (240°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (60°·s ⁻¹)	None	-	-	-	-	-
MT	IsokPF (180°·s ⁻¹)	DLR*	0.457	0.209	84.88	0.010	IP*, NDLR*, SUP*, ST*
	IsokPF (240°·s ⁻¹)	DLR*	0.398	0.158	72.32	0.027	IP*, NDLR*
	IsokPF (60°·s ⁻¹)	DLR* ^d	0.358	0.129	139.81	0.048	None
PA	IsokPF (180°·s ⁻¹)	ST*	0.447	0.200	85.35	0.012	IP*, NDLR*, DLR*, SUP*
	IsokPF (240°·s ⁻¹)	DLR*	0.379	0.144	72.95	0.035	None
	IsokPF (60°⋅s ⁻¹)	None	-	-	-	-	-
SFT	IsokPF (180°·s ⁻¹)	None	-	-	-	-	-
	IsokPF (240°·s ⁻¹)	None	-	-	-	-	-

Associations between ultrasound-derived morphological characteristics and isometric and isokinetic outcome measures based on the rest position having the greatest shared variance with the dependent variable after correcting for dynamometer arm length. The morphological variable is presented first, followed by the rest position that best predicts the dependent variable. "None" indicates that the specific measure of morphology was not a significant predictor of isometric or isokinetic performance after rest in any position. "Other Potential Positions" denotes rest positions also having a significant association with the dependent variable. *r*:

Pearson's correlation coefficient; R^2 : Shared variance; SEE: Standard error of the estimate; CSA: Cross-sectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; IsokPF ($60^{\circ} \cdot s^{-1}$): Isokinetic peak force at 60° per second; IsokPF ($180^{\circ} \cdot s^{-1}$): Isokinetic peak force at 240° per second; IsokPF ($240^{\circ} \cdot s^{-1}$): Isokinetic peak force at 240° per second; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; NDLR: Assessments taken 15 minutes after laying down in the non-dominant lateral recumbent position; SUP: Assessments taken 15 minutes after laying down in the dominant lateral recumbent position; ST: Assessments taken 15 minutes after standing up. Statistically significant ($p \le 0.05$) correlation magnitudes were quantified using the following descriptors (Hopkins et al., 2009): *Moderate

*↓*Large

Differences between correlation coefficients were examined using the Williams modification of the Hotelling test (Kenny, 1987):

^aSignificantly stronger than IP ($p \le 0.05$)

^bSignificantly stronger than NDLR ($p \le 0.05$)

^cSignificantly stronger than DLR ($p \le 0.05$)

^dSignificantly stronger than SUP ($p \le 0.05$)

^eSignificantly stronger than ST ($p \le 0.05$)

Unilateral Maximal Strength

Associations between maximal strength values and ultrasound-derived morphological characteristics are presented in Table 8. CSA, MT, and PA were significant predictors of maximal strength. CSA was the best predictor of 1-RM unilateral leg press after rest in NDLR. Nevertheless, CSA was significantly correlated with 1-RM after rest in all other positions (IP: r = 0.712, p < 0.001; DLR: r = 0.690, p < 0.001; SUP: r = 0.703, p < 0.001; ST: r = 0.698, p < 0.001). PA was the best predictor of 1-RM unilateral leg press after rest in NDLR. Nevertheless, PA was significantly correlated with 1-RM after rest in all other positions, except ST (IP: r = 0.371, p = 0.040; DLR: r = 0.359, p = 0.047; SUP: r = 0.385, p = 0.032; ST: r = 0.244, p = 0.187). MT was the best predictor of 1-RM unilateral leg press after rest in IP. Nevertheless, MT was significantly correlated with 1-RM after rest in all other positions (NDLR: r = 0.698, p < 0.001; DLR: r = 0.676, p < 0.001; SUP: r = 0.703, p < 0.001; ST: r = 0.662, p < 0.001). No other statistically significant differences were observed between rest positions for CSA, PA, or MT and 1-RM correlation coefficients (p < 0.05). UnCorEI, CorEI, and SFT were not significant predictors of unilateral 1-RM.

Table 8: Associations between Ultrasound-Derived Morphological Characteristics after Rest in Different Positions and Unilateral Strength

Morphological Variable	Strength Variable	Best Position Predictor	r	R ²	SEE	<i>p</i> -value	Other Potential Positions
CSA	1-RM Leg Press	NDLR‡	0.713	0.508	80.54	<0.001	IPŧ, SUP↓, DLR↓, ST↓
UnCorEI		None	-	-	-	-	-
CorEI		None	-	-	-	-	-
MT		IPŧ	0.722	0.522	79.42	<0.001	NDLR↓, SUP↓, DLR↓, ST↓
PA		NDLR*	0.427	0.182	103.83	0.017	IP*, SUP*, DLR*
SFT		None	-	-	-	-	-

Associations between ultrasound-derived morphological characteristics and maximal unilateral strength based on the rest position having the greatest shared variance with the dependent variable. The morphological variable is presented first, followed by the rest position that best predicts the dependent variable. "None" indicates that the specific measure of morphology was not a significant predictor of maximal strength after rest in any position. "Other Potential Positions" denotes rest positions also having a significant association with the dependent variable. *r*: Pearson's correlation coefficient; R^2 : Shared variance; SEE: Standard error of the estimate; CSA: Cross-sectional area; UnCorEI: Uncorrected echo intensity; CorEI: Corrected echo intensity; MT: Muscle thickness; PA: Pennation angle; SFT: Subcutaneous adipose tissue thickness; IP: Assessments taken immediately post laying down in the non-dominant lateral recumbent position; NDLR: Assessments taken 15 minutes after laying down in the non-dominant lateral recumbent position; ST: Assessments taken 15 minutes after standing up. Statistically significant ($p \le 0.05$) correlation magnitudes were quantified using the following descriptors (Hopkins et al., 2009): *Moderate

¹Large

[‡]Very Large

CHAPTER FIVE: DISCUSSION

Measurements of muscle morphology in the present study demonstrated high reliability and precision in all positions measured, although PA consistently provided the lowest reliability values, regardless of position. The main findings of this study suggest that CSA, UnCorEI, and CorEI of the VL differ significantly between NDLR, DLR, and SUP; however, MT, PA, and SFT appear to remain consistent. Additionally, CSA, MT, PA, and SFT were significantly greater in ST compared to NDLR, DLR, and SUP. The magnitude of the relationships between muscle morphology and performance differed between rest positions. Muscle morphology measured in the IP position appears to be the best overall predictor of performance for the majority of variables, although utilizing the NDLR and DLR positions may provide comparable, or potentially stronger results for variables such as IsokPF. Although standing measures of VL morphology have previously been reported to exhibit stronger relationships with physical performance than recumbent measures (Wagle et al., 2017), our results suggest that the relationship between muscle morphology and various performance variables in ST are weaker compared to the recumbent positions examined, specifically for IsokPF, 1-RM leg press, and for all UVJ variables except total work. ST was the best predictor of performance for only two performance variables examined: UVJ peak power (through MT) and IsokPF ($180^{\circ} \cdot s^{-1}$) (through PA), whereas the remaining significant relationships between muscle morphology and performance were better elucidated in recumbent positions.

The current investigation demonstrated that MT of the VL was significantly greater in ST compared to all recumbent positions, and CSA was significantly greater in ST compared to all recumbent positions except IP, although a trend was observed between IP and ST. Despite the

statistical significance of these findings, a smaller percentage of change was observed in CSA compared to MT, and only the changes in MT exceeded the MD. These findings align with those of Wagle et al. (2017), indicating that measurements of muscle size taken at the muscle belly may be highly influenced by changes in position in the absence of changes in CSA of the muscle as a whole. This may be a result of muscle gearing, whereby muscle fibers shorten in the longitudinal direction and expand in the transverse direction, causing the muscle fibers to rotate to a greater PA, creating a bulging effect in the center of the muscle (Azizi & Brainerd, 2007; Wakeling & Randhawa, 2014). Consistent with this, we observed a significantly greater PA in ST compared to all other positions. However, muscle gearing is typically reported during muscle contraction, when a change in the length of the muscle is induced (Azizi & Brainerd, 2007), and in the present study, careful consideration was taken to ensure that joint angle remained constant and the muscle was relaxed in all positions examined. It is apparent that changes in position can create a similar muscle-bulging effect due to the influence of gravity on muscle shape and size (Thoirs & English, 2009; Tomko et al., 2018) that may not be due to true muscle gearing. Anecdotally, we noted that the VL appeared flatter and longer in the recumbent positions when compared to ST, which may have allowed for only modest changes in CSA as compared to the larger changes in MT (Tomko et al., 2018) (Figure 13).



Figure 13: Standing (ST) Versus Supine (SUP) Rest Ultrasound Images Sample ultrasound images captured from the same participant after 15 minutes of rest in SUP and during ST. All ultrasound settings were kept consistent throughout testing for each participant.

In the present study, we observed a significant decrease in CSA after 15 minutes of rest in the recumbent position (from IP to NDLR), with no significant change in MT, which is consistent with other studies (Arroyo et al., 2018; Shea, 2017). Previous research has demonstrated that changes in body posture induce rapid fluctuations in blood flow and resulting tissue volume (Cerniglia et al., 2007; Maw et al., 1995; Tan et al., 1973; Thoirs & English, 2009; Wagle et al., 2017). Specifically, the transition from a standing to supine position causes a substantial decrease in hydrostatic pressure of the lower body and redistribution of blood out of the lower extremities to the abdomen and head, resulting in a net absorption of fluid from the interstitial fluid into the capillaries, decreasing tissue volume (Fawcett & Wynn, 1960; Hagan et al., 1978; Maw et al., 1995; Taylor et al., 1995; Thompson et al., 1928; Waterfield, 1931a). Arroyo and colleagues (2018) observed a significant decrease in VL CSA between 0 and 10 minutes and 0 and 15 minutes after transitioning from standing to recumbent positions in the absence of changes in MT, while Shea (2017) observed a similar decrease in VL CSA following 20 and 30 minutes of supine rest. These findings suggest that VL CSA may be more sensitive to fluid shifts than MT, whereas MT appears to be influenced more by changes in posture (from ST to IP) and muscle shape. Therefore, the fluid shifts may induce a change in the transverse size of the muscle rather than a change in the thickness of the muscle. However, contrasting research by Lopez et al. (2019) showed no change in rectus femoris CSA or total quadriceps femoris MT 15 minutes after transitioning from standing to a supine position. Similarly, Tomko et al. (2018) observed no change in the CSA of the rectus femoris 5 minutes after transitioning from a supine to a seated position, or from a seated to a supine position. The discrepancy in these findings may be related to the muscle examined. Specifically, Lopez et al. (2019) and Tomko et al. (2018)

examined the rectus femoris, whereas the present study and the investigations of Arroyo et al. (2018) and Shea (2017) examined the VL. Speculatively, the larger size and different structure of the VL may allow for a larger quantity of fluid shifting into and out of the muscle, or perhaps within the muscle. However, it is unlikely that fluid shifts from the intracellular space to the extracellular space occur with postural changes because changes in plasma osmolality are not induced with changes in position, and therefore fluid shifts occur primarily throughout the extracellular space (Lippi et al., 2015; Maw et al., 1995; Scharfetter et al., 1997; Shirreffs & Maughan, 1994). The discrepancies in findings may also be related to differences in populations investigated. The present study and the work of Arroyo et al. (2018) utilized young, healthy adults $(24.3 \pm 3.4 \text{ y})$, whereas Lopez et al. (2019) utilized older adults (men: $68.1 \pm 4.6 \text{ y}$, women: 66.8 ± 4.1 y). With aging, a decrease in contractile tissue along with an increase in the relative proportion of intramuscular fat and fibrous tissue is often expected. This age-related decline in muscle mass may lead to a decrease in fluid storage within the muscle (Hooper et al., 2014), which may lessen the likelihood of fluid shifts occurring in response to changes in posture (Lopez et al., 2019). This is consistent with the findings of Cerniglia et al. (2007) and Shea (2017). Cerniglia and colleagues (2007) observed no change in the CSA of low-density muscle after 15 minutes of supine rest, and Shea (2017) reported no changes in CSA of the VL in individuals who engaged in low amounts of physical activity until between 20 and 30 minutes following recumbency. In contrast, Arroyo et al. (2018) observed significant changes in VL CSA after only 10 minutes of recumbency in a sample of young healthy adults. Nevertheless, Tomko et al. (2018) observed no change in rectus femoris CSA in physically-active, young males and females. However, in this study, CSA was only measured only 5 minutes after changing position. Although fluid shifts have been shown to occur rapidly upon changes in posture (Hagan et al., 1978; Husdan et al., 1973; Stoker et al., 1966; Tan et al., 1973), these changes may not become evident within 5 minutes of position change (Arroyo et al., 2018). Additionally, participants in the Tomko et al. (2018) investigation transitioned from a seated to supine position, whereas the subjects in the current investigation and those by Arroyo et al. (2018), Lopez et al. (2019), and Shea (2017) transitioned from a standing to supine position. Previous research has demonstrated that the transition from the supine to sitting position, or from the sitting to upright position, appears to result in smaller fluid shifts than transitioning from the supine to standing position (Maxfield et al., 1941; Tan et al., 1973), which may explain the differences in these findings.

CSA was significantly lower in NDLR compared to IP in the present study, and in SUP compared to IP; however, no differences were observed between IP and DLR. These findings may indicate that fluid shifts occur to a greater extent after 15 minutes of rest in the NDLR and SUP positions, whereas rest in the DLR position may minimize fluid shifts in the dominant limb. Since hydrostatic pressure within a body part is based on its vertical height from the heart (Martin-Du Pan et al., 2004), transitioning from a supine to lateral recumbent position alters the positioning of the dominant leg relative to the heart, which may alter blood flow. For example, in the DLR position, there will be an increased hydrostatic pressure and accumulation of blood in the vessels on the dominant side of the body (Bryan, 1974; Kallet, 2015). This may allow for a greater accumulation of fluid in the tissues on the dominant side, resulting in a larger CSA in the DLR position.

We found that UnCorEI was significantly lower in ST compared to all recumbent positions, which aligns with the findings of Tomko et al. (2018), who observed a significantly

lower UnCorEI of the rectus femoris in the seated position compared to supine rest. These authors suggest that the difference in UnCorEI values may be solely attributed to the alterations in the shape and curvature of the thigh, as well as changes in joint angle upon positional change, causing the muscle to appear flatter in the supine position compared to the seated position (Tomko et al., 2018). This would have caused the deep aponeurosis of the muscle to be located more proximally to the ultrasound probe, which may have reduced the attenuation of sound waves that occurs with deeper tissues (Pillen & van Alfen, 2011; H. J. Young et al., 2015). Although the knee and hip angles were kept consistent throughout positions in the present study, previous research has suggested that an attenuation of ultrasound waves may occur in tissues that are examined at a greater depth (Pillen & van Alfen, 2011; H. J. Young et al., 2015). This attenuation can decrease the acoustic reflectivity of the ultrasound waves, therefore artificially decreasing EI values in deeper tissues (Pillen & van Alfen, 2011). With a postural change, research has demonstrated that SFT may change over time due to fluid shifts out of the subcutaneous layer (Berg et al., 1993; Hargens, 1983). The current investigation demonstrated that SFT values were significantly lower in all recumbent positions compared to ST, which supports this notion. Therefore, the greater SFT in ST may have contributed to a greater overall depth of the muscle, which may account for the decreased UnCorEI that was observed in ST. Notwithstanding, a postural change from the ST to SUP position would also result in fluid shifts from the muscle, which would likely result in a lower muscle water content, and a pursuant increase in EI (Pillen & van Alfen, 2011). A postural change from the ST to SUP position may, therefore, combine the decreased SFT and water content of muscle, which both act to increase EI. In the present study, when correcting for SFT, CorEI values obtained in ST remained

significantly lower than those obtained during IP and NDLR but were not different from SUP and DLR. These findings indicate that small and insignificant changes in SFT may have large implications for CorEI values.

The present study demonstrated that UnCorEI did not change after 15 minutes of rest in the recumbent position (from IP to NDLR), which aligns with the findings of others (Arroyo et al., 2018; Lopez et al., 2019) in the VL. In contrast, Shea (2017) observed significant increases in UnCorEI of the VL after transitioning from a standing to recumbent position, which was followed by a subsequent decline back to original values. However, when UnCorEI values were corrected for SFT, older individuals (69.3 \pm 8.3 y) had significantly elevated CorEI values over the first 20 minutes of supine rest, with values peaking 10 minutes after recumbency, whereas the CorEI values in younger individuals $(21.4 \pm 2.5 \text{ y})$ did not change over time (Shea, 2017). This is counterintuitive, considering that previous research has demonstrated that gravitational fluid shifts occur to a lesser extent in older individuals compared to younger individuals (Fu et al., 1999). However, these researchers attributed the group differences to changes in SFT, as SFT was significantly decreased in the older group from 15 to 30 minutes of recumbency, whereas no change was observed in the younger individuals (Shea, 2017). In the present investigation, SFT did not change after 15 minutes of rest in any recumbent position, which is in alignment with Shea (2017) in the younger individuals, and may explain the lack of change in both UnCorEI and CorEI over time (from IP to NDLR).

Both CorEI and UnCorEI differed between recumbent positions. UnCorEI values were significantly lower in DLR and SUP as compared to IP, and in DLR as compared to NDLR, while CorEI values were significantly lower in DLR and SUP as compared to IP and NDLR.

Previous research has suggested that fluid shifts and water content of the muscle affect EI (Pillen & van Alfen, 2011). It appears that a decrease in CSA of the muscle as a result of gravitational fluid shifts would also accompany an increase in EI (Pillen & van Alfen, 2011). Of the three measurements taken in the recumbent positions after 15 minutes of rest (NDLR, SUP, and DLR), we reported that DLR exhibited the lowest UnCorEI and the greatest CSA values. However, the greatest EI values (both UnCorEI and CorEI) were observed in IP, which had the greatest CSA of all of the recumbent positions. These findings are counterintuitive, especially considering that the IP measurements were taken immediately after transitioning from a standing position to recumbent position, and ST had the lowest EI values. Notably, the differences in UnCorEI and CorEI values did not exceed the MD. Additionally, previous research has reported that UnCorEI may increase within the first 5 minutes after changing from a standing to recumbent (Shea, 2017) or seated (Tomko et al., 2018) position, followed by a decline over time (Tomko et al., 2018), which may be a result of the rapid redistribution of body fluid out of the lower body that occurs with changes in position. Further research is necessary to elucidate these findings, but based on the current study and others (Arroyo et al., 2018; Lopez et al., 2019; Shea, 2017; Tomko et al., 2018), the association between changes in muscle EI and muscle size with postural shifts may not appear to exhibit a linear relationship, and changes in muscle EI may not directly reflect absolute changes in muscle fluid shifts, but may be more sensitive to the rate of change in fluid within the muscle (Lopez et al., 2019), SFT (Shea, 2017), or probe handling (Dankel et al., 2018). Previous research has demonstrated that tilting an ultrasound probe as little as 2° can produce significant changes in EI (Dankel et al., 2018) although all reliability values for EI were high in the present study. Furthermore, although differences in SFT between positions did not

reach statistical significance, when correcting for SFT, CorEI in SUP and DLR were not significantly different from ST, indicating that small changes in SFT can have large impacts on CorEI.

The relationship between measures of muscle size and maximal strength-producing capabilities have been well established (Andersen & Aagaard, 2006; Fukunaga et al., 2001; Hakkinen & Keskinen, 1989; Wells et al., 2014). In the present investigation, both CSA and MT were significantly correlated with UVJ PF and PP, as well as 1-RM leg press. However, only MT was significantly correlated with IsokPF, and only CSA was significantly correlated with UVJ total work. Although measures of MT and CSA appear to be highly correlated (Varanoske et al., 2017b), an investigation by Wells and colleagues (2014) suggested that increases in lower-body strength following a resistance training program may be better elucidated by changes in MT rather than changes in CSA, and therefore quantification of both may be necessary for a comprehensive understanding of muscle function. However, neither CSA nor MT was a significant predictor of UVJ height, peak velocity, or RPD in the present study, which is consistent with research indicating that peak velocity and RPD are related primarily to muscle architecture, fiber-type distribution, and efferent neural drive rather than muscle size (Andersen, Andersen, Zebis, & Aagaard, 2010; Bottinelli, Canepari, Pellegrino, & Reggiani, 1996; Harridge et al., 1996; Maffiuletti et al., 2016; Zaras et al., 2016). In the present study, PA was significantly associated with all jump variables except for total work, all isokinetic variables, and 1-RM leg press. Muscles with greater PA contain greater amounts of contractile protein arranged in parallel within a given volume, resulting in an increased ability muscle to produce force (Blazevich et al., 2006). Additionally, muscles with longer FL (and concomitant lower PA) typically contain a
greater amount of series elastic material, and therefore may display a slower RFD because of the time it takes to take up the series elastic slack prior to true muscle contraction (Blazevich et al., 2009; Maffiuletti et al., 2016). Therefore, it is apparent that muscle architecture may affect both maximal strength as well as RFD.

In addition to muscle size and architecture, EI has been shown to be related to force production (Cadore et al., 2012; Fukumoto et al., 2012; Jajtner et al., 2015; Mangine, Fukuda, et al., 2014; Mangine et al., 2015; Mangine, Hoffman, et al., 2014; Melvin et al., 2014; Scanlon et al., 2014). Muscles with lower EI values typically contain lower amounts of non-contractile tissue, including intramuscular fibrous tissue, connective tissue, and/or fat, as these tissues increase the number of reflections within the muscle (Pillen et al., 2009; Pillen & van Alfen, 2011; Watanabe et al., 2013). In the present investigation, CorEI was significantly correlated with UVJ height and peak velocity, whereas UnCorEI was not a significant predictor of any UVJ variable, which may indicate that CorEI may be preferred over UnCorEI when examining jump performance. However, neither CorEI nor UnCorEI was associated with isometric performance or 1-RM leg press, indicating that other factors may underlie the quantification of EI.

In contrast to previous research stating that stronger relationships may be observed between standing measures of muscle morphology and performance (Wagle et al., 2017), the majority of performance variables measured in the present investigation demonstrated greater relationships with recumbent measures of muscle morphology than during ST. A potential explanation for the discrepancy in these findings is that the types of performance tests administered in each study differed: the tests in the study of Wagle et al. (2017) were all conducted in the upright position, whereas in the present investigation, the UVJ was conducted

in the upright position, the isometric/isokinetic measures were completed while the participant was seated, and the 1-RM leg press was completed while the participant was in a reclined seated position. Wagle et al. (2017) suggested that the ability of muscle morphology to predict performance may be a factor of how the muscle is analyzed relative to the position in which the muscle is utilized. Therefore, measurements taken during ST may reflect muscle function only during upright activities. However, in the current study, ST was the greatest predictor of only UVJ peak power through MT. Further, ST measurements of CorEI provided only moderate relationships with UVJ height, whereas CorEI in all other positions provided large relationships with UVJ height. Similarly, IP measurements of PA were significantly more correlated with UVJ peak power and RPD than in ST, and DLR measurements of PA were significantly more correlated with UVJ PF than in ST. ST did not provide significant correlations with UVJ height, PF, or peak velocity; however, PA in select recumbent positions did. In the seated position, no measure of morphology in ST was a significant predictor of IsokPF (240°·s⁻¹), although other measures in recumbent positions were. Also, despite the report of the interpretation of the magnitude of correlation coefficients between muscle morphology and performance in the investigation by Wagle et al. (2017), many of the correlations did not reach statistical significance; thus the results should be reported with caution.

Wagle et al. (2017) reported stronger relationships between standing measures of muscle size with all isometric variables compared to supine measurements; however, this positional difference subsided during dynamic 1-RM squats. Due to the influence of joint angle and muscle length on morphology (Hacker et al., 2016; Maganaris, 2001; Myers et al., 2013; Narici et al., 1996), the ability of muscle morphology to predict performance may be related to the type of exercise assessment utilized and the actions of the muscle during the test. Although we did not administer a test of upright isometric strength, no measures of muscle size in any position were significantly related to any of the isometric variables analyzed while seated. As the ultrasound assessments were completed while in the recumbent position or while standing and were not completed while seated, this may support the findings of Wagle et al. (2017), who suggested that the ability of muscle morphology to predict performance may be related to the position in which the muscle is analyzed. Additionally, previous research has shown that, while the rectus femoris contributes to only modest amounts of knee extension torque, changes in muscle length (as induced by a seated position in comparison to a supine position with knees flexed) have a considerable effect on increasing knee extension torque (Maffiuletti & Lepers, 2003). Therefore, the isometric and isokinetic variables examined in this investigation may have been better elucidated by examining both rectus femoris and VL muscle morphology. Further, all strength assessments in the investigation of Wagle et al. (2017) involved the use of both limbs, however only right VL morphology was assessed, whereas the present investigation examined muscle morphology and performance in the dominant limb. Additionally, although Wagle et al. (2017) reported that the joint angle was standardized for both the standing and recumbent positions, the standing position did not involve resting the leg against a device or plinth, and therefore some degree of muscle contraction may have been required to retain the joint angle during the assessment, which may have affected measures of muscle morphology.

In general, morphology of the VL assessed after IP appears to be the best predictor of physical performance. All muscle morphological characteristics that were significant predictors of UVJ performance and 1-RM leg press included IP as a rest position. Although IP provided comparable relationships with UVJ performance variables to DLR and NDLR, PA after rest in IP was the only significant predictor of UVJ height and peak velocity, and MT in IP provided very large relationships with 1-RM leg press, whereas the other positions provided large relationships. Additionally, although PA after rest in DLR was the only significant predictor of IsokPF ($60^{\circ} \cdot s^{-1}$) and IsokPF ($240^{\circ} \cdot s^{-1}$), the magnitude of these relationships were not different from those provided by IP. Therefore, it is evident that waiting for fluid shifts to occur prior to ultrasound assessment of the VL may not be necessary when predicting performance, and instead may rather diminish the ability of muscle morphological characteristics to predict function (Arroyo et al., 2018; Cerniglia et al., 2007). Further research is necessary to elucidate whether similar positioning is necessary to accurately evaluate changes in muscle morphology and concomitant changes in muscle function throughout the course of a training program.

Notably, VL morphology taken after rest in SUP was not the best predictor of any of the performance variables. As this is typically the rest position utilized in most previous reports of ultrasonography, (Jajtner et al., 2013; Mangine, Fukuda, et al., 2014; Mangine, Hoffman, et al., 2014; Scanlon et al., 2014; Varanoske et al., 2017a, 2017b; Wells et al., 2014), future investigations may want to avoid using this position prior to ultrasound assessment in order to obtain the best prediction of VL muscle function. Nevertheless, many researchers wish to analyze more than just the VL in the assessment of lower-body muscle morphology, which often require subjects to lay in different positions. Specifically, during the analysis of the rectus femoris, subjects are typically positioned in the SUP position. As rest in the SUP position has been shown to diminish the relationships between VL morphology and performance to a greater extent than rest in the NDLR and DLR positions, researchers may wish to analyze both the right

and left VL muscles immediately after transitioning to a recumbent position, followed by analysis of the rectus femoris muscles. Although previous research has demonstrated that the recuts femoris may be less sensitive to postural-induced fluid shifts (Lopez et al., 2019; Tomko et al., 2018), future research may seek to examine the effects of changes in position on the rectus femoris and its relationship with performance. The weaker relationships between the recumbent measures of muscle morphology compared to standing measures as reported by Wagle et al. (2017) may also be a factor of the SUP position utilized prior to testing, although they do not directly report which position subjects were instructed to lay in prior to ultrasound assessments.

In conclusion, VL CSA, UnCorEI, and CorEI differ after rest in different recumbent positions; however, MT, PA, and SFT appear to remain consistent. All measures of muscle morphology in ST are different from those obtained after 15 minutes of rest in the recumbent positions except for CorEI. Although different magnitudes of relationships were observed between muscle morphological characteristics measured after rest in different positions and performance variables, muscle morphology in IP most consistently provides the best predictor of unilateral lower-body performance. Additionally, some measures of muscle morphology in ST provided significantly weaker relationships with IsokPF, 1-RM leg press, and all UVJ variables except total work when compared to the recumbent positions. Thus, researchers and practitioners should consider evaluation of muscle morphology of the lower-body immediately after laying down to predict force and power production. This positioning also necessitates fewer requirements on the technician and the subject and therefore may be preferred over standing ultrasounds in many settings.

APPENDIX A: APPROVAL OF HUMAN RESEARCH



University of Central Florida Institutional Review Board Office of Research & Commercialization 12201 Research Parkway, Suite 501 Orlando, Florida 32826-3246 Telephone: 407-823-2901 or 407-882-2276 www.research.ucf.edu/compliance/irb.html

Approval of Human Research

From: UCF Institutional Review Board #1 FWA00000351, IRB00001138

To: Adam J Wells and Co-PI: Alyssa Nancy Varanoske

Date: September 05, 2018

Dear Researcher:

On 09/05/2018 the IRB approved the following human participant research until 09/04/2019 inclusive:

Type of Review:	UCF Initial Review Submission Form
200	Expedited Review
Project Title:	Effect of Rest Position on Ultrasound-Derived Morphological
STREET, STREET	Characteristics of the Vastus Lateralis and Lower-Body Force
	Production
Investigator:	Adam J Wells
IRB Number:	BIO-18-14303
Funding Agency:	
Grant Title:	
Research ID:	N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form <u>cannot</u> be used to extend the approval period of a study. All forms may be completed and submitted online at <u>https://iris.research.ucf.edu</u>.

If continuing review approval is not granted before the expiration date of 09/04/2019, approval of this research expires on that date. <u>When you have completed your research, please submit a</u> <u>Study Closure request in iRIS so that IRB records will be accurate</u>.

<u>Use of the approved, stamped consent document(s) is required</u>. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

This letter is signed by:

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Signature applied by Racine Jacques on 09/05/2018 02:32:03 PM EDT

Designated Reviewer

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University of Central Florida Institutional Review Board Office of Research & Commercialization 12201 Research Parkway, Suite 501 Orlando, Florida 32826-3246 Telephone: 407-823-2901 or 407-882-2276 www.research.ucf.edu/compliance/irb.html

Approval of Human Research

From: UCF Institutional Review Board #1 FWA00000351, IRB00001138

To: Adam J Wells and Co-PI: Alyssa Nancy Varanoske

Date: September 11, 2018

Dear Researcher:

On 09/11/2018 the IRB approved the following modifications until 09/04/2019 inclusive:

Type of Review:	IRB Addendum and Modification Request Form
	Expedited Review
Modification Type:	Added Research Personnel; Minor changes to study population and procedures.
Project Title:	Effect of Rest Position on Ultrasound-Derived Morphological Characteristics of the Vastus Lateralis and Lower-Body Force Production
Investigator:	Adam J Wells
IRB Number:	BIO-18-14303
Funding Agency: Grant Title:	
Research ID:	N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form <u>cannot</u> be used to extend the approval period of a study. All forms may be completed and submitted online at <u>https://ins.research.ucf.edu</u>.

If continuing review approval is not granted before the expiration date of 09/04/2019, approval of this research expires on that date. <u>When you have completed your research</u>, please submit a <u>Study Closure request in iRIS so that IRB records will be accurate</u>.

<u>Use of the approved, stamped consent document(s) is required.</u> The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

Page 1 of 2

This letter is signed by:

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Signature applied by Racine Jacques on 09/11/2018 08:45:15 AM EDT

Designated Reviewer

Page 2 of 2



University of Central Florida Institutional Review Board Office of Research & Commercialization 12201 Research Parkway, Suite 501 Orlando, Florida 32826-3246 Telephone: 407-823-2901 or 407-882-2276 www.research.ucf.edu/compliance/urb.html

Approval of Human Research

From: UCF Institutional Review Board #1 FWA00000351, IRB00001138

To: Adam J Wells and Co-PI-Alyssa Nancy Varanoske

Date: September 21, 2018

Dear Researcher:

On 09/21/2018 the IRB approved the following modifications until 09/04/2019 inclusive:

Type of Review.	IRB Addendum and Modification Request Form
	Expedited Review
Modification Type:	Additional ultrasound assessment; minor change to study procedure
Project Title:	Effect of Rest Position on Ultrasound-Derived Morphological Characteristics of the Vastus Lateralis and Lower-Body Force Production
Investigator	Adam J Wells
IRB Number:	BIO-18-14303
Funding Agency: Grant Title:	
Research ID:	N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for research that was previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form <u>cannot</u> be used to extend the approval period of a study. All forms may be completed and submitted online at <u>https://inis.research.ucf.edu</u>.

If continuing review approval is not granted before the expiration date of 09/04/2019, approval of this research expires on that date. <u>When you have completed your research, please submit a</u> Study Closure request in iRIS so that IRB records will be accurate.

Use of the approved, stamped consent document(s) is required. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

All data, including signed consent forms if applicable, must be retained and secured per protocol for a minimum of five years (six if HIPAA applies) past the completion of this research. Any links to the identification of participants should be maintained and secured per protocol. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

Page 1 of 2

This letter is signed by:

grg

Signature applied by Racine Jacques on 09/21/2018 11:44:45 AM EDT

Designated Reviewer

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APPENDIX B: APPROVED INFORMED CONSENT

Permission to Take Part in a Human Research Study Page 1 of 9 University of Central Florida Effect of Rest Position on Ultrasound-Derived Morphological Characteristics of the Vastus Lateralis and Lower-Body Force Production Informed Consent Principal Investigator(s): Adam J. Wells, Ph.D. **Co-Investigators** Alyssa N. Varanoske, M.S. Sub-Investigator(s): Nicholas A. Coker, M.S. Cheyanne L. Frosti, B.S. Bri-ana Johnson, B.S. Investigational Site(s): University of Central Florida College of Health Professions and Sciences Institute of Exercise Physiology and Wellness



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Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are an apparently healthy, resistance-trained male aged 18-35. For this study, resistance-trained is defined as training at least 3 times/week for at least 1 year. Additionally, you are not an amputee, have a pacemaker, have any chronic illness causing you to seek medical care, are not sedentary, have not taken performance-enhancing drugs in the last 6 months, can complete all of the testing visits, are able to complete all of the testing visits, and can complete all of the exercise assessments on the familiarization day.

What should I know about a research study?

- Someone will explain this research study to you.
- · Whether or not you take part is up to you.
- · You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- · You can ask all the questions you want before you decide.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to Alyssa Varanoske (Co-Investigator) at (407) 823-2367 or by e-mail at Alyssa Varanoske@ucf.edu, or Dr. Adam Wells (Principal Investigator) at (407) 823-3906 or by e-mail at Adam.Wells@ucf.edu.

This research has been reviewed and approved by an Institutional Review Board ("IRB"). You may talk to them at 407-823-2901or irb@ucf.edu if:

- · Your questions, concerns, or complaints are not being answered by the research team.
- · You cannot reach the research team.
- · You want to talk to someone besides the research team.
- · You have questions about your rights as a research subject.
- · You want to get information or provide input about this research.

Why is this research being done?

Ultrasounds have been used to measure muscle size and quality. Ultrasound imaging of the lower body usually requires individuals to lay down for a period of 15-minutes. However, there is no consensus on which position to lay in, and this may affect the muscle image. Therefore, the relationship between ultrasound and athletic performance may also be affected. The purpose of this study is to examine if muscle characteristics of the lower body change after laying on different sides of the body and to determine which position has the greatest association with athletic performance.

How long will the research last?

We expect that you will be in this research study for approximately 1-2 weeks, consisting of 3 visits to the Human Performance Laboratory. The first visit will consist of filling out paperwork. The second visit will consist of a familiarization with all of the exercise testing assessments. The familiarization visit should last approximately 1-2 hours. You will then be asked to come in for a third visit at least 72 hours after your familiarization visit, which will consist of anthropometric measurements, ultrasound image analysis, and exercise testing assessments. The assessments completed on testing day will last approximately 4 hours.



How many people will be studied?

We expect about 36 people here will be in this research study, with a maximum of 44 people enrolled.

What happens if I say yes, I want to be in this research?

You will be asked to report to the University of Central Florida's Human Performance Laboratory on three separate occasions. The first visit will be a preliminary visit to complete the informed consent form and determine eligibility through completion of a Medical History and Activity Questionnaire (MHAQ). Physical Activity Readiness Questionnaire (PAR-Q+), and to address any questions that you may have.

The second visit to the Human Performance Laboratory will be a familiarization session. During familiarization, you will become acquainted with all exercise testing assessments. During this visit, you will complete a standardized warm-up, followed by the assessments that you will complete during your testing session (Vertical Jump, Isometric/Isokinetic Force, Unilateral Leg Press).

Following a period of at least 72 hours from the familiarization session and/or 72 hours from any other vigorous lower-body exercise, you will then return to the Human Performance Lab for a testing session, which will consist of the same tests completed during the familiarization day. In addition, you will complete anthropometric testing (height, weight, body composition) and we will take images of your leg muscles using ultrasound.

Specific Procedures:

Familiarization Visit: During the familiarization, you will become familiarized with the
exercise assessment protocols that will be used during the testing session.

Prior to the exercise assessments, you will be required to complete a standardized dynamic warmup. You will first pedal on a cycle ergometer for 5-minutes at a self-selected pace. You will then complete ten body-weight squats, ten body-weight walking lunges, ten dynamic walking hamstring stretches, ten dynamic walking quadriceps stretches, ten arm circles, and ten arm swings.

Vertical Jump (VJ) Assessment:

You will be asked to perform a unilateral countermovement jump on a force
plate, while standing on your dominant leg. You will be instructed to perform a
countermovement jump whereby you will be required to bend at the knee and
extend to maximize the height of each jump while keeping your hands on your
hips. You will perform three consecutive unilateral countermovement jumps
with three minutes of rest in between each jump.

Biodex Assessments:

You will be seated in an isokinetic dynamometer and strapped into the chair at the waist, shoulders, and across the thigh to complete a series of isokinetic strength assessments. You will be tested on your dominant leg, which will be secured to the dynamometer arm. Your range of motion will be assessed. You will then complete three warm-up repetitions. After the warm-up, you will be instructed to perform two maximal voluntary contractions while your leg stays in place, and you will be instructed to hold the contraction for 6 seconds. You will also be asked to perform three sets of three isokinetic contractions while the dynamometer arm moves at different but constant speeds. Each isokinetic testing set will consist of maximal concentric knee extension and passive knee



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flexion. Between testing sets, 3-minutes of rest will be provided. A sample depiction of the Biodex assessment is included below.



o Unilateral Leg Press Assessment:

You will be familiarized with the leg press assessment through proper instruction and technique. You will be asked to complete a unilateral leg press with the dominant leg, using a weight that you can successfully lift no more than 10 times. You will perform two warm-up sets. Two to four subsequent trials will be performed to determine a repetition maximum (RM). If you complete more than 10 repetitions of the exercise with a given external load, you will be provided with 3-minutes of rest, and weight will be added to the external apparatus until you can complete only 10 or fewer repetitions in one set. If you do not the range of motion criteria, the trial will be discarded.

Testing Visit:

- Hydration Status:
 - You will be instructed to be properly hydrated prior to coming to the laboratory prior to your testing visit. Upon arrival to the laboratory, you will be asked to provide a small urine sample in a sterile container. Urine samples will be analyzed for hydration status. If you are not properly hydrated at the time of assessment, you will be asked to drink water and will then asked to provide another urine sample until properly hydrated. There are no risks associated with the measurement of hydration status.
- o Anthropometric Measurements:
 - Body Mass and Height: Body mass and height will be measured using a scale. There are no risks or discomforts associated with the measurement of body weight and height.
 - Body Composition: Body composition will be assessed via bioelectrical impedance analysis (BIA), in which a small current will be sent through the body to measure electrical impedance. You will be asked to refrain from



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consuming food for 4 hours and caffeine/alcohol for 24 hours prior to the visit. Upon testing, you will be asked to remove your footwear, socks, and jewelry. You will be asked to stand on the platform with your heels placed on the circular rear sole electrodes and pick up the handles of the device. The whole procedure will take approximately 2-minutes, and there are no risks or discomforts associated with the use of BIA.

Ultrasound Assessments:

 During the testing visit, five rounds of non-invasive muscle ultrasound images will be captured from the dominant leg vastus lateralis (VL) muscle, which is a muscle of the thigh, by a Graduate student who has been trained in ultrasonography techniques and assessment. Of the five rounds of ultrasound assessments completed, the order of rounds 1/2, 3, and 4 will be randomized. In the first round, you will be asked to lay on a table on your left or right side for examination of the opposite leg, with your legs stacked together. Your legs will be positioned to allow a bend in the knees. Prior to image collection, all anatomical locations of interest will be identified using standardized landmarks. A mark will be made on the surface of your skin, and ultrasound gel will be applied to your leg to help capture the image. You will then be asked to remain in this position for a period of 15-minutes. After the 15-minute duration has elapsed, the ultrasound assessments from the first round will be repeated. Following the first two rounds of ultrasound assessments, you will be asked to stand for a period of 15 minutes. After the 15-minute duration has elapsed, you will be asked to lay on your back on a table for a period of 15-minutes. After this 15-minute duration has elapsed, you will be instructed to lay on your left or right side to go through another round of ultrasound assessments, identical to those used in the first round. Following the third round of assessments, you will be asked to stand for a period of 15 minutes. After the 15-minute duration has elapsed, you will be asked to lay on the opposite side that you were originally positioned on an examination table for a period of 15-minutes. After this 15minute duration has elapsed, you will be instructed to lay back on the original side to go through another round of ultrasound assessments, identical to those used in the first round. Following the fourth round of assessments, you will be asked to stand for a period of 15 minutes. After the 15-minute duration has elapsed, you will be asked to stand on your non-dominant limb for ultrasound analysis of the VL of the dominant limb in the standing position. Ultrasound assessments will be captured while you are standing on the non-dominant limb that are identical to those captured in the first round. Ultrasound images will be later analyzed for muscle characteristics. There are no potential risks associated with ultrasonography. A sample depiction of ultrasound image capture is included below.



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Prior to the exercise assessments, you will be provided with a standardized snack (kcal: 150; protein: 2g; carbohydrates: 23g; fat: 5g) to prevent the effects of low blood sugar on exercise performance. You will then be required to complete a standardized dynamic warm-up. You will first pedal on a cycle ergometer for 5-minutes at a self-selected pace. You will then complete ten body-weight squats, ten body-weight walking lunges, ten dynamic walking hamstring stretches, ten dynamic walking quadriceps stretches, ten arm circles, and ten arm swings.

- Vertical Jump Assessment:
 - The VJ assessments to be completed during the testing session will be identical to those completed during the familiarization session.
- Biodex Assessments:
 - The isokinetic and isometric assessments to be completed during the testing session will be identical to the familiarization session.
- Unilateral Leg Press Assessment:
 - The unilateral leg press assessment to be completed during the testing session will utilize the external apparatus weight that was established in the familiarization session.





You are free to withdraw your consent and discontinue participation in this study at any time without prejudice or penalty. Your decision to participate or not participate in this study will in no way affect your continued enrollment, grades, employment or your relationship with the individuals who may have an interest in this study.

What happens if I say yes, but I change my mind later?

You can leave the research at any time it will not be held against you. If you decide to leave the research, contact the investigator so that the investigator can remove you from the study schedule. Discontinuation of participation may occur at any time. You have the right to discontinue participation without penalty, regardless of the status of the study. If you decide to leave the study, your data will



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not be included in analyses for publication. Data that is collected prior to withdrawal from the study will be discarded and not used or distributed to anyone.

Is there any way being in this study could be bad for me?

No risks are associated with anthropometric or ultrasonography testing. The exercise assessments carry the same inherent risks as participating in any physical activity, such as muscle soreness and fatigue and possibly muscle strains, and/or joint sprains. To minimize these risks, you will be instructed on appropriate technique for the performance assessments, and you will be required to complete a warmup prior to completing the assessments. Although rare, there is a possibility of a more serious injury when performing unilateral assessments, and individuals with pre-existing, undiagnosed joint conditions may be more prone to these events. However, individuals with known joint conditions will be excluded from participation in the study per the PAR-Q+ and MHAQ. Additionally, all personnel involved in this study are Masters and Doctoral Research Assistants in the University of Central Florida's Human Performance Laboratory. All personnel are CPR certified through the American Red Cross, and many have First Aid certifications through the American Red Cross. Furthermore, all research personnel involved in data collection are experienced in the administration of the proposed assessments, and all Doctoral students involved in this study are Certified Strength and Conditioning Specialists through the National Strength and Conditioning Association. You will be instructed to immediately stop and report any injury or discomfort associated with the performance assessments to a member of the investigative team. The extent of the injury/discomfort, as well as your ability to continue with the study, will be subsequently be determined by the investigative team. If it is deemed that the discomfort/injury will prevent you from completing the study, or if the injury/discomfort may be exacerbated by further participation in the study, the investigative team will suspend your participation in the study.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

All paperwork related to this study will be stored in a locked cabinet during and following the investigation, and all electronically-entered data will be saved in an encrypted file.

Your participant folder and ultrasound images will be marked with an ID number to protect against a breach of confidentiality; your name and ID numbers will be stored separately and apart.

Access to research-related data, paperwork, and records will be limited to appropriate laboratory personnel only.

Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include:

- · Inability to adhere to the study protocol (ex: exercise tests)
- · Failure to adhere to any requirements
- · Failure to complete all visits to the Human Performance Lab
- · You refuse to take part in either familiarization or experimental trial assessment measures



What else do I need to know?

If you need medical care because of taking part in this research study, it will be provided by your primary care physician or the emergency medical system. Generally, this care will be billed to you, your insurance, or other third party. The University of Central Florida's Institute of Exercise Physiology and Wellness has no program to pay for medical care for research-related injury.

If you believe you have been injured during participation in this research project, you may file a claim with UCF Environmental Health & Safety, Risk and Insurance Office, P.O. Box 163500, Orlando, FL 32816-3500, (407) 823-6300. The University of Central Florida is an agency of the State of Florida for purposes of sovereign immunity and the university's and the state's liability for personal injury or property damage is extremely limited under Florida law. Accordingly, the university's and the state's ability to compensate you for any personal injury or property damage suffered during this research project is very limited.

No individual results will be published or shared with any third person or party, including the study sponsor. Individual results will remain confidential and only be relayed to participants upon request following the conclusion of all data collection and analyses.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of subject

Printed name of subject

Signature of person obtaining consent

Date

Date

Printed name of person obtaining consent

My signature below documents that the information in the consent document and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

Signature of witness to consent process

Date

Printed name of person witnessing consent process



APPENDIX C: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q+)

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

1) Has your doctor ever said that you have a heart condition OOB high blood pressure O?	YES	NC
This you doctor even suid that you have a hear condition of ontright blood pressure of		C
2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?		
3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 month Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	³⁷ D	C
4) Have you ever been diagnosed with another chronic medical condition (other than heart dise or high blood pressure)? PLEASE LIST CONDITION(S) HERE:	se 🛛	C
5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(5) AND MEDICATIONS HERE:	0	С
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physical active? Please answer NO if you had a problem in the past, but it <i>does not limit your current ability</i> to be physically PLEASE LIST CONDITION(5) HERE:	ly tive.	c
		C
7) Has your doctor ever said that you should only do medically supervised physical activity? If you answered NO to all of the questions above, you are cleared for physical activit Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete	ages 2 an	d 3
 7) Has your doctor ever said that you should only do medically supervised physical activity? If you answered NO to all of the questions above, you are cleared for physical activity Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Start becoming much more physically active – start slowly and build up gradually. Follow International Physical Activity Guidelines for your age (www.who.int/dietphysical You may take part in a health and fitness appraisal. If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal of consult a qualified exercise professional before engaging in this intensity of exercise. 	activity/en	/). ise,

Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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1.	Do you have Arthritis, Osteoporosis, or Back Problems?	
la.	If the above condition(s) is/are present, answer questions 1a-1c If NO go to question 2 Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?	YES NO
1b.	(Answer NO if you are not currently taking medications or other treatments) Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer,	UNSCOUND AND A DOMESTICS
	displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES NO
1C.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES NO
2.	Do you currently have Cancer of any kind?	
	If the above condition(s) is/are present, answer questions 2a-2b If NO go to question 3	
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	
2b,	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES NO
3.	Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure Diagnosed Abnormality of Heart Rhythm	2,
	If the above condition(s) is/are present, answer questions 3a-3d If NO 🗌 go to question 4	
3a,	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	YES NO
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES NO
3C.	Do you have chronic heart failure?	YES NO
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	
4.	Do you have High Blood Pressure?	
	If the above condition(s) is/are present, answer questions 4a-4b If NO go to question 5	
4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	YES NO
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	YES NO
5.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	
	If the above condition(s) is/are present, answer questions 5a-5e If NO 🗍 go to question 6	
5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician- prescribed therapies?	YES NO
sb.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	
5C.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	
Sd.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES NO
Se.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES NO

1.	Do you have Arthritis, Osteoporosis, or Back Problems?	
la.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?	YES NO
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the opional relumna).	
IC.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES NO
2	Do you currently have Cancer of any kind?	In so the second
£.	If the above condition(s) is/are present, answer questions 2a-2b If NO go to question 3	
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	
2b,	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES NO
3.	Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure Diagnosed Abnormality of Heart Rhythm	2,
	If the above condition(s) is/are present, answer questions 3a-3d If NO go to question 4	
3a,	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	YES NO
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES NO
3C.	Do you have chronic heart failure?	YES NO
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES NO
4.	Do you have High Blood Pressure?	
	If the above condition(s) is/are present, answer questions 4a-4b If NO go to question 5	
4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	YES NO
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	YES NO
5.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	
	If the above condition(s) is/are present, answer questions 5a-5e If NO 🗌 go to question 6	
5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician- prescribed therapies?	YES NO
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES NO
5C.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	YES NO
sd.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES NO
Se,	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES NO

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1	If you answered NO to all of the follow-ty you are ready to become more physical It is advised that you consult a gualified exercis activity plan to meet your health needs.	up questions about your medical condition, ly active - sign the PARTICIPANT DECLARATION below: se professional to help you develop a safe and effective physical
۲	You are encouraged to start slowly and build u 3-5 days per week including aerobic and muscl	p gradually - 20 to 60 minutes of low to moderate intensity exercise, le strengthening exercises.
۲	As you progress, you should aim to accumulate	150 minutes or more of moderate intensity physical activity per week
•	If you are over the age of 45 yr and NOT accust qualified exercise professional before engaging	romed to regular vigorous to maximal effort exercise, consult a g in this intensity of exercise.
	If you answered YES to one or more of You should seek further information before becomin the specially designed online screening and exercise visit a qualified exercise professional to work through	f the follow-up questions about your medical condition of more physically active or engaging in a fitness appraisal. You should complete recommendations program - the ePARmed-X+ at www.eparmedx.com and/o in the ePARmed-X+ and for further information.
A	Delay becoming more active if:	
1	You have a temporary illness such as a cold or f	ever; it is best to wait until you feel better.
V	You are pregnant - talk to your health care prace and/or complete the ePARmed-X+ at www.ep	titioner, your physician, a qualified exercise professional, armedx.com before becoming more physically active.
4	Your health changes - talk to your doctor or qu activity program.	alified exercise professional before continuing with any physical
1		
- All -	PARTIC	IPANT DECLARATION
All p If yo prov	PARTIC persons who have completed the PAR-Q+ please ou are less than the legal age required for conser vider must also sign this form.	IPANT DECLARATION e read and sign the declaration below. nt or require the assent of a care provider, your parent, guardian or care
All ; if yo prov i, thi phys coni or o to lo Trus	PARTIC persons who have completed the PAR-Q+ please ou are less than the legal age required for conservider must also sign this form. e undersigned, have read, understood to my full sical activity clearance is valid for a maximum of dition changes. I also acknowledge that a Truste other designate) may retain a copy of this form for pocal, national, and international guidelines reganst stee maintains the privacy of the information an	IPANT DECLARATION e read and sign the declaration below. Int or require the assent of a care provider, your parent, guardian or care satisfaction and completed this questionnaire. I acknowledge that this f 12 months from the date it is completed and becomes invalid if my re (such as my employer, community/fitness centre, health care provider or their records. In these instances, the Trustee will be required to adhere rding the storage of personal health information ensuring that the d does not misuse or wrongfully disclose such information.
All p If yc prov I, the phys cons or o to lc Trus	PARTIC persons who have completed the PAR-Q+ please ou are less than the legal age required for conser- vider must also sign this form. e undersigned, have read, understood to my full sical activity clearance is valid for a maximum or dition changes. I also acknowledge that a Truste other designate) may retain a copy of this form fo pocal, national, and international guidelines regan stee maintains the privacy of the information an PANT ID	IPANT DECLARATION e read and sign the declaration below. Int or require the assent of a care provider, your parent, guardian or care satisfaction and completed this questionnaire. I acknowledge that this f 12 months from the date it is completed and becomes invalid if my re (such as my employer, community/fitness centre, health care provider or their records. In these instances, the Trustee will be required to adhere rding the storage of personal health information ensuring that the d does not misuse or wrongfully disclose such information. DATE
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APPENDIX D: MEDICAL HISTORY AND ACTIVITY QUESTIONNAIRE (MHAQ)

connuc	ntial Medical and Activity History Questionnaire
Participant #	Date:
What is your age?	What is your birth month/year?/
Are you willing to main the study) for the entire	ntain the same dietary, supplement, and exercise habits (besides what is done in time that you are enrolled in the study?
Are you currently invo perform? List all activi	lved in resistance training (lifting weights, using machines) that you consistently ties in the past year.
Pleas <mark>e list any sports/a</mark> e activities in the past ye	ctivities that you have a history of and are currently involved with. List all ar.

List any medications, herbals, or supple you currently take or have taken the las	ements (vitamins, ergogenic aids, performance-enhancing drugs) st two months:
Medication/Supplement	Reason for medication/supplement
12	
a	
Are you allergic to any medications? If	f yes, please list medications and reaction.
Please list any allergies, including food	allergies that you may have.
Have you ever been been talized? If we	a alagaa amalaja
nave you ever been nosphanzeu? II ye	s, please explain.
Year of hospitalization	<u>Reason</u>
Year of hospitalization	Reason
Year of hospitalization	<u>Reason</u>
Year of hospitalization	Reason
Year of hospitalization	Reason
Year of hospitalization	s, please explain. <u>Reason</u>
Year of hospitalization	hat have caused you to seek medical care.
Year of hospitalization	hat have caused you to seek medical care.
Year of hospitalization	hat have caused you to seek medical care.
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Have you ever had (or do you have now) active malignant disease or cancer. If yes, please explain.

Have you ever had (or do you have scheduled) any procedure Iodine, Banum, or Nuclear Medicine Isotopes? (CT and PET scans are examples) If yes, please specify the date of the procedure.

Have you ever had (or do you have now) any of the following? If "yes", please provide an explanation next to the question. Please circle questions that you do not know the answer to.

Cystic fibrosis	yes	no
Water retention problems	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High cholesterol	yes	no
Rheumatic fever	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Inflammatory bowel disease	yes	no

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Gastrointestinal disease	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Dementia	yes	no
Artificial limb	yes	no
Alzheimer's	yes	no
Uncontrolled diarrhea/nausea/vomiting	yes	no
Range of motion restrictions	yes	no
Cardiovascular Disease		
Peripheral vascular disease	yes	no
Cerebrovascular disease	yes	no
Coronary artery disease	yes	no
Aortic stenosis	yes	no
Congestive heart failure	yes	no
Artenial fibrilation	yes	no
"Heart block"	yes	no
Myocardial infarction (Heart attack)	yes	no
Poorly controlled hypertension	yes	no
Heart pacemaker	yes	no
High blood pressure	yes	110
Heart murmur	yes	no
Pulmonary disease		
Chronic obstructive pulmonary disease	yes	no
Asthma	yes	no
Interstitial lung disease	yes	no
Emphysema	yes	no
Ascites/ pleural effusion	yes	no

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Metabolic disorder		
Diabetes mellitus (type 1, type 2)	yes	no
Diabetes insipidus	yes	no
Thyroid disorders	yes	no
Renal disease	yes	no
Liver disease	yes	no
Immunodeficiency disorder	yes	no

Any others (specify):

Do you smoke cigarettes or use any other tobacco products?	yes	no
Do you have a history of drug or alcohol dependency?	yes	no
Has your doctor ever said you have a heart condition and that you should only do physical activity recommended by a	140,000-00	
doctor?	yes	no
Do you feel any pain in your chest when you do physical activity?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeats?	yes	no
Do you ever have any arm or jaw discomfort, nausea, or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you lose your balance because of dizziness or		
do you ever lose consciousness?	yes	no
Are you pregnant?	yes	no
Is there a chance that you may be pregnant?	yes	no
Have you ever had any tingling or numbness in		
your arms or legs?	yes	110
Has a member of your family or close relative		

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died of heart problems or sudden death before the age of 50?	yes	no
Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?	yes	no
Do you have a bone or joint problem that could be made worse by a change in your physical activity?	yes	no
Has a health care practitioner ever denied or restricted your participation in exercise for any problem?	yes	no
Do you know any other reason why you should not do physical activity?	yes	no

APPENDIX E: TESTING DAY DATA COLLECTION SHEET

2	Ultrasound F	lest Study	
	Familiari	zation	
Informed Consent	PAR-Q+	MHAQ	
Date://	Time:		
Dominant Leg: L R (circle on	e)		
Warm-Up			
Unilateral Vertical Jump (at least 3)			
Biodex Seat height (vertical): Ankle position: Left/right position: Front/back position: For/aft position: For/aft position: For/aft position: MVIC 1 3-min rest MVIC 2 3-min rest Isokinetic @ 60 °/s x 3 3-min rest Isokinetic @ 180 °/s x 3 3-min rest Isokinetic @ 240 °/s x 3			
3-RM Estimated Leg Press Warm-Up 1 Load (8-10 reps): <i>1-min rest</i> Warm-Up 2 Load (6-8 reps): 2-min rest Warm-Up 2 Load (4-6 reps):			
3-RM Attempt 1 Load (3 reps) 3-min rest 3-RM Attempt 2 Load (3 reps) 3-min rest			
3-RM Attempt 3 Load (3 reps) Brzycki equation: Weight / [1.0278 – (0.	0278 x Number of re	petitions)]	
	Ultrasound Rest Study Testing Day	ŧ,	
---	--------------------------------------	----	
Date: / / Tim	ne: Age:years DOB://		
Last meal: >4 hours?	Y N		
24-hour Alcohol Abstention: Y	N 24-hour Caffeine Abstention: Y N		
Last time exercising:	>72 hours? Y N		
USG: (1) (2)(3)		
Mark Leg: Leg length:	:cm		
Height:cm	Body Mass: kg		
BIA Percent Body Fat:	% Print BIA Sheet:		
Supine: Non-Dominant Lateral Recumbe Standing:5 Supine: Time laying down: Plus 15-min time: Actual time at begin Non-Dominant Lateral Recum Time laying down: IP time of assessme Plus 15-min time: Actual time at begin Dominant Lateral Recum Time laying down: Plus 15-min time:	ent:		
Actual time at begin	inning of assessment:		
Time standing:			
Plus 15-min time: Actual time at begi	inning of assessment:		

Subject #: Ultrasound Rest Study Testing Day Warm-Up Biodex **Unilateral Vertical Jump** Isokinetic @ 60 °/s x 3: Jump 1: Avg. Peak Torque: _____N Avg. Mean Torque: _____N Height: cm N Peak Force: W 3-min rest Peak Power: Isokinetic @ 180 °/s x 3: Total Work: I Avg. Peak Torque: _____ N Avg. Mean Torque: _____ N Peak Velocity: m/s RPD: _____N/s 3-min rest 3-min rest Isokinetic @ 240 °/s x 3: Jump 2: Avg. Peak Torque: ____ N Avg. Mean Torque: ____ N Height: cm Peak Force: N Peak Power: _____ W 1-RM Leg Press Total Work: J Warm-Up 1 Load (8-10 reps): Peak Velocity: m/s 1-min rest RPD: _____N/s Warm-Up 2 Load (4-6 reps): 3-min rest 2-min rest Jump 3: Warm-Up 2 Load (2-3 reps): Height: cm N Peak Force: 3-min rest W 1-RM Attempt 1 Load (1 rep): Peak Power: 3-min rest Total Work: J 1-RM Attempt 2 Load (1 rep): Peak Velocity: m/s 3-min rest RPD: N/s 1-RM Attempt 3 Load (1 rep): Biodex MVIC 1: Peak Torque: _____N Mean Torque: N 3-min rest MVIC 2: Peak Torque: N Mean Torque: N 3-min rest

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