

# INTRAMUSCULAR FAT: WHAT CAN MUSCLE ECHO INTENSITY TELL US

by

HUI-JU YOUNG

(Under the Direction of Kevin K. McCully)

## ABSTRACT

Skeletal muscle ultrasound has recently emerged as one of the imaging techniques to examine muscle quality. Validation of the ultrasound technique and quantification of muscle echo intensity (EI) to units that are consistent with other body composition measures are necessary. **Purpose:** The purpose of these studies was to validate the ultrasound technique with high-resolution T1-weighted magnetic resonance imaging (MRI). The second purpose was to compare ultrasound-measured % intramuscular fat to other health and fitness indicators. **Methods:** Two experiments were performed. The first experiment examined four muscles in the lower extremity using both ultrasound and MRI. Muscle EI and MRI-measured % intramuscular fat of each muscle were compared and used to establish calibration equations. The second experiment applied the calibration equations to convert EI to % intramuscular fat and compared the ultrasound-measured % intramuscular fat to some health and fitness measures. **Results:** In the first study, three types of calibration equations were established based on the strong correlations found between MRI % intramuscular fat and muscle EI after correcting for subcutaneous fat thickness ( $r \geq 0.8$  in all four muscles). The ultrasound technique also demonstrated high between-day reproducibility ( $r \geq 0.8$  in all four muscles) and inter-

analyzer reliability ( $r = 0.9$  in all four muscles). In the second study, strong correlations were found between ultrasound-measured % intramuscular fat of the four muscles ( $r \geq 0.8$ ). Weak to moderate correlations were found when compared % intramuscular fat to BMI, waist/hip ratio, muscle thickness, and muscular strength. Relationships between ultrasound –measured % intramuscular fat, physical activity level, and frequency of fast food consumption were also observed. **Conclusion:** Muscle ultrasound is a practical technique to examine muscle health when other imaging techniques are not available. Future studies are needed to validate the calibration equations presented in these studies as well as establish more equations for other muscles to enhance its use in both the research and clinical settings. More studies are also needed to investigate the complex relationship between intramuscular fat and lifestyle.

**INDEX WORDS:** Muscle echo intensity, Intramuscular fat, Ultrasound, MRI, Calibration equation

INTRAMUSCULAR FAT: WHAT CAN MUSCLE ECHO INTENSITY TELL US

by

HUI-JU YOUNG

B.S.Ed., The University of Georgia, 2010

M.S., The University of Georgia, 2011

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial  
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

© 2014

Hui-Ju Young

All Rights Reserved

INTRAMUSCULAR FAT: WHAT CAN MUSCLE ECHO INTENSITY TELL US

by

HUI-JU YOUNG

Major Professor: Kevin McCully

Committee: Mary Ann Johnson  
Qun Zhao  
Nathan Jenkins

Electronic Version Approved:

Julie Coffield  
Interim Dean of the Graduate School  
The University of Georgia  
August 2014

## DEDICATION

To all my family across the world, thank you for all the countless encouragement and laughter. To my grandparents, I think about you each and every day and you will always be on my mind. To my parents, Shih-Shung Young, Chen-Ing Chen, and my sister, Shih-Ju Young, thank you for your selfless love and support. Without you, I would not be the person I am today

## ACKNOWLEDGEMENTS

I would like to first acknowledge all the participants and students from the disability wellness class for serving as my stress reliever and for giving me all the courage and motivation to move forward. I would like to thank my past and current labmates, especially Melissa Erickson, Michael Southern, Jared Brizentine, Terence Ryan, Mary Ann Reynolds, and Kristine Polley for their countless help and support. I would also like to thank the undergraduate students who worked in our lab, especially Catherine Zhang and Nehemiah Lawson for their assistance with the image analysis. I would like to thank Kim Mason from the Bio-imaging Research Center for all her help on the MRI data collection. I would like to thank Dr. Lesly White, Dr. Ted Baumgartner, and Dr. Jonathan Murrow for all the guidance and advice they have given to me. I would like to specially thank Dr. Mary Ann Johnson, Dr. Qun Zhao, and Dr. Nathan Jenkins for serving on my committee and for all the advice they have given to me and for being such great mentors. Last but not least, I would like give my greatest appreciation to Dr. Kevin McCully for bringing me into the field of research, for always being there, and for guiding me to see the importance to improve the quality of life for individuals with disabilities.

## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS .....	v
LIST OF TABLES .....	vii
LIST OF FIGURES .....	ix
CHAPTER	
1 INTRODUCTION .....	1
Purpose of the Study .....	3
Hypotheses .....	4
Significance of the Study .....	4
2 REVIEW OF LITERATURE .....	6
Obesity and Adipose Tissue.....	6
Distribution of Adipose Tissue .....	9
Techniques for Assessing Body Composition .....	10
Skeletal Muscle Ultrasound .....	13
References.....	20
3 VALIDATION OF MUSCLE ECHO INTENSITY WITH MAGNETIC RESONANCE IMAGING TECHNIQUE.....	33
Abstract .....	34
Introduction.....	35
Materials and Methods.....	36



Results.....	42
Discussion.....	45
References.....	52
4 COMPARISONS OF ULTRASOUND-MEASURED PERCENT INTRAMUSCULAR FAT TO HEALTH AND FITNESS INDICATORS....	69
Abstract.....	70
Introduction.....	71
Materials and Methods.....	72
Results.....	76
Discussion.....	80
References.....	85
5 SUMMARY AND CONCLUSIONS .....	102
References.....	109

## LIST OF TABLES

	Page
Table 3.1: Physical characteristics of the participants .....	57
Table 3.2: Outcomes of ultrasound and MRI testing .....	58
Table 3.3: Between-day reproducibility of the ultrasound technique .....	59
Table 3.4: Inter-analyzer reliability of the ultrasound technique .....	60
Table 4.1: Physical characteristics of study participants .....	90
Table 4.2: Comparisons between scanning locations .....	91
Table 4.3: Correlations of ultrasound % fat between muscle groups .....	92
Table 4.4: Correlations between ultrasound % fat and body composition measures .....	93
Table 4.5: Correlations between ultrasound % fat controlled by age and gender and other body composition measures .....	94
Table 4.6: Correlations between ultrasound % fat and physical activity level .....	95
Table 4.7: Correlations between mean ultrasound % fat, PA level and diet .....	96
Table 4.8: Correlations between mean ultrasound % fat and blood glucose and lipid profile .....	97

## LIST OF FIGURES

	Page
Figure 2.1: T <sub>1</sub> -weighted MRI images showing intramuscular fat difference between two individuals with same amount of subcutaneous fat.....	11
Figure 2.2: An ultrasound B-mode image showing the structure of skeletal muscle .....	14
Figure 2.3: A representative picture showing the action of ultrasound wave traveling down to the skeletal muscle.....	15
Figure 2.4: Representative ultrasound B-mode images comparing muscle EI between a healthy individual and an individual with severe health conditions .....	17
Figure 3.1: Correlations between subcutaneous fat thickness and muscle EI .....	61
Figure 3.2: Representative T <sub>1</sub> -weighted MRI and ultrasound B-mode images show the muscle site comparability between two imaging techniques .....	62
Figure 3.3: Correlations between MRI-measured % intramuscular fat and muscle echo intensity in the four muscle groups .....	63
Figure 3.4: Correlations between MRI-measured % intramuscular fat and muscle EI after correcting for subcutaneous fat thickness in the four muscle groups.....	64
Figure 3.5: Correlations between MRI-measured % intramuscular fat and muscle EI after correcting for subcutaneous fat thickness in the four muscle groups, separated by gender .....	65
Figure 3.6: A correlation graph between MRI-measured % intramuscular fat and corrected muscle EI of all four muscles .....	66

Figure 3.7: Differences between MRI-measured % fat and ultrasound % fat calculated using the three types of calibration equations .....	67
Figure 3.8: Two representative graphs showing variability of muscle EIs between two different scanning sites within a muscle (A) as well as variability of % intramuscular fat between the three MRI slices (B).....	68
Figure 4.1: Correlation graphs that demonstrate the relationship between ultrasound-measured intramuscular fat and BMI (A) and between ultrasound-measured intramuscular fat and waist and hip ratio (B) .....	98
Figure 4.2: Correlation graphs that demonstrate the relationship between ultrasound-measured intramuscular and muscular strength .....	99
Figure 4.3: Comparisons between ultrasound-measured intramuscular fat of three physical activity levels: low, moderate, and vigorous, and three levels of fast food consumption: < 1 time/week, 1 time/week, and >1 time/week.....	100
Figure 4.4: Comparisons between ultrasound-measured intramuscular fat of two physical activity levels: low + moderate group and vigorous, and two levels of fast food consumption frequency: $\leq 1$ time/week and $> 1$ time/week .....	101

## CHAPTER 1

### INTRODUCTION

Obesity is associated with many chronic diseases and disabilities. It is one of the leading causes of morbidity and mortality in the world. Previous studies have indicated that increased total adiposity is correlated with reduced skeletal muscle mass and insulin resistance in both children and adults, which can contribute to higher risk of cardiovascular and metabolic disease (16, 80). A growing body of research suggests that obesity is no longer just about the number and volume of adipocytes (13, 14). As different health risk profiles are presented in individuals with the same amount of body fat, we now know that the distribution of adipose tissue also plays a crucial role in determining the health consequences associated with obesity (37, 45). For example, impaired insulin sensitivity and higher risk of type-2 diabetes have been found to be associated with increased fat depots within or between skeletal muscle fibers (70, 91). All of the evidence together indicates the importance to examine the distribution of adipose tissue.

Skeletal muscle ultrasound has recently emerged as one of the imaging techniques to measure muscle quality or muscle health (75). When a beam of ultrasound waves travels into a muscle and encounters different tissues within the muscle, attenuation of the ultrasound waves occurs due to reflection, scattering, refraction, and absorption (52, 75, 96). The amount of reflection, scattering, refraction, and absorption is based on the density of a tissue and the acoustic impedance between two different tissues (52, 59). A

lean muscle tissue has low echogenicity while intramuscular fat and connective tissue have high echogenicity. As a result, when a muscle contains more adipose or fibrous tissue, it appears to be brighter on an ultrasound brightness mode (B-mode) scan when compared to a muscle with less adipose or fibrous tissue. In the past, quantification of muscle echo intensity (EI) was based on visual evaluation, which largely depended on the experience of the evaluators and the results could then be less accurate and more subjective. Approximately a decade ago, studies began to use gray-scale histogram to quantify muscle EI with mean pixel intensity as a more reliable and objective analysis method (9, 20, 75). The same principle applies: a muscle with higher mean pixel intensity indicates more intramuscular fat or fibrous tissue within that muscle.

Ultrasound is relatively cheap, portable, and can be applied on all individuals (71). For individuals who cannot undergo magnetic resonance imaging (MRI), computed tomography (CT) scan, or dual-energy X-ray absorptiometry (DXA) scan due to factors such as metal implant, radiation exposure, or muscle spasticity, the ultrasound technique can be utilized as an alternative way to assess their muscle health. Previous studies have shown that muscle EI is negatively associated with muscular strength, muscular power, and cardiovascular health in people across all age groups (9, 20). These findings suggest the potential capability of the ultrasound technique for classifying individuals based on their fitness and functionality. While a growing number of studies have been utilizing this ultrasound technique, to our knowledge, no study has validated muscle EI to % intramuscular fat obtained from other imaging techniques. Animal biopsy studies have shown positive correlations between intramuscular lipid content, fibrous tissue, and muscle EI (33, 39, 67, 74). While the findings are informative, questions such as the

representativeness of biopsy sample of the entire muscle group as well as the confirmation of the results obtained from animal studies in human subjects remain to be addressed. Moreover, the current gray-scale analysis method reports mean pixel intensity in arbitrary units, which makes comparison between the results obtained from the ultrasound technique and other body or muscle composition measures difficult. In order to enhance the application of this ultrasound technique in both the research and clinical settings, quantifying muscle EI to a physiological unit that allows it to be comparable to other body and muscle composition techniques is necessary.

## **Purposes**

There is a need to increase our understanding of the ultrasound EI technique. With the low cost, practicality, and portability of ultrasound technology, improvement of the ultrasound technique can make it a valuable imaging tool for assessment of muscle health. The goals of this research study were to compare the ultrasound technique with MRI, and to establish calibration equations to allow for better comparisons between the ultrasound technique and other body and muscle composition measures. Two experiments were performed. The purpose of the first experiment was to provide validation for the ultrasound technique by examining the relationship between muscle EI and % intramuscular fat measured with MRI in four muscles: rectus femoris, bicep femoris, tibialis anterior, and medial gastrocnemius. Tests of reproducibility and reliability of the ultrasound technique were performed. The first experiment also aimed to establish calibration equations to quantify muscle EI into % intramuscular fat. The purpose of the second experiment was to examine the associations between %

intramuscular fat measured with ultrasound, other body composition measures, physical activity level, dietary intake, muscular strength, and blood glucose and lipid profile. The results of the second experiment were also compared to previous literature.

## **Hypotheses**

The hypotheses for the first experiment are:

- H<sub>1</sub>) Muscle EI of the four muscles in the lower extremity will be significantly correlated with their % intramuscular fat measured with MRI.
- H<sub>2</sub>) The ultrasound EI technique will be reproducible, both between testing days and between image-analyzing investigators.

The hypotheses for the second experiment are:

- H<sub>1</sub>) The % intramuscular fat measured with ultrasound will be strongly correlated between the four muscles, consistent with previous literature.
- H<sub>2</sub>) The % intramuscular fat measured with ultrasound will be positively associated with BMI, waist-hip ratio, and muscle thickness, comparable to previous literature.
- H<sub>3</sub>) Higher % intramuscular fat will be associated with lower muscular strength, more physical activity, and poorer diet.

## **Significance of the Study**

Although studies have reported the correlations of muscle EI measured with other health and fitness parameters, to our knowledge, no study has examined the relationship between muscle EI and % intramuscular fat measured with other imaging techniques on human subjects. In addition, the key limitation to previous studies is the lack of



compatible measurement units between the results obtained from the ultrasound and other body or muscle composition techniques. The present study aims to validate this ultrasound technique as well as establish calibration methods to quantify muscle EI into % intramuscular fat. The success of this study will increase our knowledge on muscle EI in human skeletal muscle. It will also enhance the practicality of this ultrasound technique in the field of obesity research.

## CHAPTER 2

### REVIEW OF LITERATURE

Obesity is considered to be one of the major contributors to many health conditions and disabilities. Evidence has suggested the importance of identifying distribution of fat depots, given its association with different health risk profiles (1, 13, 14, 26). Skeletal muscle is the largest organ in the human body (11). Increased fat depots within skeletal muscle have been shown to be associated with reduced lean muscle mass and muscular strength, and thus affects the functionality of an individual (54, 85, 93). Furthermore, previous studies have suggested that increased intramuscular fat alters normal muscle metabolism, which contributes to several pathologies such as insulin resistance and type 2 diabetes (72, 85, 92). This review of literature will describe obesity and adipose tissue, distribution of adipose tissue, common non-invasive techniques used to assess body composition, and a skeletal muscle ultrasound technique used for examination of muscle health. A discussion on the limitations of the current ultrasound technique will also be presented.

#### **Obesity and Adipose Tissue**

Obesity is generally defined as having excessive body fat (13). It is one of the leading causes of morbidity and mortality in the world. According to the World Health Organization (99), approximately 35% of adults worldwide were overweight in

2008. Among them, 11% were classified as obese. Obesity is associated with several secondary health conditions such as cardiovascular diseases, hypertension, metabolic syndrome, cancers, and many other disabilities (29, 31, 50, 64, 78) and thus making prevention and early interventions critical.

A combination of contributing factors leads to obesity. The equation of obesity is rather complex, but it can also be explained in a simplified definition: energy balance (38, 65). A positive energy balance means that the calories that an individual consumes are more than what the individual spends. When a positive energy balance is maintained for a period of time, overweight or obese can occur (21, 38). The energy balance equation consists of two major factors: physical activity and energy intake. As explained by the equation, sedentary lifestyle can lead to obesity (46). Physical inactivity can lead to loss in muscle mass and accumulation of lipid within muscle, and thus contributing to functional decline and chronic diseases (54, 58, 63, 85, 93). Leitzmann et al. examined the level of physical activity in 252925 males and females using self-reported questionnaires. They concluded that individuals who did not meet the physical activity guidelines were predisposed to higher risk of mortality when compared to their physically active counterparts (57). Unhealthy diet can also lead to obesity and metabolic conditions (84, 95). Several studies have reported a positive correlation between fast food consumption and body mass index among children and teenagers (10, 19). High fat diet has also been found to be associated with larger waist circumference (66, 94). In addition, studies have also shown a significant increase in intramuscular triglyceride content with high-fat feeding in both animals and humans (66, 87, 88). As Davey stated (12), the environment we

live in often provides more opportunities for people to consume more than what they actually need. Factors such as larger portion size, cheap fast food items, and the convenience of purchasing prepackaged food can all promote unfavorable eating habits.

Does this mean that individuals who are overweight or obese should always be considered as unfit? Obesity paradox is used to describe a phenomenon that obese individuals with chronic health conditions have better survival rate when compared to their normal weight counterparts (18, 61). Lavie et al. studied 209 patients with heart failure and reported that the patients with higher total body fat and BMI demonstrated higher survival rate when compared to those with normal BMI (55). In addition, as McAuley et al. stated in their review paper, people who were obese but physically fit were at no higher risk for cardiovascular disease and all-cause mortality when compared to their non-obese counterparts (61). These studies suggest that fitness serves as a better predictor of mortality. In addition, as McAuley et al. pointed out in their paper, factors such as blood pressure, lipid profile, insulin sensitivity, and other health markers that are often related to higher BMI need to be considered before determining the presence of healthy obesity (61). In other words, although being obese can have negative effect on health, defining obesity by the total adiposity can be misleading. As mentioned earlier, physical inactivity and unhealthy diet are associated with increased lipid content within muscle and insulin resistance. These all together support the importance of examining fat distribution.

There are different types of fat. The predominant type of fat found in human adults is white fat, which comprises adipocytes, pre-adipocytes, macrophages,

endothelial cells, fibroblasts, and leukocytes (30, 47, 100). Adipose tissue or adipocytes have long been recognized as glycogen and fat storage cells (47, 100). Their additional roles in metabolic control and as endocrine organs were not identified until recently (30). Adipose tissue secretes different types of adipokines such as leptin and adiponectin (2). Leptin was identified by its role on increasing metabolism and promote weight loss. Reduction in adiponectin concentration was also found to be associated with excessive secretion of insulin and insulin resistant (2, 3). Adipose tissues can also produce proinflammatory proteins or cytokines such as interleukin-6 and tumornecrosis factor- $\alpha$  (2). Studies have suggested that increased expression of those cytokines is related to obesity (48, 79). Furthermore, previous literature has indicated that macrophages in adipose tissue are responsible for the increased expression of those cytokines. This finding suggests a role for adipose tissue inflammation in developing insulin resistance and health complications associated with obesity (22, 36, 68).

### **Distribution of Adipose Tissue**

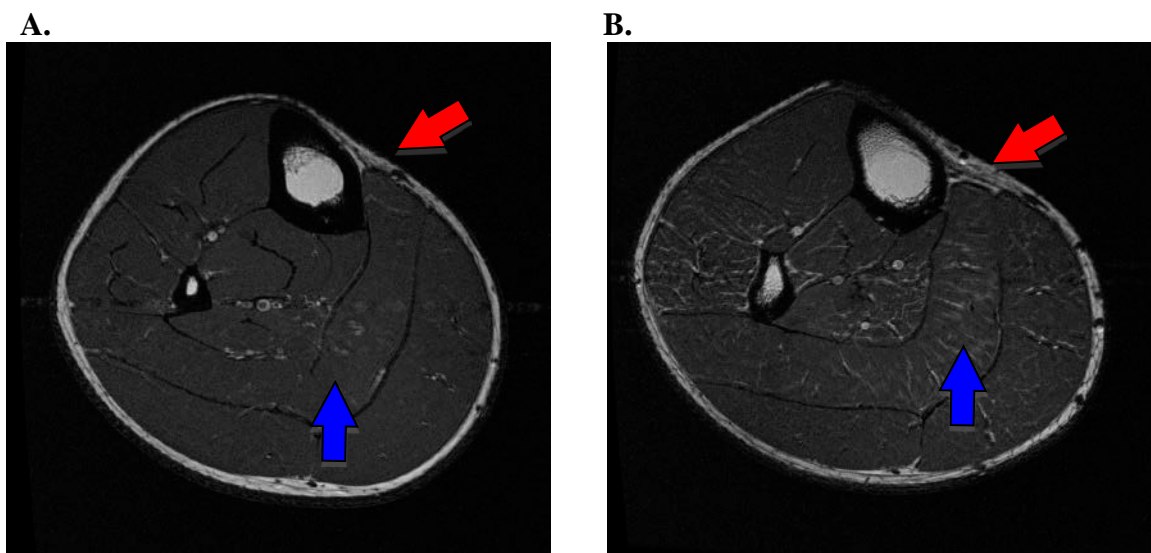
The associations of increased total adiposity and health consequences such as cardiovascular disease, diabetes, and impaired mobility have been well studied (31, 41, 62). Previous studies have suggested that the locations of fat depots are associated with different disease risk profiles (37, 45). The distribution of adipose tissue results from many factors such as gender, age, genotype, lifestyle, hormones, and drugs (43), which makes risk stratification of obesity difficult. Previous studies have suggested that fat depots in the upper trunk and abdominal area are associated with unfavorable

health profiles (23, 43, 89). Despres et al. reported significantly different disease profiles on obese subjects who had the same amount of body fat. They found an abnormal metabolic risk profile in individuals with higher visceral adipose tissue while those with higher subcutaneous adipose tissue demonstrated normal health profile (13, 14). Koster et al. found higher abdominal visceral fat and less thigh subcutaneous fat in older obese people with metabolic syndrome when compared to those without metabolic syndrome, given both groups had the same amount of body fat mass (51). Studies have also found that fat depots within or between skeletal muscle fibers are associated with reduced muscle mass and increased insulin resistance, and can contribute to developing type 2 diabetes (70). Thamer et al. utilized proton magnetic resonance spectroscopy to examine lipids within muscle fibers and found a negative correlation between intramuscular fat and insulin sensitivity in untrained subjects (91). These findings highlight the importance to examining the locations of fat depots for better prediction of health risk profiles.

### **Techniques of Assessing Body Composition**

Early studies utilized biopsy to investigate the relationship between intramyocellular lipid and insulin sensitivity in both animal and human subjects. Storlien et al. reported a negative correlation between insulin sensitivity and mean muscle triglyceride accumulation in rats after high-fat feeding (90). Pan et al investigated vastus lateralis muscles using percutaneous biopsy and found that a significant relationship between increased muscle triglyceride and impaired insulin action in humans (70). While biopsies provide direct examination of muscle lipid content, this technique was limited

by its invasive nature. Several simple methods have been developed to assess body composition. These include but are not limited to BMI, waist circumference, and waist-to-hip ratio (15). BMI is perhaps the most commonly used method to classify individuals into different categories based on the combination of their body weight and height (49). BMI, expressed in  $\text{kg}/\text{m}^2$ , is a simple method that can give a general idea regarding the body composition of an individual at no cost. However, it does not provide information on fat distribution. Waist circumference and waist-to-hip ratio are measures of central adiposity, which are easy to obtain and cost-effective. They are believed to be better predictors than BMI for obesity-related health conditions by providing a good estimation on general body fat distribution (56). The lack of the ability to determine the actual locations of fat depots limits their usage. As demonstrated by Figure 2.1A and 1B, individuals can have significantly different intramuscular lipid content (blue arrows) while having similar amount of subcutaneous fat (red arrows).



**Figure 2.1**

When this is the case, the body composition results obtained from the methods mentioned above can be misleading. Utilizing techniques that can indicate the locations of fat deposits is important, as different fat distribution is associated with different health risk profiles.

Non-invasive techniques such as dual-energy X-ray absorptiometry (DXA), computed tomography (CT), proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$  MRS), and magnetic resonance imaging (MRI) have been utilized to measure total body composition as well as regional fat and intramuscular lipid content (32, 43). Each technique has its own advantages and limitations. DXA has been widely used in the research setting due to its relatively low cost. However, its % body fat result is obtained by using some generalized equations that convert body density or resistance into % fat, and thus is considered to be an indirect measure (40). It also contains a small dose of radiation that is not suitable for individuals who are pregnant or cannot go under radiation. CT imaging utilizes the attenuation characteristics of different tissues to examine the composition of skeletal muscle (6, 8, 25, 28). Fat has lower attenuation coefficient than other tissues and thus can be distinguished by CT imaging technique (5). Unfortunately, CT scans involve ionizing radiation and thus are not a preferred option for repeated measurements (75, 98).  $^1\text{H}$  MRS distinguishes lipids contained within and outside of muscle fibers by utilizing their different signal attributable to protons (7). Krssak et al. used localized  $^1\text{H}$  MRS to examine in human soleus muscle and found an inverse correlation between intramyocellular lipid concentration and insulin sensitivity (53). While  $^1\text{H}$  MRS has the advantage of providing chemical composition of the examined muscle tissues, it is limited by its

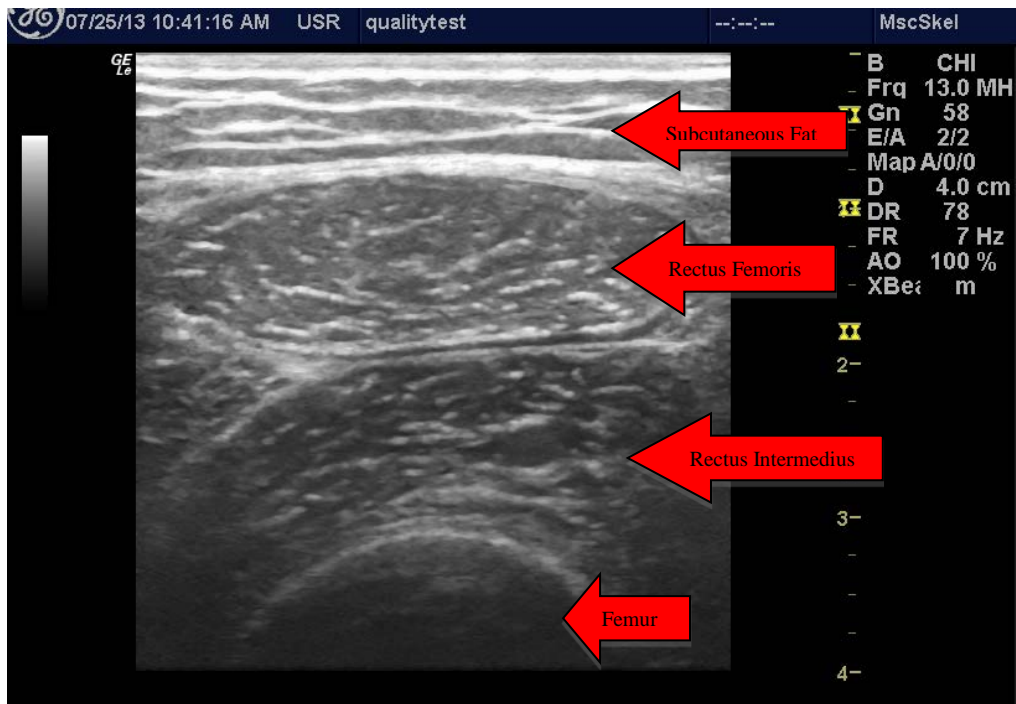


practicality (69). MRI allows visualization and quantification of lipid content within and between muscle groups non-invasively (43, 75). As every cell in human body contains protons, MRI works by aligning the protons to a large magnetic field followed by proton excitation with radio frequency pulses. It then detects the time it takes for the protons to relax back to the original direction (17). There are two common types of MRI images: T<sub>1</sub> and T<sub>2</sub> weighted images. T<sub>1</sub> weighted images detect the longitudinal relaxation time of different tissues in the body while T<sub>2</sub> weighted images detect the transverse relaxation time of the tissues (17). As Goodpaster et al. pointed out the advantages of MRI in their paper, this imaging technique did not depend on the muscle fiber orientation, nor was it limited to certain muscle groups with each scan (27). Although MRI and CT are currently the preferred imaging techniques as they provide detailed information of body and muscle structure and composition, they are very expensive and thus can limit their use in both research and clinical settings. In addition, both imaging techniques are also not suitable for individuals who have metal implant, uncontrolled muscular movement (i.e., spasticity), or those who are pregnant or morbidly obese due to the nature of the techniques and radiation exposure (24). Therefore, providing another imaging option for those individuals is needed.

### **Skeletal Muscle Ultrasound**

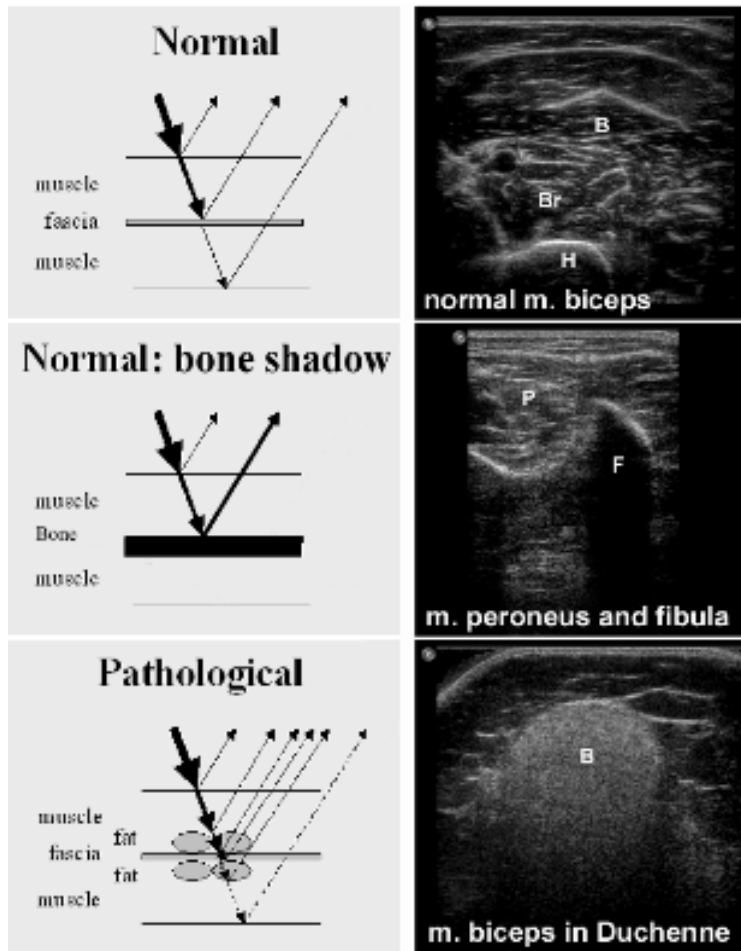
Ultrasound has been widely used for examination of the skeletal muscle structure (34, 43, 52, 71, 73, 75). With the utilization of ultrasound brightness mode (B-mode), the structure of a body compartment such as muscles, bone, nerves, and

blood vessels can be easily distinguished (71). Figure 2.2 represents a B-mode image that contains skin, subcutaneous fat, rectus femoris, rectus intermedius, and femur of an individual's thigh (starting from top to bottom).



**Figure 2.2**

Ultrasound can also be used to measure the overall quality of a muscle (75). In 1980, Heckmatt J.Z. et al. discovered that healthy and diseased muscles had different sonographic appearance (34). Reflection of ultrasound beams occurs when a sound wave encounters tissues with different densities or acoustic impedances (75, 83). As can be seen in Figure 2.3, when ultrasound travels down to the skeletal muscle and goes through different tissues such as muscle and fascia, the amount of reflection is based on the different acoustical impedance between the two tissues.



**Figure 2.3** (Adapted from Pillen and Alfen, 2011, Skeletal muscle ultrasound)

Echogenicity describes the amount of reflection caused by different degrees of acoustic impedance in tissues (44, 75). Anechoic is used to describe tissues such as lumens of blood vessels that produce no echo. Anechoic tissues appear black on the B-mode ultrasound image. Subcutaneous fat is considered to be hypoechoic, which creates weak reflection back to the transducer and appears to be grey on ultrasound B-mode images. In addition, tissues that are deep down to the structure may be hypoechoic due to the physical attenuation of ultrasound beam (52, 75, 77, 98). Tissues that are hyperechoic have strong reflection and have bright appearance on the

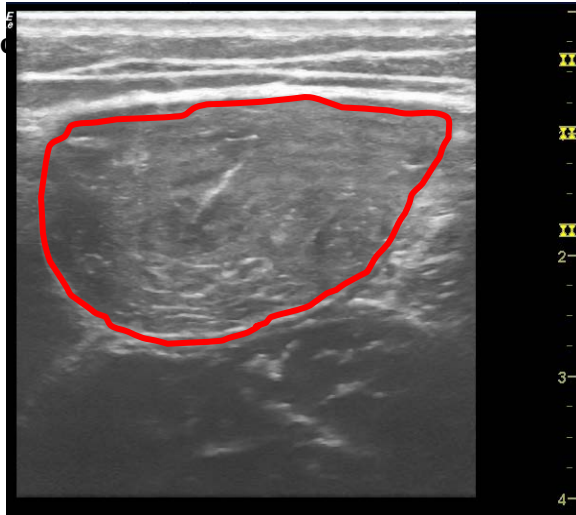
B-mode images. Bone, fascia, nerves, and tendons are examples of hyperechoic tissues (75). As a result, a diseased muscle that contains more intramuscular fat and connective tissue would appear brighter (higher echo intensity) than a normal healthy muscle (34, 73, 82).

In the past, evaluation of muscle echo intensity was largely done by using a visual assessment called Heckmatt's scale (35). The Heckmatt's scale evaluates muscle quality based on 4 grades. Grade I indicates a normal muscle composition while grade IV suggests an abnormal muscle composition with increased muscle echo intensity and reduced bone echo (35, 77). Evaluation of muscle quality using Heckmatt's scale can be easily performed; however, increased evaluation inaccuracy can be found in early stages of a neuromuscular disease when only a slight change in muscle structure was present (75). Zuberi et al. examined 100 children using the visual evaluation technique and concluded a sensitivity of 78% in detecting the presence of neuromuscular disease (101). Factors such as the experience of the evaluators, age and gender of the subjects, and differences between muscle groups can also reduce the reliability and specificity of the ultrasound results (60, 75, 81, 83).

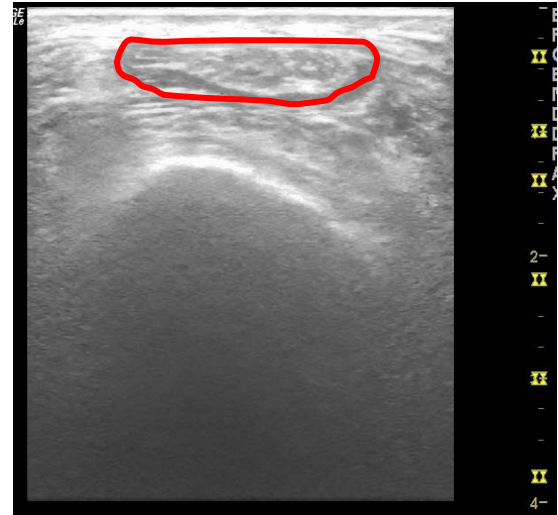
A more objective and reliable quantification of muscle echo intensity has been established using computer-aided gray scale (75, 77). This analysis technique is performed with a standard histogram function by examining the mean pixel intensity of a muscle of interest. As explained previously, the assumption is that the higher the mean pixel intensity or echo intensity of the muscle of interest, the worse the muscle quality (9, 20, 75). Figure 2.4A and 2.4B demonstrate the rectus femoris muscle of a healthy individual (A) and an individual with severe health conditions (B). Despite

the difference in muscle size, the individual with severe health conditions also has brighter muscle appearance (higher muscle echo intensity) when compared to the healthy individual. This suggests the muscle of the individual with severe health conditions contains more intramuscular fat and/or fibrous tissue.

**A. RF of a healthy person**



**B. RF of a person with health**



**Figure 2.4**

Previous studies have shown that skeletal muscle quality determined by ultrasound echo intensity is negatively correlated with muscular strength, muscular power, and cardiovascular health in people across different age groups (20, 83). Fukumoto et al. investigated the association of muscular strength and muscle echo intensity. They found a significant negative correlation between the echo intensity and knee extensor isometric strength, after controlling for age or muscle thickness (20). In another study conducted by Cadore et al., the relationship between muscle echo intensity and muscular strength was confirmed. In addition, they also reported

significant correlations between muscle echo intensity and cardiorespiratory fitness measured with workloads at the ventilation threshold (9).

Studies have also reported the normal values for muscle thickness and echo intensity in different muscle groups for both children and adults (4, 86), making it possible to compare the echo intensity values obtained from different studies. However, for studies that use different ultrasound devices or settings, a new set of normal values must be established. Several studies have established techniques to address this issue. Pillen et al. established a correction factor that makes comparisons of echo intensity obtained from different ultrasound devices possible (76). While this is a very important finding to improve the application of muscle ultrasound in the clinical settings, this technique requires a phantom that is currently not commercially available. Another technique proposed by Hughes et al. and Wallace et al. used the raw backscattered radio-frequency time-domain signal to calculate echo intensity (42, 97); however, this technique is currently not practical as the raw radio-frequency is usually not accessible to the public.

Although many studies have investigated the associations of muscle echo intensity with other outcomes such as muscular strength, power, and cardiovascular health, the ultrasound technique possesses some limitations. One of limitations is that the ultrasound technique cannot distinguish whether the change in echo intensity is caused by adipose tissue or by fibrous tissues currently. Although previous studies have suggested strong correlation between muscle echo intensity and interstitial fibrous tissue measured with biopsy samples in animal models (39, 74), testing the translatability of their findings to human is required. Another limitation is that it has

not been validated. Reimers et al. examined biopsy samples of 86 muscles and reported a positive correlation between muscle echo intensity and intramuscular lipid content (83). While the findings are exciting, questions such as the representative of muscle biopsy samples as well as the accuracy of visual echo intensity evaluation need to be addressed. In addition, the ultrasound technique reports its outcome measures in arbitrary echo intensity units, which makes comparisons between the ultrasound technique and other body and muscle composition techniques difficult.

## References

1. **Addison O, Marcus RL, LaStayo PC, and Ryan AS.** Intermuscular Fat: A Review of the Consequences and Causes. *International Journal of Endocrinology* 2014: 11, 2014.
2. **Antuna-Puente B, Feve B, Fellahi S, and Bastard JP.** Adipokines: The missing link between insulin resistance and obesity. *Diabetes & Metabolism* 34: 2-11, 2008.
3. **Arner P.** Insulin resistance in type 2 diabetes -- role of the adipokines. *Current Molecular Medicine* 5: 333-339, 2005.
4. **Arts IMP, Pillen S, Schelhaas HJ, Overeem S, and Zwarts MJ.** Normal values for quantitative muscle ultrasonography in adults. *Muscle & Nerve* 41: 32-41, 2010.
5. **Ashwell M, Cole TJ, and Dixon AK.** Obesity: new insight into the anthropometric classification of fat distribution shown by computed tomography. *BMJ* 290: 1692-1694, 1985.
6. **Beasley LE, Koster A, Newman AB, Javaid MK, Ferrucci L, Kritchevsky SB, Kuller LH, Pahor M, Schaap LA, Visser M, Rubin SM, Goodpaster BH, Harris TB, and The Health ABCs.** Inflammation and Race and Gender Differences in Computerized Tomography-measured Adipose Depots. *Obesity* 17: 1062-1069, 2009.
7. **Boesch C, De combaz J, Slotboom J, and Kreis R.** Observation of intramyocellular lipids by means of H magnetic resonance spectroscopy. *Proceedings of the Nutrition Society* 58: 841-850, 1999.
8. **Borkan GA, Gerzof SG, Robbins AH, Hults DE, Silbert CK, and Silbert JE.** Assessment of abdominal fat content by computed tomography. *The American Journal of Clinical Nutrition* 36: 172-177, 1982.
9. **Cadore EL, Izquierdo M, Conceição M, Radaelli Rg, Pinto RS, Baroni BM, Vaz MAI, Alberton CL, Pinto SS, Cunha G, Bottaro M, and Kruel LFM.** Echo intensity is



associated with skeletal muscle power and cardiovascular performance in elderly men.

*Experimental Gerontology* 47: 473-478, 2012.

10. **Chiang P-H, Wahlqvist ML, Lee M-S, Huang L-Y, Chen H-H, and Huang ST-Y.**

Fast-food outlets and walkability in school neighbourhoods predict fatness in boys and height in girls: a Taiwanese population study. *Public Health Nutrition* 14: 1601-1609, 2011.

11. **Close GL, Kayani A, Vasilaki A, and McArdle A.** Skeletal Muscle Damage with Exercise and Aging. *Sports Medicine* 35: 413-427, 2005.

12. **Davey RC.** The obesity epidemic: too much food for thought? *British Journal of Sports Medicine* 38: 360-363, 2004.

13. **Després J.** Body Fat Distribution and Risk of Cardiovascular Disease: An Update. *Circulation* 126: 1301-1313, 2012.

14. **Després J, Moorjani S, Lupien P, Tremblay A, Nadeau A, and Bouchard C.** Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* 10: 497-511, 1990.

15. **Deurenberg P, Weststrate JA, and Seidell JC.** Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr* 65: 105-114, 1991.

16. **Dietz WH.** Health Consequences of Obesity in Youth: Childhood Predictors of Adult Disease. *Pediatrics* 101: 518-525, 1998.

17. **Fleckenstein JL, Crues III JV, and Reimers CD.** *Muscle Imaging in Health and Disease*. NY: Springer, 1996.

18. **Fleischmann E, Teal N, Dudley J, May W, Bower JD, and Salahudeen AK.** Influence of excess weight on mortality and hospital stay in 1346 hemodialysis patients. *Kidney International* 55: 1560-1567, 1999.

19. **Fox MK, Dodd AH, Wilson A, and Gleason PM.** Association between School Food Environment and Practices and Body Mass Index of US Public School Children. *Journal of the American Dietetic Association* 109: S108-S117, 2009.
20. **Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, Kimura M, and Ichihashi N.** Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur J Appl Physiol* 112: 1519-1525, 2012.
21. **Gesta S, Tseng Y-H, and Kahn CR.** Developmental Origin of Fat: Tracking Obesity to Its Source. *Cell* 131: 242-256, 2007.
22. **Goldstein Barry J., and Rosarao Scalia.** Adiponectin: A Novel Adipokine Linking Adipocytes and Vascular Function. *The Journal of Clinical Endocrinology & Metabolism* 89: 2563-2568, 2004.
23. **Goodpaster BH.** Measuring body fat distribution and content in humans. *Current Opinion in Clinical Nutrition & Metabolic Care* 5: 481-487, 2002.
24. **Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E, and Newman AB.** Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *Journal of Applied Physiology* 90: 2157-2165, 2001.
25. **Goodpaster BH, Kelley DE, Thaete FL, He J, and Ross R.** Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *Journal of Applied Physiology* 89: 104-110, 2000.
26. **Goodpaster BH, Krishnaswami S, Harris TB, and et al.** Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. *Archives of Internal Medicine* 165: 777-783, 2005.

27. **Goodpaster BH, Stenger VA, Boada F, McKolanis T, Davis D, Ross R, and Kelley DE.** Skeletal muscle lipid concentration quantified by magnetic resonance imaging. *The American Journal of Clinical Nutrition* 79: 748-754, 2004.
28. **Goodpaster BH, Thaete FL, and Kelley DE.** Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *The American Journal of Clinical Nutrition* 71: 885-892, 2000.
29. **Goya Wannamethee S, Gerald Shaper A, Whincup PH, and Walker M.** Overweight and obesity and the burden of disease and disability in elderly men. *International Journal Of Obesity And Related Metabolic Disorders: Journal Of The International Association For The Study Of Obesity* 28: 1374-1382, 2004.
30. **Greenberg AS, and Obin MS.** Obesity and the role of adipose tissue in inflammation and metabolism. *The American Journal of Clinical Nutrition* 83: 461S-465S, 2006.
31. **Grundy SM.** Obesity, Metabolic Syndrome, and Cardiovascular Disease. *Journal of Clinical Endocrinology & Metabolism* 89: 2595-2600, 2004.
32. **Haarbo J, Gotfredsen A, Hassager C, and Christiansen C.** Validation of body composition by dual energy X-ray absorptiometry (DEXA). *Clinical Physiology* 11: 331-341, 1991.
33. **Hassen A, Wilson DE, Amin VR, Rouse GH, and Hays CL.** Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science* 79: 11-18, 2001.
34. **Heckmatt J, Dubowitz V, and Leeman S.** Detection Of pathological change in dystrophic muscle with B-scan ultrasound imaging *The Lancet* 315: 1389-1390, 1980.

35. **Heckmatt J, Leeman S, and Dubowitz V.** Ultrasound imaging in the diagnosis of muscle disease. *Journal of Pediatric* 101: 656-660, 1982.
36. **Heilbronn LK, and Campbell LV.** Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Current Pharmaceutical Design* 14: 1225-1230, 2008.
37. **Hermsdorff HH, and Monteiro JB.** [Visceral, subcutaneous or intramuscular fat: where is the problem?]. *Arquivos brasileiros de endocrinologia e metabologia* 48: 803-811, 2004.
38. **Hill JO.** Understanding and Addressing the Epidemic of Obesity: An Energy Balance Perspective. *Endocrine Reviews* 27: 750-761, 2006.
39. **Hu C-F, Chen CP-C, Tsai W-C, Hu L-L, Hsu C-C, Tseng S-T, and Shau Y-W.** Quantification of Skeletal Muscle Fibrosis at Different Healing Stages Using Sonography: A Morphologic and Histologic Study in an Animal Model. *Journal of Ultrasound in Medicine* 31: 43-48, 2012.
40. **Hu HH, Nayak KS, and Goran MI.** Assessment of abdominal adipose tissue and organ fat content by magnetic resonance imaging. *Obesity Reviews* 12: e504-e515, 2011.
41. **Hubert HB, Feinleib M, McNamara PM, and Castelli WP.** Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 67: 968-977, 1983.
42. **Hughes MS, Marsh JN, Wallace KD, Donahue TA, Connolly AM, Lanza GM, and Wickline SA.** Sensitive Ultrasonic Detection of Dystrophic Skeletal Muscle in Patients with Duchenne Muscular Dystrophy Using an Entropy-Based Signal Receiver. *Ultrasound in medicine & biology* 33: 1236-1243, 2007.

43. **Iacobellis G.** Imaging of visceral adipose tissue: an emerging diagnostic tool and therapeutic target. *Current Drug Targets Cardiovascular & Haematological Disorders* 5: 345-353, 2005.
44. **Ihnatsenka B, and Boezaart AP.** Ultrasound: Basic understanding and learning the language *International Journal of Shoulder Surgery* 4: 55-62, 2010.
45. **Ingram KH, Lara-Castro C, Gower BA, Makowsky R, Allison DB, Newcomer BR, Munoz AJ, Beasley TM, Lawrence JC, Lopez-Ben R, Rigsby DY, and Garvey WT.** Intramyocellular lipid and insulin resistance: differential relationships in European and African Americans. *Obesity (Silver Spring, Md)* 19: 1469-1475, 2011.
46. **Jebb SA, and Moore MS.** Contribution of a sedentary lifestyle and inactivity to the etiology of overweight and obesity: current evidence and research issues. *MEDICINE AND SCIENCE IN SPORTS AND EXERCISE* 31: S534-541, 1999.
47. **Juge-Aubry CE, Henrichot E, and Meier CA.** Adipose tissue: a regulator of inflammation. *Best Practice & Research Clinical Endocrinology & Metabolism* 19: 547-566, 2005.
48. **Kern PA, Ranganathan S, Li C, Wood L, and Ranganathan G.** *Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance.* 2001, p. E745-E751.
49. **Keys A, Fidanza F, Karvonen MJ, Kimura N, and Taylor HL.** Indices of relative weight and obesity. *Journal of Chronic Diseases* 25: 329-343, 1972.
50. **Kopelman P.** Health risks associated with overweight and obesity. *Obesity Reviews* 8: 13-17, 2007.

51. **Koster A, Stenholm S, Alley DE, Kim LJ, Simonsick EM, Kanaya AM, Visser M, Houston DK, Nicklas BJ, Tylavsky FA, Satterfield S, Goodpaster BH, Ferrucci L, Harris TB, and For the Health ABCS.** Body Fat Distribution and Inflammation Among Obese Older Adults With and Without Metabolic Syndrome. *Obesity* 18: 2354-2361, 2010.
52. **Kremkau F.** *Diagnostic ultrasound: principles and instruments.* Philadelphia: W. B. Saunders Company, 1998.
53. **Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Shulman GI, and Roden M.** Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a <sup>1</sup>H NMR spectroscopy study. *Diabetologia* 42: 113-116, 1999.
54. **Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, Corsi AM, Rantanen T, Guralnik JM, and Ferrucci L.** Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *Journal of Applied Physiology* 95: 1851-1860, 2003.
55. **Lavie CJ, Osman AF, Milani RV, and Mehra MR.** Body composition and prognosis in chronic systolic heart failure: the obesity paradox. *The American Journal of Cardiology* 91: 891-894, 2003.
56. **Lee CMY, Huxley RR, Wildman RP, and Woodward M.** Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. *Journal of clinical epidemiology* 61: 646-653, 2008.
57. **Leitzmann MF, Park Y, Blair A, and et al.** Physical activity recommendations and decreased risk of mortality. *Archives of Internal Medicine* 167: 2453-2460, 2007.

58. **Manini TM, Clark BC, Nalls MA, Goodpaster BH, Ploutz-Snyder LL, and Harris TB.** Reduced physical activity increases intermuscular adipose tissue in healthy young adults. *The American Journal of Clinical Nutrition* 85: 377-384, 2007.
59. **Martin K.** *Diagnostic Ultrasound: Physics and Equipment.* Cambridge, NY: Cambridge University Press, 2010.
60. **Maurits NM, Bollen AE, Windhausen A, De Jager AEJ, and Van Der Hoeven JH.** Muscle ultrasound analysis: normal values and differentiation between myopathies and neuropathies. *Ultrasound in medicine & biology* 29: 215-225, 2003.
61. **McAuley PA, and Blair SN.** Obesity paradoxes. *Journal of Sports Sciences* 29: 773-782, 2011.
62. **Mokdad AH, Ford ES, Bowman BA, and et al.** Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 289: 76-79, 2003.
63. **Muscaritoli M, Anker SD, Argil<sup>√</sup>cs J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, Fearon KC, Laviano A, Maggio M, Fanelli FR, Schneider SM, Schols A, and Sieber CC.** Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by Special Interest Groups (SIG) ,Äúcachexia-anorexia in chronic wasting diseases,Äù and ,Äúnutrition in geriatrics,Äù. *Clinical Nutrition* 29: 154-159, 2010.
64. **Must A, Spadano J, Coakley EH, Field AE, Colditz G, and Dietz WH.** The disease burden associated with overweight and obesity. *JAMA* 282: 1523-1529, 1999.
65. **National Heart L, and Blood Institute.** Healthy Weight Basics National Institute of Health. <http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/healthy-weight-basics/balance.htm>. [June 10, 2014].

66. **Newby P, Muller D, Hallfrisch J, Qiao N, Andres R, and Tucker KL.** Dietary patterns and changes in body mass index and waist circumference in adults. *The American Journal of Clinical Nutrition* 77: 1417-1425, 2003.
67. **Newcom DW, Baas TJ, and Lampe JF.** Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal of Animal Science* 80: 3046-3052, 2002.
68. **Nishimura S., Manabe I., and Nagai R.** Adipose tissue inflammation in obesity and metabolic syndrome. *Discovery Medicine* 8: 55-60, 2009.
69. **Pagani E, Bizzi A, Di Salle F, De Stefano N, and Filippi M.** Basic concepts of advanced MRI techniques. *Neurol Sci* 29: 290-295, 2008.
70. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, and Storlien LH.** Skeletal Muscle Triglyceride Levels Are Inversely Related to Insulin Action. *Diabetes* 46: 983-988, 1997.
71. **Pettrons P.** Ultrasound of muscles. *Eur Radiol* 12: 35-43, 2002.
72. **Phillips DIW, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, and Taylor R.** Intramuscular triglyceride and muscle insulin sensitivity: Evidence for a relationship in nondiabetic subjects. *Metabolism - Clinical and Experimental* 45: 947-950.
73. **Pillen S, Arts IMP, and Zwarts MJ.** Muscle ultrasound in neuromuscular disorders. *Muscle & Nerve* 37: 679-693, 2008.
74. **Pillen S, Tak RO, Zwarts MJ, Lammens MMY, Verrijp KN, Arts IMP, van der Laak JA, Hoogerbrugge PM, van Engelen BGM, and Verrips A.** Skeletal Muscle Ultrasound: Correlation Between Fibrous Tissue and Echo Intensity. *Ultrasound in medicine & biology* 35: 443-446, 2009.



75. **Pillen S, and van Alfen N.** Skeletal muscle ultrasound. *Neurological Research* 33: 1016-1024, 2011.
76. **Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, and Zwarts MJ.** Quantitative gray-scale analysis in skeletal muscle ultrasound: A comparison study of two ultrasound devices. *Muscle & Nerve* 39: 781-786, 2009.
77. **Pillen S, van Keimpema M, Nievelstein RAJ, Verrips A, van Kruijsbergen-Raijmann W, and Zwarts MJ.** Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound in medicine & biology* 32: 1315-1321, 2006.
78. **Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, and Eckel RH.** Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss: An Update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease From the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 113: 898-918, 2006.
79. **Pradhan AD, Manson JE, Rifai N, Buring JE, and Ridker PM.** C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286: 327-334, 2001.
80. **Reilly JJ, Methven E, McDowell ZC, Hacking B, Alexander D, Stewart L, and Kelnar CJH.** Health consequences of obesity. *Archives of Disease in Childhood* 88: 748-752, 2003.
81. **Reimers C, Fleckenstein J, Witt T, W. M-F, and DE. P.** Muscular ultrasound in idiopathic inflammatory myopathies of adults. *Journal of Neurological Science* 116: 82-92, 1993.

82. **Reimers CD, Schlotter B, Eicke BM, and Witt TN.** Calf enlargement in neuromuscular diseases: a quantitative ultrasound study in 350 patients and review of the literature. *Journal of the neurological sciences* 143: 46-56, 1996.
83. **Reimers K, Reimers CD, Wagner S, Paetzke I, and Pongratz DE.** Skeletal muscle sonography: a correlative study of echogenicity and morphology. *Journal of Ultrasound in Medicine* 12: 73-77, 1993.
84. **Ryan AS.** Insulin Resistance with Aging: Effects of Diet and Exercise. *Sports Medicine* 30: 327-346, 2000.
85. **Ryan AS, Buscemi A, Forrester L, Hafer-Macko CE, and Ivey FM.** Atrophy and Intramuscular Fat in Specific Muscles of the Thigh: Associated Weakness and Hyperinsulinemia in Stroke Survivors. *Neurorehabilitation and Neural Repair* 25: 865-872, 2011.
86. **Scholten RR, Pillen S, Verrips A, and Zwarts MJ.** Quantitative ultrasonography of skeletal muscles in children: Normal values. *Muscle & Nerve* 27: 693-698, 2003.
87. **Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WHM, Nicolay K, and Schrauwen P.** Intramyocellular Lipid Content and Molecular Adaptations in Response to a 1-Week High-Fat Diet. *Obesity Research* 13: 2088-2094, 2005.
88. **Shaw CS, Clark J, and Wagenmakers AJM.** The Effect of Exercise and Nutrition on Intramuscular Fat Metabolism and Insulin Sensitivity. *Annual Review of Nutrition* 30: 13-34, 2010.

89. **Shen W, Wang Z, Punyanita M, Lei J, Shinav A, Kral J, Imielinska C, Ross R, and Heymsfield S.** Adipose tissue quantification by imaging methods: a proposed classification. *Obesity Research* 11: 5-16, 2003.
90. **Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, and Kraegen EW.** Influence of Dietary Fat Composition on Development of Insulin Resistance in Rats: Relationship to Muscle Triglyceride and  $\omega$ -3 Fatty Acids in Muscle Phospholipid. *Diabetes* 40: 280-289, 1991.
91. **Thamer C, Machann Jr, Bachmann O, Haap M, Dahl D, Wietek B, Tschritter O, Niess A, Brechtel K, Fritsche A, Claussen C, Jacob S, Schick F, H $\ddot{u}$ ring H-U, and Stumvoll M.** Intramyocellular Lipids: Anthropometric Determinants and Relationships with Maximal Aerobic Capacity and Insulin Sensitivity. *Journal of Clinical Endocrinology & Metabolism* 88: 1785-1791, 2003.
92. **Therkelsen KE, Pedley A, Speliotes EK, Massaro JM, Murabito J, Hoffmann U, and Fox CS.** Intramuscular Fat and Associations With Metabolic Risk Factors in the Framingham Heart Study. *Arteriosclerosis, Thrombosis, and Vascular Biology* 33: 863-870, 2013.
93. **Thomas DR.** Loss of skeletal muscle mass in aging: Examining the relationship of starvation, sarcopenia and cachexia. *Clinical Nutrition* 26: 389-399, 2007.
94. **Toeller M, Buyken AE, Heitkamp G, Cathelineau G, Ferriss B, and Michel G.** Nutrient intakes as predictors of body weight in European people with type 1 diabetes. *International Journal Of Obesity And Related Metabolic Disorders: Journal Of The International Association For The Study Of Obesity* 25: 1815-1822, 2001.

95. **Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellesse AA, Tapsell LC, Nilsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, and Storlien LH.** Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 44: 312-319, 2001.
96. **Wagner DR.** Ultrasound as a Tool to Assess Body Fat. *Journal of Obesity* 2013: 9, 2013.
97. **Wallace K, Marsh J, Baldwin S, Connolly A, Keeling R, Lanza G, Wickline S, and Hughes M.** Sensitive ultrasonic delineation of steroid treatment in living dystrophic mice with energy-based and entropy -based radio frequency signal processing. *IEEE Trans Ultrason Ferroelectr Freq Control* 54: 2291-2299, 2007.
98. **Wattjes M, Kley R, and Fischer D.** Neuromuscular imaging in inherited muscle diseases. *Eur Radiol* 20: 2447-2460, 2010.
99. **WHO Media centre.** Obesity and overweight World Health Organization. <http://www.who.int/mediacentre/factsheets/fs311/en/>. [June 28, 2014].
100. **Wozniak S, Gee L, Wachtel M, and Frezza E.** Adipose Tissue: The New Endocrine Organ? A Review Article. *Dig Dis Sci* 54: 1847-1856, 2009.
101. **Zuberi SM, Matta N, Nawaz S, Stephenson JBP, McWilliam RC, and Hollman A.** Muscle ultrasound in the assessment of suspected neuromuscular disease in childhood. *Neuromuscular Disorders* 9: 203-207, 1999.

CHAPTER 3  
VALIDATION OF MUSCLE ECHO INTENSITY WITH MAGNETIC  
RESONANCE IMAGING TECHNIQUE

---

<sup>1</sup>Young, H-J., Jenkins, N.T., Zhao, Q., McCully, K.K. To be submitted to Journal of Ultrasound in Medicine.

## **Abstract**

**Purpose:** Previous studies have used ultrasound to measure muscle quality, with the data presented as muscle echo intensity (EI). The purpose of this study was to compare muscle EI to high-resolution T<sub>1</sub>-weighted magnetic resonance imaging (MRI) as well as to establish calibration equations to estimate % intramuscular fat from EI. **Methods:** Thirty-one participants (14 males, 17 females) between the ages of 20 to 61 underwent both ultrasound and MRI testing on four muscles: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG). Muscle EI and MRI-measured % intramuscular fat of each muscle were compared and used to establish the calibration equations. **Results:** Three types of calibration equations: group, muscle specific, and sex specific, were established based on the strong correlations found between MRI % intramuscular fat and muscle EI after correcting for subcutaneous fat thickness ( $r = 0.9$  in RF,  $r = 0.8$  in BF,  $r = 0.8$  in TA,  $r = 0.8$  in MG). The ultrasound technique demonstrated high between-day reproducibility ( $r = 0.9$  in RF,  $r = 0.7$  in BF,  $r = 0.9$  in TA,  $r = 0.8$  in MG) and high inter-analyzer reliability was observed in all muscle groups ( $r = 0.9$  in RF,  $r = 0.9$  in BF,  $r = 0.9$  in TA,  $r = 0.9$  in MG). **Conclusion:** The current study suggests that muscle ultrasound is a practical and reproducible technique that can be used as another imaging technique for examination of % intramuscular fat. Variability of muscle EIs and % intramuscular fat was found between different sites within a muscle, suggesting the need to examine multiple sites for a comprehensive muscle composition. Future ultrasound studies should incorporate % intramuscular fat measures to allow for comparisons with other body composition techniques as well as are needed establish more equations for other muscle groups to enhance its use in both research and clinical settings.

### *Keywords:*

Muscle ultrasound, T1-weighted MRI, muscle EI, intramuscular fat, muscle composition

## **Introduction**

Overweight and obesity are associated with many secondary health conditions (5, 6, 20). Recent studies have suggested that adipocytes deposited in different areas of the body have different physiological activities, such that the health risks of obesity are closely related to the location of fat depots in addition to the total amount of adipose tissue (4, 9). For example, visceral abdominal fat has been found to be related to dyslipidemia, glucose intolerance, and higher risk of cardiovascular disease (19). Increased fat depots within skeletal muscle has also been found to be associated with functional decline and metabolic disorders (27). These studies have indicated the importance of examining the distribution of adipose tissue and deposition of ectopic fat.

Muscle ultrasound has recently emerged as an imaging technique to measure muscle quality (17). Lean muscle tissue has low echogenicity while intramuscular fat and connective tissue have high echogenicity (12). This technique quantifies total muscle echo intensity (EI) using gray scale analysis with the assumption that the higher the mean pixel intensity of a muscle region of interest, the lower the muscle quality (i.e. more intramuscular fat) (2, 3, 17). Muscle ultrasound is a low cost and easily accessible technology that can be applied on individuals who cannot undergo other imaging technologies such as magnetic resonance imaging (MRI), computed tomography (CT), and dual-energy X-ray absorptiometry (DXA) for examination of their muscle health. Several studies have shown that increased EI is negatively correlated with muscular strength and cardiovascular health in people across different age groups (2, 3). Some studies have also established analytical methods to improve the consistency and compatibility of this ultrasound technique across different ultrasound devices (18).

Ultrasound has been recognized as a valuable tool to evaluate marbling in cattle and swine with the establishment of % intramuscular fat prediction equations (7, 13, 14). To our knowledge, such studies have not been conducted in human subjects due to the impracticality of obtaining biopsy samples in humans. In addition, no study has compared muscle EI to MRI, an imaging technique that provides a comprehensive picture of the structure and composition of human skeletal muscle. The other limitation of the current ultrasound technique is the use of arbitrary EI units as an outcome measure, which makes it difficult to compare ultrasound to other body and muscle composition techniques. Although utilizing the prediction equations established from animal subjects can be convenient, the equations are not necessarily appropriate for human subjects. The purpose of the present study was to compare EI from the ultrasound technique with % intramuscular fat measurements derived from high-resolution T<sub>1</sub>-weighted MRI images. The study also aimed to establish muscle specific calibration equations that can be used in humans to quantify muscle EI into % intramuscular fat.

## **Materials and Methods**

### **Participants**

Thirty-one participants (14 males, 17 females) between the ages of 20 to 61 were recruited in this study. Participants with a range of body mass index (BMI) and physical activity level were recruited to provide a range of body adiposity. The study consisted of an ultrasound test and a MRI test. Ten participants underwent an additional ultrasound test on a separate day to assess the reliability and reproducibility of the ultrasound technique. The study was approved by the Institutional Review Board. We certify that all applicable instructional and governmental regulations concerning the ethical use of



human volunteers were followed during the course of this study. Written informed consent was obtained from all participants prior to any data collection and after a detailed description of the study was provided. Data was collected from March 2014 to April 2014.

### Study Design

All participants completed two or three test sessions. The first and second sessions involved an ultrasound test and a MRI test. The third session was an optional session to test reproducibility of the ultrasound technique. Tests on individual participants were completed within a one-week period.

### *Ultrasound Experimental Protocol*

An ultrasound test was performed on four muscles of the lower extremity: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG) with an ultrasound-imaging device (LOGIQ e; GE Healthcare UK Ltd., Chalfont, Buckinghamshire, England). The dominant leg was tested. Each participant was instructed to rest supine on an exam table. Ultrasound Brightness mode (B-mode) with musculoskeletal scanning preset and a multi-frequency linear transducer (8-12 MHz) were used. Gain and transducer frequency were adjusted to 58-dB and 8 MHz, respectively. Scanning depth was set to be 4 cm with 1.27 cm slice thickness and an apparent spatial resolution of 80  $\mu\text{m}/\text{pixel}$ . The scanning depth was only increased when testing participants with greater subcutaneous fat to allow for capturing enough muscle area. Time gain compensation was adjusted to neutral position. Focus number and area

were increased to ensure all muscles groups being scanned were covered and consistent across all participants. Other ultrasound settings stayed unchanged from the preset.

Before starting the ultrasound test, the upper and lower leg length of each participant was measured from lateral patella base to anterior superior iliac spine and from lateral patella tip to calcaneus, respectively. Marks were made on the 1/3 and 1/4 of the anterior and posterior parts of the upper and lower legs. The purpose of the marks was to ensure that the scanning locations between ultrasound and MRI as well as between participants were consistent. A generous amount of ultrasound gel was then applied to avoid excessive pressure placed on the skin. Each scan involved a 16-second ultrasound clip on one of the marks, and each muscle was scanned twice (both 1/3 and 1/4 marks). A total of 8 scans were obtained from each participant. Each ultrasound clip was then saved into JPEG images for analysis. Muscle EI was determined by gray-scale analysis using ImageJ (21). A muscle of interest was circled manually while avoiding surrounding fascia and bone. The mean pixel intensity of the selected muscle region was obtained from each measurement and an average of 3 measurements was calculated. Subcutaneous fat thickness, muscle thickness, and area of muscle being circled were also recorded. Images were analyzed by two investigators to test for the inter-rater reliability of the ultrasound technique.

#### *Magnetic Resonance imaging (MRI) Experimental Protocol*

T<sub>1</sub>-weighted MRI images (TR = 800 ms; TE = min full) were obtained using a 3.0 Tesla whole body MR system (GE Healthcare, Waukesha, WI) at the University of Georgia Biomedical Health and Sciences Institute. Images were obtained with 1024 x

1024 matrix on both lower leg and upper leg of each participant. A field of view (FOV) of 18 cm x 18 cm (resolution of 176  $\mu\text{m}/\text{pixel}$ ) was set for the upper leg scans and 16 cm x 16 cm (resolution of 156  $\mu\text{m}/\text{pixel}$ ) for the lower leg scans unless changes were necessary (i.e. participants with larger size of thigh or calf). A volume knee coil was placed on the lower leg with the centerline of the coil aligned to the ultrasound mark (see previous section “*Ultrasound Experimental Protocol*”). A lower leg scan involved a total of 4 imaging slices with 3.0 mm slice thickness and 10.0 mm spacing. The coil was then repositioned with the centerline lined up to another ultrasound mark on the upper leg. An upper leg scan was done with the same settings as the lower leg scan except the FOV and slice spacing were changed to 18 cm x 18 cm and 15.0 mm, respectively. The entire MRI testing procedure including positioning and the upper and lower leg scans was approximately 30 minutes per participant.

MRI images were analyzed using ImageJ (21) and with a similar protocol published in a previous study (25). The muscle of interest was circled and a histogram was obtained. The pixel intensities of pure fat, pure muscle, and connective tissue were determined by visual judgment. The determined pixel intensity (DPI) of pure fat ( $DPI_{fat}$ ) was calculated by averaging the intensities of 3 selected areas of pure fat. The same procedure was followed for acquiring the DPI of pure muscle ( $DPI_{muscle}$ ) and connective tissue. The DPIs were then used to differentiate each tissue within the muscle of interest. To calculate the % intramuscular fat of a muscle, a weighted % fat ( $\%fat_{weighted}$ ) associated with each raw pixel intensity ( $PI_{raw}$ ) was first calculated using equation 3.1,

$$\%fat_{weighted} = (PI_{raw} - DPI_{muscle}) / (DPI_{fat} - DPI_{muscle}) * 100 \quad (\text{Equation 3.1})$$

Weighted fat pixel counts ( $FPC_{weighted}$ ) and weighted muscle pixel counts ( $MPC_{weighted}$ ) were then determined using the equations below,

$$FPC_{weighted} = (\%fat_{weighted} * raw\ pixel\ count) / 100 \quad (\text{Equation 3.2})$$

$$MPC_{weighted} = (raw\ pixel\ count * (100 - \%fat_{weighted})) / 100 \quad (\text{Equation 3.3})$$

Sums of  $FPC_{weighted}$  and  $MPC_{weighted}$  of a muscle were then used to calculate % intramuscular fat of a muscle using the equation presented below,

$$\% fat = FPC_{weighted\_sum} / (FPC_{weighted\_sum} + MPC_{weighted\_sum}) * 100 \quad (\text{Equation 3.4})$$

#### *Correcting for Subcutaneous Fat Thickness*

During the initial data analysis, an independent influence of subcutaneous fat thickness on muscle echo intensity was observed. To further examine this potential influence, the subcutaneous fat thickness of 5 participants was reduced by applying four different levels of external pressure to the skin. Care was taken to ensure minimal change in muscle shape. Figure 3.1 demonstrates echo intensities associated with different subcutaneous fat thickness. The average of the five slopes and y-intercepts were calculated and the following equation was established, where  $cf$  = correction factor and  $x$  = subcutaneous fat thickness,

$$cf = -39.887x + 80.4148 \quad (\text{Equation 3.5})$$

When plotted 1.0cm as the subcutaneous fat into the equation demonstrated below, the  $cf$  represents the addition of echo intensity with every 1.0cm subcutaneous fat thickness,

$$cf = -39.887 (1.0cm) + 80.4148 = 40.5278 \quad (\text{Equation 3.6})$$

The  $cf$  was then applied to correct for the potential influence of subcutaneous fat on echo intensity using the following equation, where  $y_1$  = raw echo intensity,  $x$  = subcutaneous fat thickness,  $cf = 40.5278$ , and  $y_2$  = corrected echo intensity,

$$y_2 = y_1 + (x * cf) \quad (\text{Equation 3.7})$$

### *Generation of Calibration Equations*

Correlations between muscle EI and MRI % fat were obtained and the linear regression equations were used to generate calibration equations. Different types of calibration equations were established by combining all the muscle groups (group equation) as well as examining separately based on muscle groups (muscle specific equation) and sex (sex specific equation). Ultrasound % fat calculated with each equation was compared.

### Statistical Analysis

Data are presented as means  $\pm$  SD. Correlation between ultrasound echo intensity and MRI measured % fat of each muscle was analyzed using Pearson

correlation. Ultrasound test-retest and inter-rater reliabilities were analyzed using coefficient of variation (CV) and two-way random intraclass correlation coefficients (ICC) with absolute agreement. CV of the echo intensity of the two ultrasound scanning locations and three MRI slices were also computed. Statistical analyses were performed using SPSS 19.0 (IBM®, Armonk, NY). Significance was accepted when  $p < 0.05$ .

## **Results**

### *Study Participants*

All participants completed the study without any adverse events. The physical characteristics of the participants are summarized in Table 3.1.

### *Ultrasound and MRI Results*

Representative muscle images obtained from both MR and ultrasound are demonstrated in Figure 3.2A-D. Both MRI and ultrasound images were acquired from the same location of each muscle group so that comparisons between muscle EI and MRI-measured % intramuscular fat could be made. Examined muscle area, muscle volume, and ultrasound EI of each muscle group are shown in Table 3.2. It should be noted that the entire area of BF, TA, and MG could not be fully examined due to the limitation of ultrasound window.

### *Uncorrected Echo Intensity vs. MRI-measured % Intramuscular Fat*

Comparisons between the ultrasound EI without correction for subcutaneous fat thickness and % intramuscular fat measured with MRI are shown in Figures 3.3A-D.

Moderate to strong correlations were found between ultrasound EI and MRI % fat when examining RF ( $r = 0.8$ ) and TA ( $r = 0.7$ ). Weak to moderate correlations were found in BF ( $r = 0.4$ ) and MG ( $r = 0.5$ ).

#### *Corrected Echo Intensity vs. MRI-measured % Intramuscular Fat*

Figure 3.4A-D shows the correlations between MRI % intramuscular fat and muscle EI after corrected for subcutaneous fat thickness. Stronger correlations were found in all muscle groups when compared to the ones before the correction factor was applied ( $r = 0.9$  in RF,  $r = 0.8$  in BF,  $r = 0.8$  in TA,  $r = 0.8$  in MG). When examining the correlations separately by gender (Figure 3.5A-D), similar and stronger correlations were found in all muscles in males ( $r = 0.9$  in RF,  $r = 0.8$  in BF,  $r = 0.8$  in TA,  $r = 0.9$  in MG) when compared to females ( $r = 0.8$  in RF,  $r = 0.8$  in BF,  $r = 0.6$  in TA,  $r = 0.8$  in MG). A moderate to strong correlation was found when compared ultrasound corrected EI and MRI % intramuscular fat after combining all the examined muscles ( $r = 0.7$ ).

#### Calibration Equations

Three types of calibration equations were established and all the equations are presented below where  $x = \text{raw echo intensity}$ ,  $y = \% \text{ intramuscular fat}$ , and  $z = \text{Subcutaneous fat thickness}$ ,

$$y = (0.114 * ((40 * z) + x)) + 5.231 \quad (\text{Group equation})$$

	$y = (0.093 * ((40 * z) + x)) + 4.698$	<i>(Muscle specific)</i>
<b>RF</b>	$y = (0.062 * ((40 * z) + x)) + 7.901$	<i>(Sex specific, female)</i>
	$y = (0.144 * ((40 * z) + x)) + 1.126$	<i>(Sex specific, male)</i>
	$y = (0.143 * ((40 * z) + x)) + 3.459$	<i>(Muscle specific)</i>
<b>BF</b>	$y = (0.177 * ((40 * z) + x)) + 1.823$	<i>(Sex specific, female)</i>
	$y = (0.152 * ((40 * z) + x)) + 2.368$	<i>(Sex specific, male)</i>
	$y = (0.256 * ((40 * z) + x)) - 2.991$	<i>(Muscle specific)</i>
<b>TA</b>	$y = (0.250 * ((40 * z) + x)) - 2.366$	<i>(Sex specific, female)</i>
	$y = (0.198 * ((40 * z) + x)) + 0.094$	<i>(Sex specific, male)</i>
	$y = (0.143 * ((40 * z) + x)) + 3.459$	<i>(Muscle specific)</i>
<b>MG</b>	$y = (0.177 * ((40 * z) + x)) + 1.823$	<i>(Sex specific, female)</i>
	$y = (0.152 * ((40 * z) + x)) + 2.368$	<i>(Sex specific, male)</i>

#### Comparisons Between Different Equations

Relationships between calibrated ultrasound % fat and MRI % intramuscular fat were examined. Figure 3.7A-D demonstrates difference between MRI % intramuscular fat and ultrasound % fat calculated using each equation. When converting muscle EI into % fat using the group equation, the mean and standard deviation of % fat difference were larger across all muscle groups ( $-2.41 \pm 1.13$  in RF;  $0.83 \pm 2.27$  in BF;  $1.38 \pm 2.77$  in TA;  $0.50 \pm 2.16$  in MG) when compared to that using muscle specific ( $0.00 \pm 1.02$  in RF;  $0.27 \pm 2.20$  in BF;  $0.00 \pm 2.15$  in TA;  $0.00 \pm 1.83$  in MG) and sex specific equations ( $-0.03 \pm 0.72$  in RF;  $-0.44 \pm 2.70$  in BF;  $-0.56 \pm 3.51$  in TA;  $-0.02 \pm 1.71$  in MG).

#### Within Muscle Variability

Muscle EIs between the two scanning locations were compared. The mean CV



between the two locations was 5.6 % in RF, 6.3% in BF, 5.0% in TA, and 4.8% in MG. After correcting for subcutaneous fat thickness, the mean coefficient of variation was 5.7% in RF, 8.7% in BF, 4.9% in TA, and 5.2% in MG (Figure 3.8A). Percent intramuscular fat differences between the three MRI slices were also compared (Figure 3.8B). The mean CV between the three MRI slices was 11.0 % in RF, 7.6% in BF, 5.6% in TA, and 5.1% in MG.

#### Ultrasound Reproducibility and Inter-Analyzer Reliability

The reproducibility of the ultrasound technique was examined by repeating the same testing procedure on ten participants on two different days within a week. Table 3.3 presents the results of EI, CV, and ICC. The ultrasound technique demonstrated high reproducibility between the two testing days across all muscle groups ( $r = 0.9$  in RF,  $r = 0.7$  in BF,  $r = 0.9$  in TA,  $r = 0.8$  in MG). The inter-analyzer reliability of the ultrasound technique was tested on the ultrasound images of 23 participants. The mean EI of each muscle group as well as the mean CV and ICC are presented in Table 3.4. A high inter-analyzer reliability was observed in all muscle groups ( $r = 0.9$  in RF,  $r = 0.9$  in BF,  $r = 0.9$  in TA,  $r = 0.9$  in MG).

## **Discussion**

The main finding of the present study was the generation of calibration equations to quantify muscle EI into % intramuscular fat on four muscles in the lower extremity. In this study, moderate to strong correlations were found between MRI-measured % intramuscular fat and muscle EI. This is consistent with previous literature that compared muscle EI to % intramuscular fat measured with muscle biopsy samples in animal models

(22, 24). Reimers et al. examined muscle echogenicity and biopsy samples of 86 muscles and concluded that the increased muscle EI was mainly caused by intramuscular lipid content (24). Previous studies have also reported the associations of higher muscle EI with reduced muscular strength, neuromuscular diseases, and lower cardiovascular performance (2, 3, 8, 15, 28). While muscle EI has provided valuable clinical information, the arbitrary EI units make comparisons between the ultrasound technique and other body and muscle composition techniques difficult, and thus limit its use as an alternative technique to examine muscle composition. The calibration equations established in the present study can help address this limitation.

Our study also observed an independent influence of subcutaneous fat thickness on muscle EI, given all the settings were kept consistent, and was able to develop a correction factor to correct for the potential influence. This phenomenon was reported in previous literature (18, 29). As Wattjes et al. pointed out in their review article, reflection or absorption of ultrasound sound wave made visualization of deeper tissue difficult, which could limit its application to examining superficial muscle groups (29). In the present study, a correction factor was established after examining the associations of EIs and subcutaneous fat thickness altered by applying different levels of pressure on the skin. After the correction factor was applied to raw EIs, the correlations between ultrasound EI and MRI % intramuscular fat improved. In addition, it should be noted that the present study developed one correction factor and applied it to all muscle groups.

Better correlations were observed when compared MRI % fat to corrected ultrasound EI of each muscle group than comparing MRI % fat to corrected ultrasound EI of all muscle groups. This finding is consistent with previous studies that suggested

variability in EI across different muscle groups (1, 17). Pillen & Alfen suggested the differences in fibrous tissue distribution and muscle fiber orientation of each muscle group, which resulted in a muscle's unique range of EI (17). In addition, a better relationship was found when compared MRI % intramuscular fat and corrected ultrasound EI of each muscle group in males to that in females. As Arts et al. reported that the relationship between age and echo intensity was gender and muscle dependent (1), our result agrees with their findings.

In this study, we reported 3 types of calibration equations: group equation, muscle specific equation, and sex specific equation. A better relationship was observed when compared MRI % fat and ultrasound % fat calculated using muscle specific equation to that calculated using group equation. This again supports the notion that the normal range of EI is muscle specific. Muscle specific and sex specific equations were compared and similar correlations were found between MRI % intramuscular fat and ultrasound % fat calculated using the two equations, suggesting the use of both equations in the future. Furthermore, a better relationship between MRI % fat and ultrasound % fat was observed in RF. A possible explanation for this is the limitation of ultrasound window. As previously mentioned, the ultrasound window was able to capture the entire area of RF but not the other muscles, which could result in potential measurement error. Future studies can address this issue by investigating the relationship between MRI % fat and ultrasound EI using an ultrasound probe with wider field of view such as the ones used to scan full sized cow when examining bigger muscle groups, or by combining ultrasound scans of different sites of a muscle to obtain a representative image of a muscle.

The present study investigated % intramuscular fat measured with MRI in 4 different muscle groups: RF, BF, TA, and MG. We found a range of approximately 13.0 to 16.0 % mean intramuscular fat from all muscle groups. Our results are comparable to % intramuscular fat reported by other studies using different imaging techniques. Kovanlikaya et al. examined the relationships between insulin levels and fat accumulation in the soleus muscle, liver, and pancreas using the three-point Dixon MRI technique in 15 young, healthy Mexican-American females and reported an average intramuscular fat of approximately 15 % in the lean group and 23 % in the obese group, classified based on BMI (11). Wren et al. (30) investigated % intramuscular fat of several muscle groups in nine boys with Duchene muscular dystrophy and found that the % intramuscular fat was highly correlated with each muscle group ( $r = 0.83$  to  $0.98$ ). Overall, the levels of intramuscular fat reported in the present study are consistent with results reported in previous studies.

In this study, we also demonstrated the high reproducibility ( $r = 0.9$  in RF,  $r = 0.7$  in BF,  $r = 0.9$  in TA,  $r = 0.8$  in MG) and inter-rater reliability ( $r = 0.9$  in RF,  $r = 0.9$  in BF,  $r = 0.9$  in TA,  $r = 0.9$  in MG) of the ultrasound technique. We reported strong ICC between day 1 and day 2 in all muscle groups. This finding is comparable to the study conducted by Reimers et al. in which they found a test-retest correlation coefficient of 0.94 for EI in calf muscle (23). A higher CV was found in BF (CV = 13.1 %) between 2 different days. A possible explanation is the difficulty to locate the same ultrasound scanning area of BF. Ultrasound has limited FOV which makes it difficult to capture the entire muscle area of larger muscle groups transversely. In addition, the present study observed significant variability of muscle EIs between two locations of each muscle

group as well as % intramuscular fat between 3 different MRI slices (Figure 3.8A & B), which indicate the importance of ensuring the consistency of scanning locations. As Scholten et al. pointed out in their study, measuring the exact muscle site was necessary to obtain comparable and reliable results across individuals (26). In this study, although care was taken to ensure the same measurement sites were scanned across different days and participants, potential discrepancy can contribute to the larger CV observed in BF. Future studies will need to ensure the scanning site consistency and consider the variability between different sites within a muscle to accurately interpret muscle composition.

The study also observed larger variability in participants with higher % intramuscular fat (above 15%) measured by MRI. We have some possible explanations for this observed high variability. As Pillen et al. reported, attenuation of the ultrasound beam occurs when the sound wave encounters different tissues such as muscle, connective tissue, and adipose tissue (17). Our hypothesis is that when the amount of intramuscular fat reaches to approximately 15 %, it would start affecting the reflection and absorption of sound non-systematically based on the distribution patterns of intramuscular fat and connective tissue. This results in underestimating the actual EI of a muscle. In addition, the present study examined the association between muscle EI and % intramuscular fat without separating the potential influence of connective tissue. Previous studies have suggested strong correlation between muscle EI and interstitial fibrous tissue measured with biopsy samples in animal models (10, 16). While less amount of connective tissue was assumed in the relatively young and healthy participants recruited in this study, any potential effect of connective tissue on EI is unknown. Future

studies are needed to establish methods such as texture analysis to identify different tissues within a muscle using ultrasound. The second explanation is the potential limitation of the current MRI % intramuscular fat analysis. In this study, we relied on manually-determined PIs to differentiate pure muscle, pure fat, and connective tissue and calculated % intramuscular fat based on DPIs of the three tissues (25). When a muscle contains high adipose tissue, it makes identification of pure muscle difficult. As a result, underestimation of intramuscular fat can occur. Further investigations that use better analytical methods such as the Dixon MRI technique are needed to validate the current manually-determined PI method (11)

Our study has some limitations. First, as mentioned previously, the potential influence of connective tissue on muscle EI was not addressed in this study. Future studies with a combination of imaging techniques and potentially muscle biopsies are needed to further examine the role of connective tissue on muscle EI. The second limitation of the study is the number of muscles being examined. The present study only examined four muscle groups in the lower extremity. While this study demonstrates the possibility to estimate % intramuscular fat from muscle EI, future studies are required to establish calibration equations for other muscle groups. Another limitation is that the calibration equations established in this study can only be applied to muscle EI obtained with the specific ultrasound device and settings used in this study. A potential solution for this is to establish correction factors or analytical techniques to convert muscle EI from one ultrasound device to another, as reported by previous studies (18, 31). Another possible solution is to develop a phantom with a range of % fat to calibrate different ultrasound devices. Nevertheless, future studies are needed to improve the compatibility

between ultrasound devices to enhance the practicality of the ultrasound technique.

In conclusion, we established calibration equations to quantify muscle EI into % intramuscular fat after assessing four different muscles in the lower extremity with high-resolution T<sub>1</sub>-weighted MRI and ultrasound. A correction factor for subcutaneous fat thickness was developed to correct for its potential influence on muscle EI. Future studies are required to test the validity and reliability of the calibration equations. In addition, variability of muscle EIs and % intramuscular fat between different ultrasound scanning sites and MRI slices was found, suggesting the need to examine multiple sites of a muscle to obtain a comprehensive composition of a muscle. Muscle ultrasound is a low cost, easily accessible, and highly reproducible technique that can serve as an option of imaging techniques to examine muscle health. More studies are required to enhance the practicality of the ultrasound technique in both research and clinical settings.

#### *Acknowledgements*

The authors would like to thank Kim Mason for her assistant in MRI data collection and the participants of this study for their enthusiastic commitment to this study.

#### *Funding*

This study was funded in part by National Institutes of Health Grant (HD-039676)

#### *Conflict of interest statement*

The authors report no conflicts of interest.

## References

1. **Arts IMP, Pillen S, Schelhaas HJ, Overeem S, and Zwarts MJ.** Normal values for quantitative muscle ultrasonography in adults. *Muscle & Nerve* 41: 32-41, 2010.
2. **Cadore EL, Izquierdo M, Conceição M, Radaelli Rg, Pinto RS, Baroni BM, Vaz MAI, Alberton CL, Pinto SS, Cunha G, Bottaro M, and Kruel LFM.** Echo intensity is associated with skeletal muscle power and cardiovascular performance in elderly men. *Experimental Gerontology* 47: 473-478, 2012.
3. **Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, Kimura M, and Ichihashi N.** Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur J Appl Physiol* 112: 1519-1525, 2012.
4. **Goodpaster BH, Krishnaswami S, Harris TB, and et al.** Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. *Archives of Internal Medicine* 165: 777-783, 2005.
5. **Goya Wannamethee S, Gerald Shaper A, Whincup PH, and Walker M.** Overweight and obesity and the burden of disease and disability in elderly men. *International Journal Of Obesity And Related Metabolic Disorders: Journal Of The International Association For The Study Of Obesity* 28: 1374-1382, 2004.
6. **Grundy SM.** Obesity, Metabolic Syndrome, and Cardiovascular Disease. *Journal of Clinical Endocrinology & Metabolism* 89: 2595-2600, 2004.
7. **Hassen A, Wilson DE, Amin VR, Rouse GH, and Hays CL.** Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science* 79: 11-18, 2001.



8. **Heckmatt J, Dubowitz V, and Leeman S.** Detection Of pathological change in dystrophic muscle with B-scan ultrasound imaging *The Lancet* 315: 1389-1390, 1980.
9. **Hernsdorff HH, and Monteiro JB.** [Visceral, subcutaneous or intramuscular fat: where is the problem?]. *Arquivos brasileiros de endocrinologia e metabologia* 48: 803-811, 2004.
10. **Hu C-F, Chen CP-C, Tsai W-C, Hu L-L, Hsu C-C, Tseng S-T, and Shau Y-W.** Quantification of Skeletal Muscle Fibrosis at Different Healing Stages Using Sonography: A Morphologic and Histologic Study in an Animal Model. *Journal of Ultrasound in Medicine* 31: 43-48, 2012.
11. **Kovanlikaya A, Mittelman S, Ward A, Geffner M, Dorey F, and Gilsanz V.** Obesity and fat quantification in lean tissues using three-point Dixon MR imaging. *Pediatr Radiol* 35: 601-607, 2005.
12. **Mayans D, Cartwright MS, and Walker FO.** Neuromuscular Ultrasonography: Quantifying Muscle and Nerve Measurements. *Physical Medicine and Rehabilitation Clinics of North America* 23: 133-148, 2012.
13. **Newcom DW, Baas TJ, and Lampe JF.** Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal of Animal Science* 80: 3046-3052, 2002.
14. **Parish JA, Rhinehart JD, and C. VR.** Ultrasound Scanning Beef Cattle for Body Composition Starkville, MS: Mississippi State University Extension Service, 2011.
15. **Pillen S, Arts IMP, and Zwarts MJ.** Muscle ultrasound in neuromuscular disorders. *Muscle & Nerve* 37: 679-693, 2008.

16. **Pillen S, Tak RO, Zwarts MJ, Lammens MMY, Verrijp KN, Arts IMP, van der Laak JA, Hoogerbrugge PM, van Engelen BGM, and Verrips A.** Skeletal Muscle Ultrasound: Correlation Between Fibrous Tissue and Echo Intensity. *Ultrasound in medicine & biology* 35: 443-446, 2009.
17. **Pillen S, and van Alfen N.** Skeletal muscle ultrasound. *Neurological Research* 33: 1016-1024, 2011.
18. **Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, and Zwarts MJ.** Quantitative gray-scale analysis in skeletal muscle ultrasound: A comparison study of two ultrasound devices. *Muscle & Nerve* 39: 781-786, 2009.
19. **Pillen S, van Keimpema M, Nievelstein RAJ, Verrips A, van Kruijsbergen-Raijmann W, and Zwarts MJ.** Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound in medicine & biology* 32: 1315-1321, 2006.
20. **Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, and Eckel RH.** Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss: An Update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease From the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 113: 898-918, 2006.
21. **Rasband WS.** ImageJ. Bethesda, Maryland, USA: U.S. National Institutes of Health, 1997-2014.
22. **Reimers CD, Fleckenstein JL, Witt TN, Muller-Felber W, and Pongratz DE.** Muscular ultrasound in idiopathic inflammatory myopathies of adults. *Journal of the neurological sciences* 116: 82-92, 1993.

23. **Reimers CD, Schlotter B, Eicke BM, and Witt TN.** Calf enlargement in neuromuscular diseases: a quantitative ultrasound study in 350 patients and review of the literature. *Journal of the neurological sciences* 143: 46-56, 1996.
24. **Reimers K, Reimers CD, Wagner S, Paetzke I, and Pongratz DE.** Skeletal muscle sonography: a correlative study of echogenicity and morphology. *Journal of Ultrasound in Medicine* 12: 73-77, 1993.
25. **Ryan TE, Brizendine JT, Backus D, and McCully KK.** Electrically Induced Resistance Training in Individuals With Motor Complete Spinal Cord Injury. *Archives of Physical Medicine and Rehabilitation* 94: 2166-2173, 2013.
26. **Scholten RR, Pillen S, Verrips A, and Zwarts MJ.** Quantitative ultrasonography of skeletal muscles in children: Normal values. *Muscle & Nerve* 27: 693-698, 2003.
27. **Shaw CS, Clark J, and Wagenmakers AJM.** The Effect of Exercise and Nutrition on Intramuscular Fat Metabolism and Insulin Sensitivity. *Annual Review of Nutrition* 30: 13-34, 2010.
28. **Watanabe Y, Yamada Y, Fukumoto Y, Ishihara T, Yokoyama K, Yoshida T, Miyake M, Yamagata E, and Kimura M.** Echo intensity obtained from ultrasonography images reflecting muscle strength in elderly men. *Clinical Interventions in Aging* 8: 993-998, 2013.
29. **Wattjes M, Kley R, and Fischer D.** Neuromuscular imaging in inherited muscle diseases. *Eur Radiol* 20: 2447-2460, 2010.
30. **Wren TAL, Bluml S, Tseng-Ong L, and Gilsanz V.** Three-Point Technique of Fat Quantification of Muscle Tissue as a Marker of Disease Progression in Duchenne

Muscular Dystrophy: Preliminary Study. *American Journal of Roentgenology* 190: W8-W12, 2008.

31. **Zaidman CM, Holland MR, Anderson CC, and Pestronk A.** Calibrated quantitative ultrasound imaging of skeletal muscle using backscatter analysis. *Muscle & Nerve* 38: 893-898, 2008.

**Table 3.1. Physical characteristics of participants**

	<b>Male (n = 14)</b>	<b>Female (n = 17)</b>
<b>Age, yrs</b>	27.9 ± 14.9 (20-64)	21.9 ± 2.5 (20-29)
<b>Height, cm</b>	181.3 ± 7.8 (170-198.1)	165.3 ± 5.5 (157.5-175)
<b>Weight, kg</b>	77.6 ± 11.1 (62.6-95.8)	63.7 ± 9.3 (46.8-90.3)
<b>BMI, kg/m<sup>2</sup></b>	23.4 ± 3.5 (19.4-29.4)	23.6 ± 2.6 (18.9-33.1)

Note: Values are expressed as mean ± SD (range). BMI = body mass index

**Table 3.2. Outcomes of ultrasound and MRI testing**

	<b>Rectus Femoris (n= 28)</b>	<b>Biceps Femoris (n= 27)</b>	<b>Tibialis Anterior (n= 27)</b>	<b>Medial Gastrocnemius (n=26)</b>
<b>Ultrasound</b>				
<i>Examined Muscle Area, cm<sup>2</sup></i>	4.8 ± 1.4	13.2 ± 2.1	10.1 ± 1.5	8.8 ± 1.6
<i>Examined Muscle Volume, cm<sup>3</sup></i>	5.5 ± 2.5	14.6 ± 6.2	11.2 ± 4.7	11.1 ± 2.0
<i>Echo intensity, AU</i>	55.1 ± 7.4 (37.0-66.3)	42.6 ± 7.3 (23.1-56.9)	56.1 ± 8.0 (32.8-68.8)	51.5 ± 8.5 (32.2-65.9)
<b>MRI</b>				
<i>Examined Muscle Area, cm<sup>2</sup></i>	4.3 ± 1.5	16.0 ± 3.6	15.9 ± 2.9	16.9 ± 4.8
<i>Examined Muscle Volume, cm<sup>3</sup></i>	1.3 ± 0.5	4.8 ± 1.1	4.8 ± 0.9	5.1 ± 1.4
<i>Intramuscular fat, %</i>	13.1 ± 2.5 (7.8-16.3)	15.6 ± 4.6 (7.5-21.7)	14.7 ± 4.3 (8.5-25.3)	14.6 ± 4.8 (7.9-30.1)

Note: Values are expressed as mean ± SD (range). AU = arbitrary unit. MRI = magnetic resonance imaging.

**Table 3.3. Between-day reproducibility of the ultrasound technique**

	Day 1	Day 2	Mean CV (%)	ICC (95% CI)
<b>Muscle Echo Intensity</b>				
<i>Rectus Femoris, AU</i>	56.9 ± 6.9	57.7 ± 9.8	3.3 ± 3.0	0.91 (0.64-0.98)
<i>Biceps Femoris, AU</i>	42.0 ± 9.0	41.0 ± 11.2	13.1 ± 8.7	0.71 (-0.28-0.93)
<i>Tibialis Anterior, AU</i>	57.9 ± 5.5	58.1 ± 4.6	2.6 ± 1.6	0.90 (0.57-0.97)
<i>Medial Gastrocnemius, AU</i>	54.5 ± 7.5	54.7 ± 7.2	5.6 ± 4.9	0.80 (0.12-0.95)

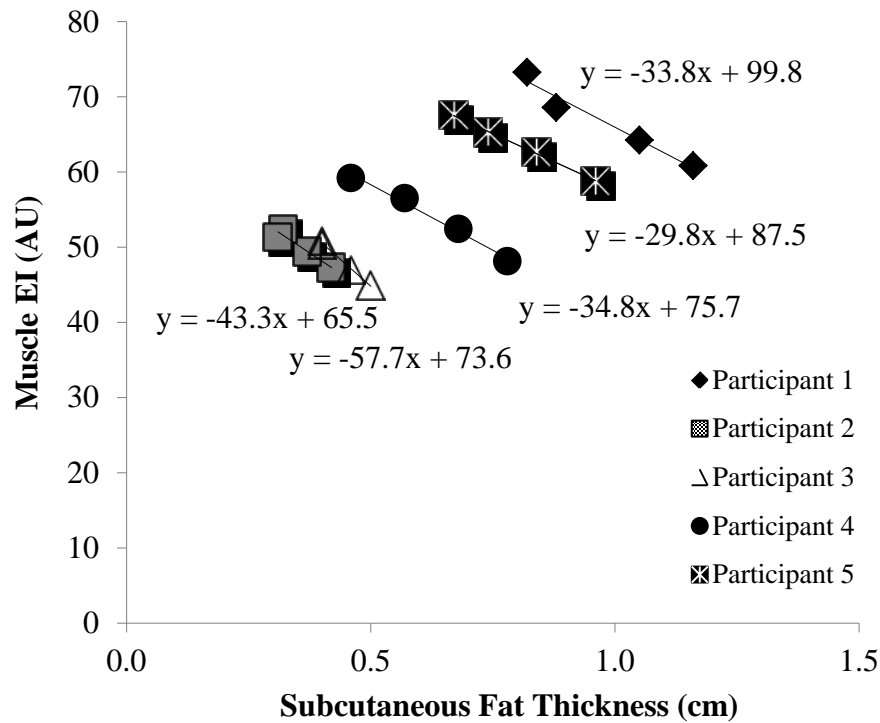
Note: Values are expressed as mean ± SD (range). AU = arbitrary units. CV = coefficient of variation. ICC = intraclass correlation coefficient.

**Table 3.4. Inter-analyzer reliability of the ultrasound technique**

	<b>Rater1</b>	<b>Rater 2</b>	<b>Mean CV (%)</b>	<b>ICC (95% CI)</b>
<b>Muscle Echo Intensity</b>				
<i>Rectus Femoris, AU</i>	53.2 ± 7.3	50.8 ± 7.3	4.3 ± 2.6	0.94 (0.66-0.98)
<i>Biceps Femoris, AU</i>	40.4 ± 7.5	39.1 ± 8.0	4.5 ± 2.7	0.97 (0.88-0.99)
<i>Tibialis Anterior, AU</i>	57.6 ± 8.7	55.1 ± 9.2	3.5 ± 2.2	0.97 (0.89-0.99)
<i>Medial Gastrocnemius, AU</i>	52.2 ± 9.1	51.4 ± 8.6	3.7 ± 3.1	0.97 (0.28-0.99)

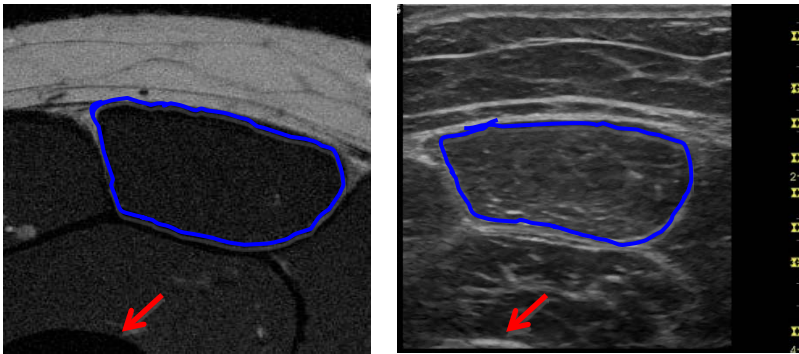
Note: Values are expressed as mean ± SD (range). AU = arbitrary units. CV = coefficient of variation. ICC = intraclass correlation coefficient.



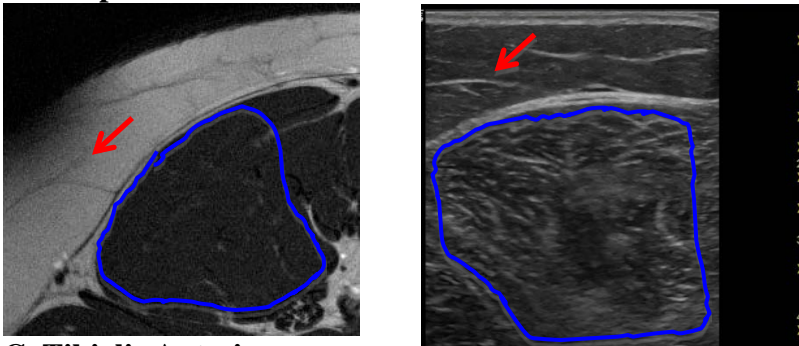


**Figure 3.1.** Correlations between subcutaneous fat thickness and muscle echo intensity (EI). Lower muscle EI is associated with higher subcutaneous fat thickness, suggesting an independent influence of subcutaneous fat thickness on muscle EI. The regression equations were averaged and used to establish a correction factor for subcutaneous fat thickness.

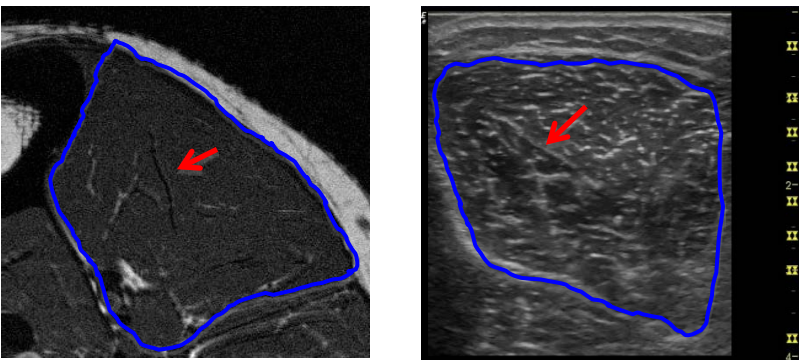
**A. Rectus Femoris**



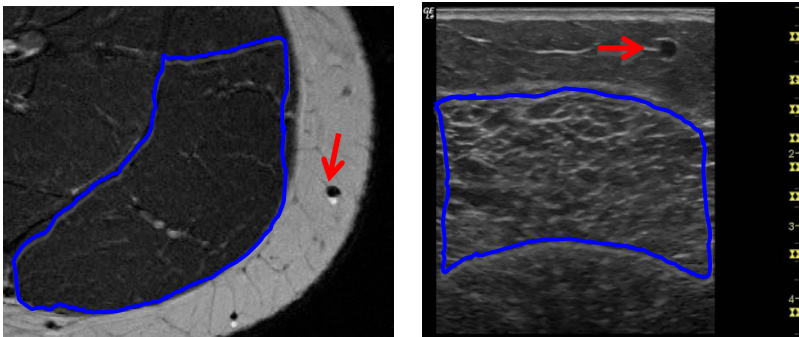
**B. Biceps Femoris**



**C. Tibialis Anterior**



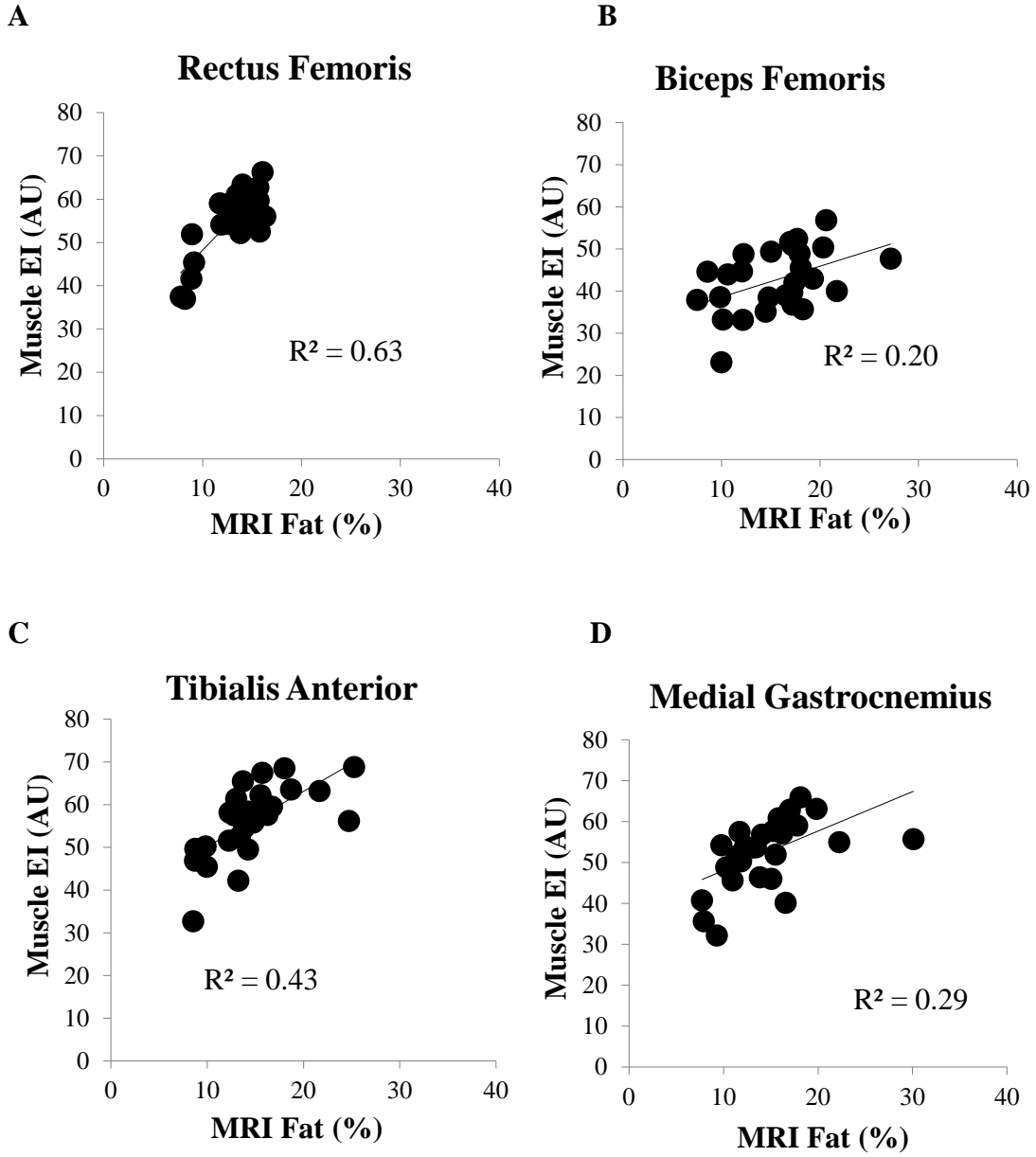
**D. Medial Gastrocnemius**



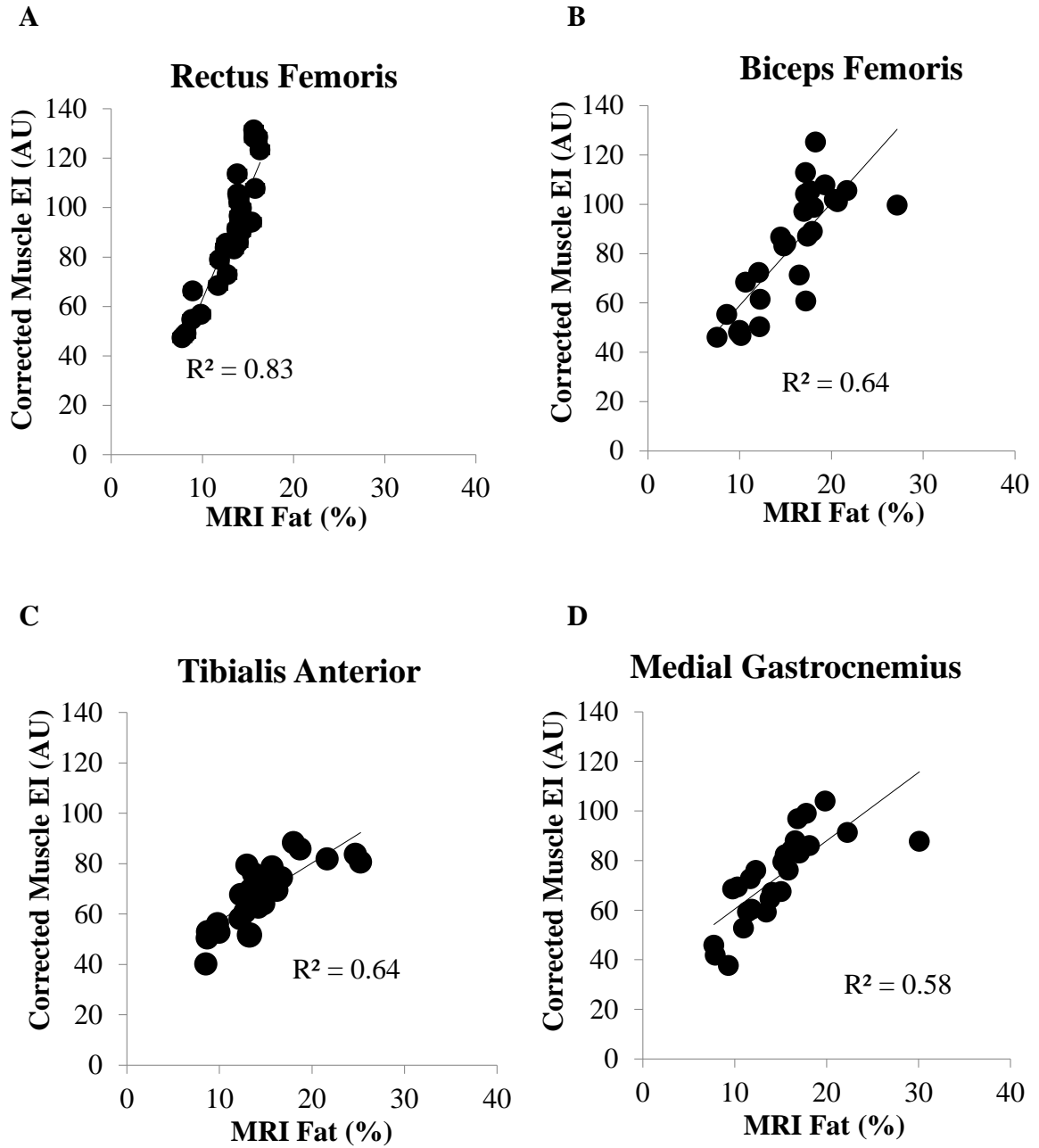
**MRI Images**

**Ultrasound Images**

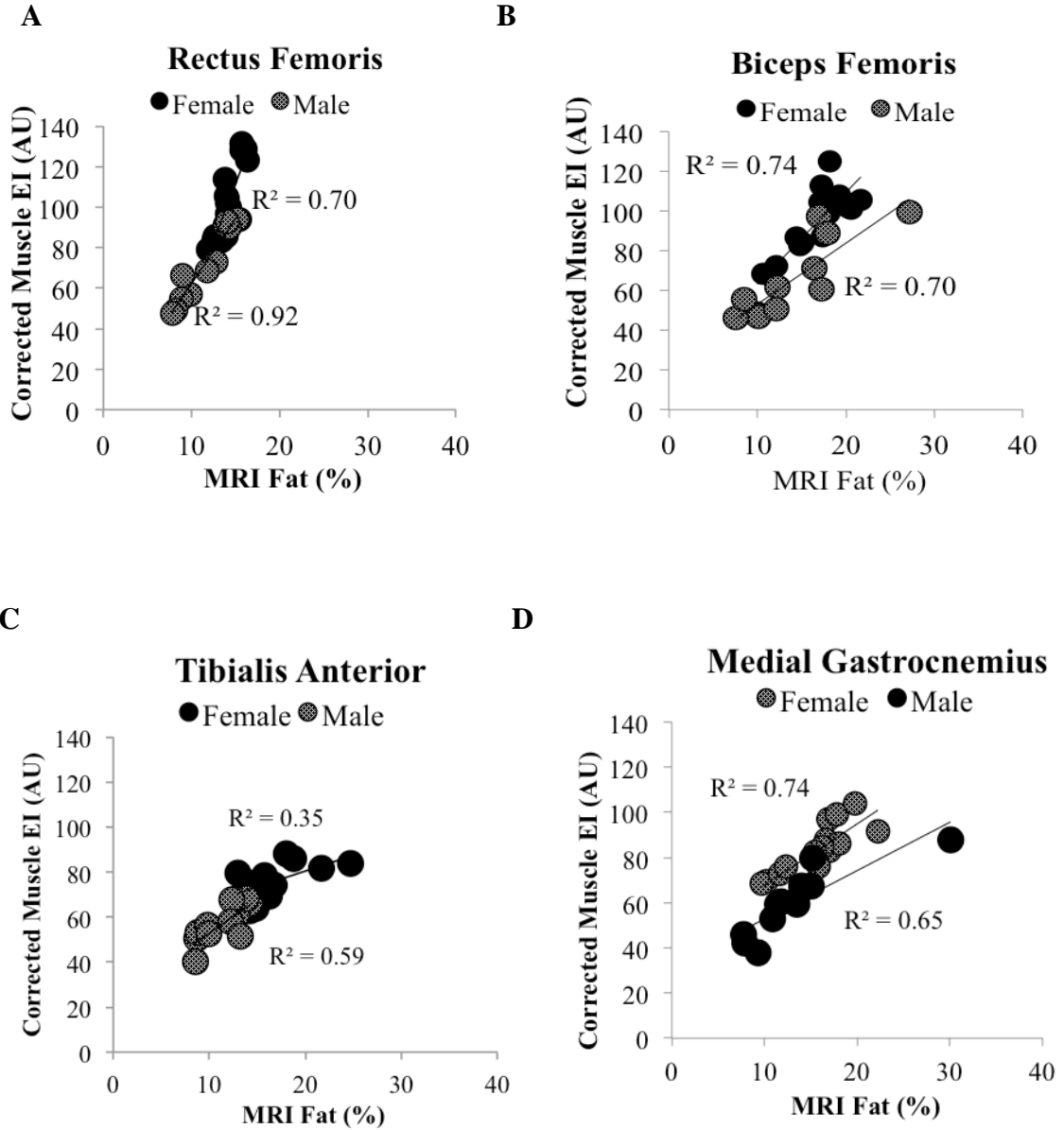
**Figure 3.2.** Representative  $T_1$ -weighted MRI and ultrasound B-mode images demonstrate the muscle site comparability between two imaging techniques. Red arrows indicate the lined-up anatomical appearance.



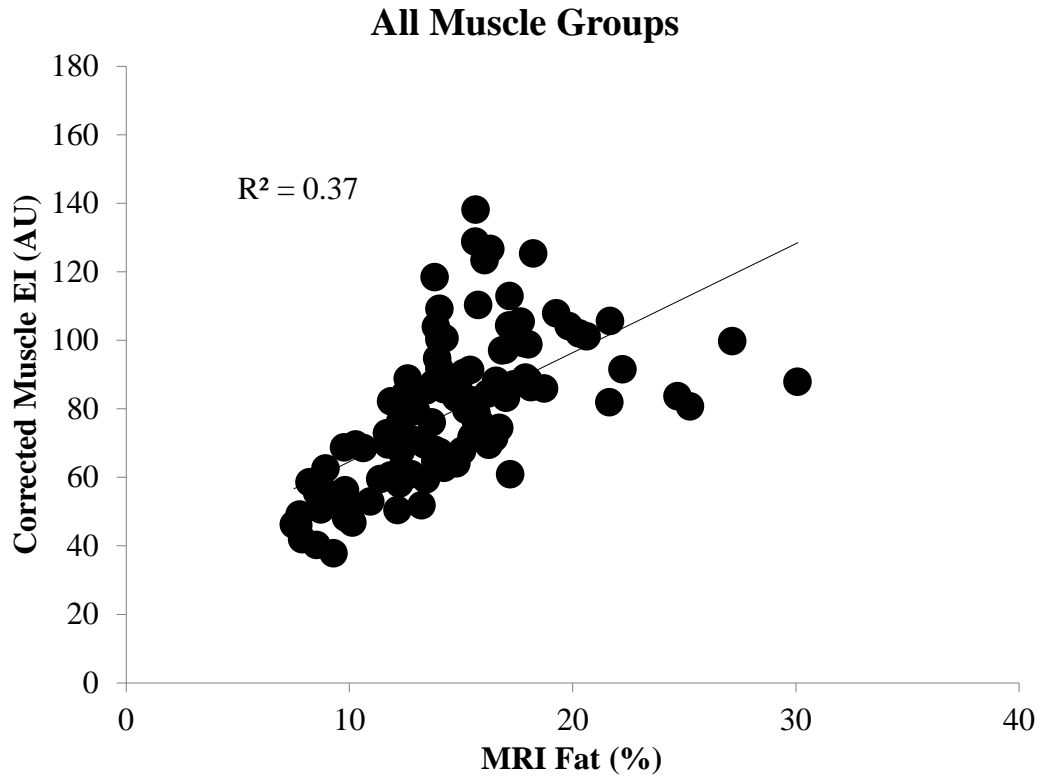
**Figure 3.3.** Correlations between MRI-measured % intramuscular fat and muscle echo intensity (EI) in the four muscle groups.



**Figure 3.4.** Correlations between MRI-measured % intramuscular fat and muscle echo intensity (EI) after correcting for subcutaneous fat thickness in the four muscle groups.

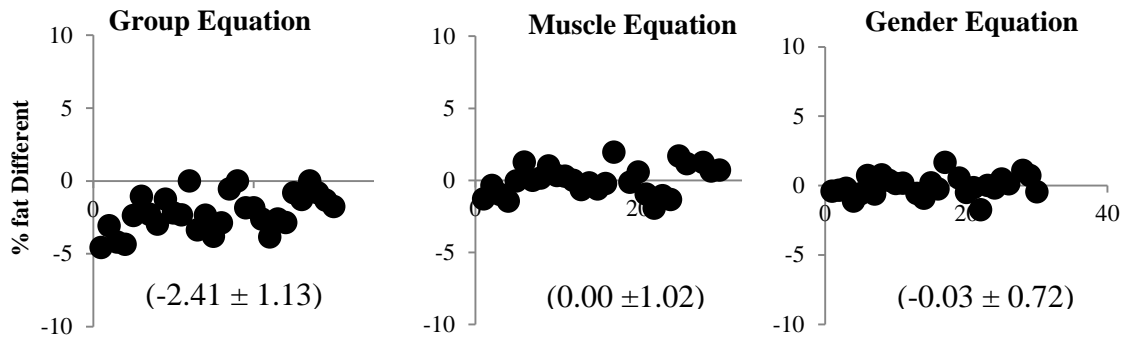


**Figure 3.5.** Correlations between MRI-measured % intramuscular fat and muscle echo intensity (EI) after correcting for subcutaneous fat thickness in the four muscle groups, separated by gender.

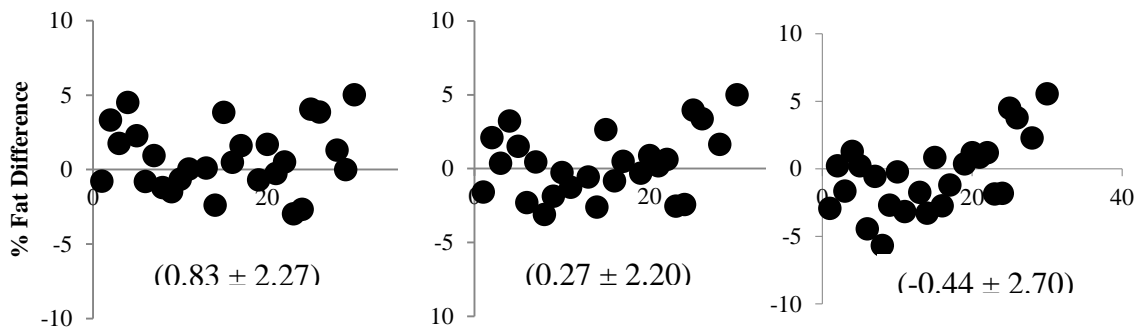


**Figure 3.6.** A correlation graph between MRI-measured % intramuscular fat and corrected muscle echo intensity (EI) of all four muscles.

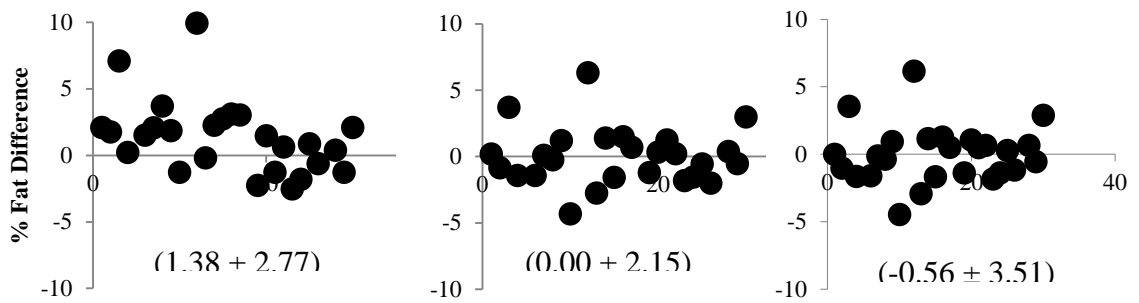
### A. Rectus Femoris



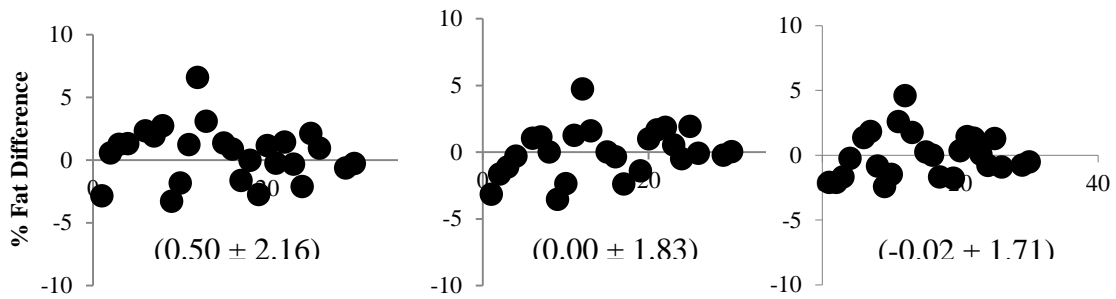
### B. Biceps Femoris



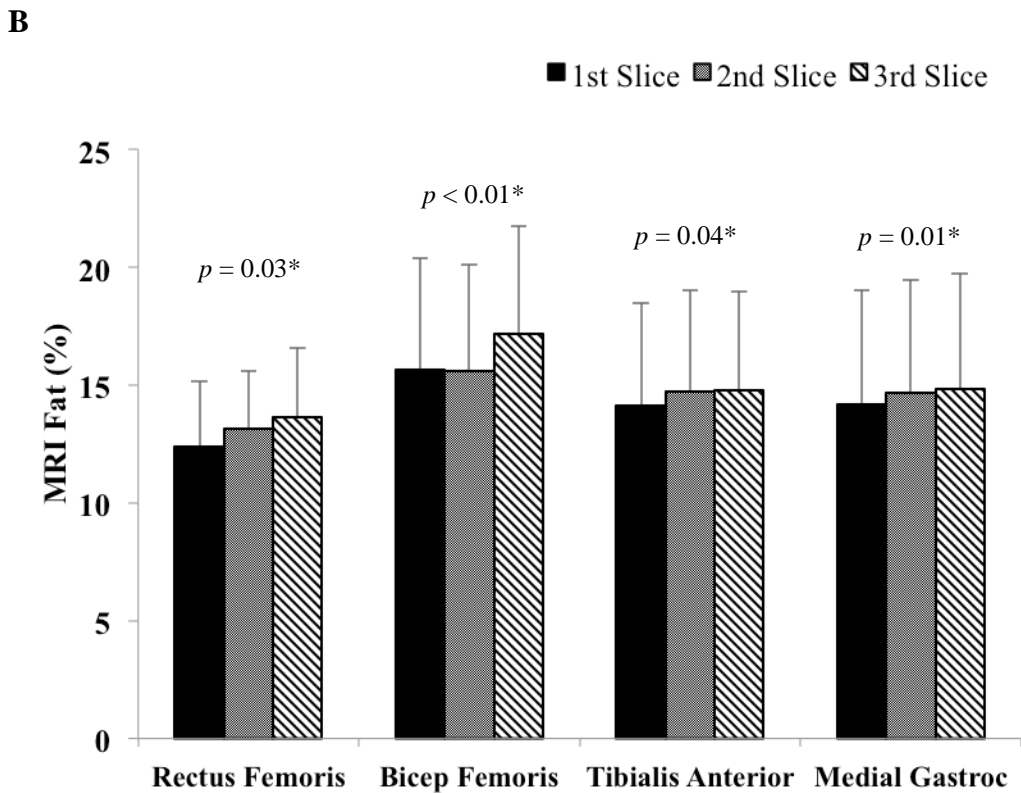
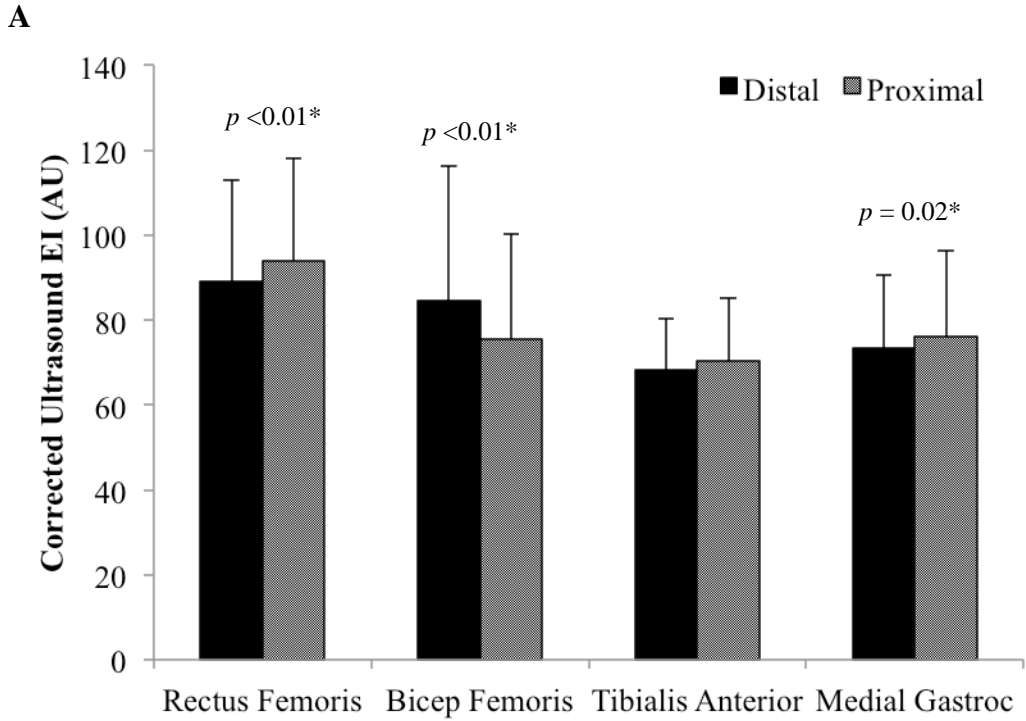
### C. Tibialis Anterior



### D. Medial Gastrocnemius



**Figure 3.7.** Differences between MRI-measured % fat and ultrasound % fat calculated using the three types of calibration equations.



**Figure 3.8.** Two representative graphs showing variability of muscle EIs between two different scanning sites within a muscle (A) as well as variability of % intramuscular fat between the three MRI slices (B).



**CHAPTER 4**

**COMPARISONS OF ULTRASOUND MEASURED PERCENT  
INTRAMUSCULAR FAT TO FITNESS AND HEALTH INDICATORS**

---

<sup>2</sup>Young, HJ, McCully, K.K. To be submitted to Journal of Ultrasound in Medicine.

## **Abstract**

**Purpose:** Previous studies have suggested that intramuscular fat content is inversely associated with health and fitness indicators. Recently, a newly established calibration method was proposed to estimate intramuscular fat with muscle echo intensity. The purpose of this study was to examine the ultrasound-measured % intramuscular fat of four muscles: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG), and to compare the results to other body composition measures, muscular strength, physical activity level, intake of fast food and sugary beverages, and blood lipids profile and glucose level.

**Methods:** Forty-two participants (16 males, 26 females) between the ages of 19 to 68 underwent a health and fitness screening interview, a finger-stick blood test, an ultrasound test, and a muscular strength test. Muscle echo intensity was quantified into % intramuscular fat using sex specific equations. **Results:** Strong correlations were found when compared % intramuscular fat across the four muscles ( $r \geq 0.8$ ). Weak to moderate correlations were found between % intramuscular fat and BMI ( $r \geq 0.2$ ), waist/hip ratio ( $r \geq 0.2$ ), muscle thickness ( $r = -0.5$  in RF,  $r = -0.4$  in TA,  $r = -0.7$  in MG), and muscular strength normalized by body weight (leg extension:  $r = 0.4$ , leg flexion:  $r = -0.5$ ). Potential relationships between % intramuscular fat, physical activity level, and frequency of fast food consumption were observed. **Conclusion:** The weak to moderate relationships between ultrasound-measured % intramuscular fat and other health and fitness indicators are consistent with previous literature, which suggests the use of the ultrasound technique for examination of intramuscular fat.

### *Keywords:*

Ultrasound, Echo Intensity, Intramuscular Fat, Calibration Equation

## **Introduction**

Obesity is associated with many health conditions such as cardiovascular disease and type-2 diabetes mellitus, which leads to increased morbidity and mortality in the world (11, 12, 22). While obesity in general is considered to be unhealthy, it has been observed that obese individuals do not always present higher risk of cardiovascular and metabolic disease, and similarly, lean individuals are not always exempt from those diseases (7, 8, 15, 16). Previous studies have suggested the importance of examining the distribution of adiposity (1, 7, 13). Koster et al. (16) reported in their study that individuals with metabolic syndrome were found to have higher abdominal visceral fat and lower thigh subcutaneous fat when compared to those without, which indicates the location of adipose tissue is important in determining health risks. In addition, increased skeletal muscle fat infiltration has been found to be related to insulin resistance and higher risk of diabetes (14, 19, 20). These results support the need to evaluate intramuscular fat as an independent health risk factor.

There are several non-invasive imaging techniques that have been used to assess intramuscular fat (21, 26, 28). Muscle ultrasound technique has recently emerged as an imaging technique to evaluate muscle quality (21). As assessed by computer-aided gray-scale analysis, muscle with high echogenicity indicates more intramuscular fat or connective tissue within the muscle. When compared to other imaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT), ultrasound is a much cheaper and easily accessible technology, making it a potentially valuable imaging tool to examine body and muscle composition. One limitation of the current ultrasound technique is that the data is typically reported in arbitrary echo intensity (EI) units, which

makes comparisons between ultrasound and other body composition techniques difficult. A novel approach that uses calibration equations to quantify muscle EI into % intramuscular fat has recently been established in our laboratory (Young et al, under review).

The purpose of this study was to use ultrasound to measure muscle %fat in four muscles in the lower extremity: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG), and to investigate the associations between ultrasound-measured % intramuscular fat and other health and fitness indicators.

## **Materials and Methods**

### *Participants*

Forty-two participants (16 males, 26 females) between the ages of 19 to 68 were recruited in this study. Participants with a wide range of body mass index (BMI) and physical activity level were recruited. Exclusion criteria were based on the following conditions: 1) any known medical condition that would be unsafe for participation in the study, and 2) any cognitive impairment such that informed consent cannot be obtained. The study was approved by the Institutional Review Board. We certify that all applicable instructional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this study. Written informed consent was obtained from all subjects prior to data collection and after a detailed description of the study was provided. Data was collected from March 2014 to May 2014.

### *Experimental Protocol*

All participants were asked to complete one testing session. The session was consisted of a health and fitness screening interview, a blood lipid profile and glucose

test, an ultrasound test, and a muscular strength test of the lower extremity. All tests and measurements were conducted in the Department of Kinesiology, Exercise Muscle Physiology Laboratory.

#### *Health and Fitness Interview & Lipid Profile Blood Test*

All participants completed a health and fitness screening interview in which their physical characteristics, health history, and frequency of fast food and sugary beverage consumptions were recorded. The International Physical Activity Questionnaire (IPAQ, short format) was used to evaluate physical activity level. Total MET per week was then calculated based on the published Guidelines for the data processing and analysis of the IPAQ.

Blood lipid profile and glucose level were measured using a portable blood lipid analyzer and test cassette system (Cholestech LDX) (2, 4). Participants were instructed to come in fast and were seated during the blood sampling procedure. Prior to the finger prick, the blood-sampling site was cleansed with an alcohol swab and was allowed to dry thoroughly. A 35  $\mu$ l of blood was collected using a capillary tube. The blood sample was then placed into a cassette for analysis. The analysis took no longer than 10 minutes and the total cholesterol (TC), triglyceride, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and glucose level were obtained.

#### *Lower Extremity Strength Test*

The maximal isometric strength of the knee extensors and flexors was measured using a Biodex system. Participants were positioned on the dynamometer with the knee of their dominant leg flexed at 60 °. Other body parts were fixed with shoulder, waist,

and thigh straps and a sensor pad was positioned in the lower leg. Isometric strength was measured with three 5-second leg extensions and flexions, separated by a 15-second rest period. Participants were instructed to give their best effort during the test and the consistency of the three trials was evaluated for quality control. Strength measurements were made prior to or on a different day from the ultrasound measurements.

### *Ultrasound Testing*

Four muscles in the lower extremity: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and medial gastrocnemius (MG) were assessed with an ultrasound imaging device (LOGIQ e; GE Healthcare UK Ltd., Chalfont, Buckinghamshire, England). The dominant leg of all the participants was tested. Each participant was instructed to rest supine on an exam table. The upper leg length of each participant was measured from lateral patella base to anterior superior iliac spine, and the lower leg length was measured from lateral patella tip to calcaneus.

Ultrasound Brightness mode (B-mode) with musculoskeletal scanning preset and a multi-frequency linear transducer (8-12 MHz) were used. Gain and transducer frequency were adjusted to 58-dB and 8 Hz, respectively. Scanning depth was set to be 4cm and was only changed when testing participants with greater subcutaneous fat to ensure enough muscle area was captured. Time gain compensation was adjusted to neutral position. Focus number and area were adjusted to cover the entire muscle being examined as well as to make sure the consistency of focus area across all participants. Other ultrasound settings were maintained at manufacturer's default settings. Marks were made on the 1/3 and 1/4 (distal and proximal to the knee) of the anterior and

posterior parts of the upper and lower legs. The purpose of the marks was to ensure the consistency of scanning locations across all participants. To start the ultrasound test, a generous amount of ultrasound gel was then applied to the ultrasound probe avoid excessive pressure placed on the skin. Each scan involved a 16-second ultrasound clip on one of the marks. Each muscle was scanned twice on the distal and proximal sites, and a total of 8 scans was obtained from each participant. Each ultrasound clip was then saved into JPEG images for analysis.

#### *Ultrasound-measured % Intramuscular Fat Analysis*

Muscle EI was determined by gray-scale analysis using ImageJ (24). A muscle was manually circled while executing best effort to avoid surrounding fascia and bone. The mean pixel intensity of the selected muscle was obtained from each measurement and the average of 3 measurements was calculated. Subcutaneous fat thickness was also analyzed. The % intramuscular fat of each muscle group was then calculated with measured muscle EI and subcutaneous fat thickness using the sex specific equations presented below where  $x = \text{raw echo intensity}$ ,  $y = \text{\% intramuscular fat}$ , and  $z = \text{Subcutaneous fat thickness}$ ,

$$RF: \quad y = (0.062 * ((40 * z) + x)) + 7.901 \text{ (Female)} \quad \text{(Equation 4.1.1)}$$

$$y = (0.144 * ((40 * z) + x)) + 1.126 \text{ (Male)} \quad \text{(Equation 4.1.2)}$$

$$BF: \quad y = (0.177 * ((40 * z) + x)) + 1.823 \text{ (Female)} \quad \text{(Equation 4.2.1)}$$

$$y = (0.152 * ((40 * z) + x)) + 2.368 \text{ (Male)} \quad \text{(Equation 4.2.2)}$$

$$TA: \quad y = (0.250 * ((40 * z) + x)) - 2.366 \text{ (Female)} \quad \text{(Equation 4.3.1)}$$

$$y = (0.198 * ((40 * z) + x)) + 0.094 \text{ (Male)} \quad \text{(Equation 4.3.2)}$$

$$MG: \quad y = (0.177 * ((40 * z) + x)) + 1.823 \text{ (Female)} \quad \text{(Equation 4.4.1)}$$

$$y = (0.152 * ((40 * z) + x)) + 2.368 \text{ (Male)} \quad \text{(Equation 4.4.2)}$$

### Statistical analysis

Data are presented as means  $\pm$  SD. Relationships between ultrasound-measured % intramuscular fat and all the other outcome variables were analyzed using Pearson correlation coefficient. Coefficient of variation (CV) was calculated to examine the differences between the distal and proximal scanning sites, and the differences were compared using a two-tailed Student's *t*-test for paired samples. Participants were classified into three groups based on their physical activity level (low, moderate, vigorous) and three other groups based on their frequency of fast food consumption (<1 time/wk, 1 time/wk, and >1 time/wk). One-way analysis of variance (ANOVA) with within-subjects factor (physical activity level and frequency of fast food consumption) was performed. Statistical analyses were performed using SPSS 19.0 (IBM®, Armonk, NY). Significance was accepted when  $p < 0.05$ .

## **Results**

### Study Participants

All participants completed the study without any adverse events. The physical characteristics, presence of chronic disease, lower extremity muscular strength, blood lipid profile, and blood glucose level of the participants are summarized in Table 4.1.

### Comparisons between Scanning Locations & Muscle Groups



The ultrasound EI and subcutaneous fat thickness of each muscle group are shown in Table 4.2A. When compared EI and subcutaneous fat thickness of the distal and proximal sites of each muscle, the mean CV between the two sites was 6.2 % in RF, 5.7 % in BF, 2.6 % in TA, and 4.6 % in MG. When examining the subcutaneous fat thickness, the mean CV between the two sites was 7.8 % in RF, 19.3 % in BF, 18.0 % in TA, and 19.3 % in MG. Both EI and subcutaneous fat thickness of each muscle group showed significant difference or approaching to significant difference between the two scanning sites. Table 4.2B demonstrates ultrasound-measured % intramuscular fat of each muscle group. The mean CV between the distal and proximal sites was 3.6 % in RF, 7.2 % in BF, 5.4 % in TA, and 6.0 % in MG. Statistically significant but small differences were found in all muscle groups except TA when comparisons of % intramuscular fat between the two scanning sites were made ( $0.4 \pm 0.7$  in RF,  $p < 0.01$ ;  $1.6 \pm 2.0$  in BF,  $p < 0.01$ ;  $0.3 \pm 1.5$  in TA,  $p = 0.3$ ;  $0.6 \pm 1.7$  in MG,  $p = 0.02$ ). The ultrasound-measured % intramuscular fat was also compared across each muscle group and significant correlations were found in all comparisons (Table 4.3). A stronger correlation was found when compared RF % intramuscular fat to MG % intramuscular fat ( $r = 0.8$ ) and BF % intramuscular fat to TA % intramuscular fat ( $r = 0.8$ ).

#### Ultrasound % Fat v.s BMI, Waist/Hip Ratio, and Muscle Thickness

Relationships between ultrasound-measured % intramuscular fat of each muscle group and body mass index (BMI), waist/hip ratio, and muscle thickness are summarized in Tables 4.4. Significant correlations were found between BMI and ultrasound-measured % fat in all muscle groups except TA ( $r = 0.3$  in RF,  $r = 0.4$  in BF,  $r = 0.2$  in

TA,  $r = 0.4$  in MG). When using age and gender as control variables (Table 4.5), BMI and % intramuscular fat showed significant positive correlations in all muscle groups ( $r = 0.3$  in RF,  $r = 0.6$  in BF,  $r = 0.3$  in TA,  $r = 0.4$  in MG). A significant correlation was found when compared RF % intramuscular fat to waist/hip ratio ( $r = 0.4$ ,  $p = 0.03$ ), but no relationship was found in other muscles. With age and gender as control variables, a weaker correlation between RF % intramuscular fat and waist/hip ratio was observed ( $r = 0.2$ ). When compared % intramuscular fat to muscle thickness, a negative correlation was found in all muscle groups ( $r = -0.5$  in RF,  $r = -0.4$  in TA,  $r = -0.7$  in MG). With age and gender as control variables, the % intramuscular fat and muscle thickness of RF and MG showed slightly weaker but still significant correlations ( $r = 0.4$  in RF,  $r = 0.6$  in MG). BF muscle thickness was not obtained in every participant due to the limitation of ultrasound window and thus was not reported. A mean % intramuscular fat was also calculated by averaging % intramuscular fat of all the muscle groups. When examining the association between BMI and the mean % intramuscular fat (Figure 4.1), a moderate and significant correlation was found ( $r = 0.4$ ,  $p < 0.01$ ). No relationship was found when compared the mean % intramuscular fat to waist/hip ratio ( $r = 0.09$ ,  $p = 0.3$ ).

#### Ultrasound % Fat vs. Muscular Strength

Figure 4.2 shows the associations between muscular strength and ultrasound-measured % intramuscular fat. No significant relationship was found when compared the RF % intramuscular fat to the peak torque of leg extension ( $r = -0.3$ ,  $p = 0.08$ ). When examining the correlation between the BF % intramuscular fat and the peak torque of leg flexion, a moderate negative correlation was found ( $r = -0.4$ ,  $p < 0.01$ ). When the peak

torque was normalized by body weight, better and significant correlations were found between RF % fat and leg extension strength ( $r = -0.4, p = 0.01$ ) and between BF % fat and leg flexion strength ( $r = -0.5, p < 0.01$ ). When normalizing the peak torque with muscle area, a positive correlation was found between RF % fat and leg extension strength ( $r = 0.4, p = 0.02$ ). The relationship between BF % fat and leg flexion strength was diminished after normalized by muscle area ( $r = 0.1, p = 0.5$ ).

#### Ultrasound % Fat vs. Physical Activity Level & Energy Intake

The relationship between ultrasound-measured % intramuscular fat and physical activity level was examined (Table 4.6). Significant and negative correlations were found when compared total MET to RF % intramuscular fat ( $r = -0.3, p = 0.03$ ) and MG % intramuscular fat ( $r = -0.4, p = 0.01$ ). Non-significant weak relationships were found when compared total MET to BF % intramuscular fat ( $r = -0.2, p = 0.3$ ) and TA % intramuscular fat ( $r = -0.2, p = 0.2$ ). When compared the mean % intramuscular fat to walking MET, total MET, frequency of sugary beverage consumption, and frequency of fast food consumption (Table 4.7), weak and non-significant correlations were found between the mean % fat and total MET ( $r = -0.3, p = 0.06$ ) and between the mean % fat and fast food consumption ( $r = -0.2, p = 0.2$ ). No relationship was found when compared the mean % intramuscular fat to walking MET and sugary beverage consumption. To further examining the relationship between % intramuscular fat and physical activity level, participants were divided into 3 groups based on their total MET: low, moderate, and vigorous (Figure 4.3A). A one-way ANOVA was performed to test the relationship between % intramuscular fat and the three physical activity levels. The ANOVA was

significant,  $F(2, 39) = 3.934, p = 0.028$ . Participants were again divided into 3 groups based on their frequency of fast food consumption: < 1 time/wk, 1 time/wk, and > 1 time/wk (Figure 4.3B). A one-way ANOVA was performed to examine the relationship between % intramuscular fat and fast food consumption frequency. The ANOVA was not significant,  $F(2, 39) = 1.931, p = 0.159$ . When combined moderate and vigorous physical activity groups together ( $14.1 \pm 2.9, n = 37$ ) and compared to the low physical activity group ( $18.0 \pm 2.5, n = 5$ ), a significant difference was found between groups ( $p = 0.01$ ). When compared  $\leq 1$  time per week fast food consumption group to the > 1 time per week group, a significant difference was observed between the two groups ( $p = 0.01$ ).

#### Ultrasound % Fat vs. Blood Lipid Profile and Glucose Level

The mean % intramuscular fat was compared to blood lipid profile and glucose and the results were shown in Table 4.8. No significant relationship was found when compared the mean % intramuscular fat to total cholesterol and blood glucose level. Weak and non-significant correlations were found when compared mean % intramuscular fat to HDL ( $r = 0.2$ ), triglyceride ( $r = 0.1$ ), and LDL ( $r = -0.1$ ).

## **Discussion**

The present study is the first to report % intramuscular fat measured with muscle ultrasound in four muscles of the lower extremity. The study also showed consistent findings when compared to previous literature that examined the correlations of intramuscular fat across different muscles as well as the relationships between % intramuscular fat measured with other techniques and BMI, waist to hip ratio, muscle

thickness, and fast food consumption.

In this study, moderate to strong correlations in ultrasound-measured % intramuscular fat were found across all the muscles examined. Wren et al. (29) investigated the % intramuscular fat between different muscles and reported strong correlations between each muscle group ( $r > 0.8$ ). Goodpaster et al. utilized fat-selective MRI technique to examine intramuscular lipid content of different muscles in sixteen normal weight and obese subjects (10). They reported a correlation of 0.66 between the lipid content of MG and TA. The present study found a correlation of 0.71 between MG % intramuscular fat and TA % intramuscular fat. Similar correlations were also observed between other muscle groups. The results are consistent with the previous studies.

The present study suggested a positive correlation between BMI and ultrasound-measured % intramuscular fat. Kovanlikaya et al. examined % fat in the liver, muscle, and pancreas in 15 healthy Mexican-American females and concluded a strong correlation between BMI and % fat in the soleus muscle (17). Goodpaster et al. (10) examined intramuscular fat using MRI and reported a positive correlation between BMI and the lipid content of MG ( $r = 0.62$ ) but no relationship between BMI and lipid content of the TA ( $r = 0.11$ ) and soleus ( $r = 0.12$ ). The present study found a weaker correlation between BMI and MG % intramuscular fat when compared to the correlation that Goodpaster et al. reported. A possible explanation is that the ultrasound didn't capture the entire area of MG, which could result in overestimating or underestimating the actual % intramuscular fat. When examining the relationship between % intramuscular fat and waist/hip ratio, a significant correlation was observed in RF but not the other muscle groups, both before and after controlling for age and gender. Thamer et al. reported a

positive correlation between waist-hip ratio and intramuscular lipid content of the soleus muscle but not the TA muscle (5). Although the present study did not examine soleus muscle, it is suggested that type-1 muscle fibers were more predominant in the quadriceps, similar to soleus, when compared to TA, MG, and BF (23). The current finding was supported. In addition, significant correlations were found between % intramuscular fat and muscle thickness, both before and after controlling for age and gender. This finding is comparable to previous ultrasound studies that suggested the association between decreased muscle EI and increased muscle thickness (3, 9). Ryan et al. (25) compared the affected and non-affected legs in stroke patients and reported a negative correlation between intramuscular fat and muscle area of the thigh, consistent with our results.

In this study, an interesting relationship was observed between % intramuscular fat and physical activity level. Based on our finding, a significant correlation was found when compared % intramuscular fat in the RF and MG to physical activity level measured with total MET. On the other hand, no relationship was found when compared % intramuscular fat in the BF and TA to physical activity level, both measured with walking MET and total MET. A possible explanation is that the quadriceps and calves are the primary muscles for performing the majority of daily activity, which contribute to their better associations with physical activity level. In addition, when classified participants into 3 groups based on their physical activity level, participants in the low physical activity group had significantly higher % intramuscular fat when compared to participants in the moderate and vigorous groups. This finding suggests a potential curvilinear relationship between intramuscular lipid content and physical activity level.

Studies with larger sample size will be needed to test this hypothesis. Another interesting finding was the association between fast food consumption and % intramuscular fat. When classified the participants into 3 groups based on their fast food consumption frequency, participants who consumed fast food more than once per week had significantly higher % intramuscular fat when compared to participants who consumed fast food less than one time per week. Although this result is comparable to previous studies that observed the association between higher intramuscular lipid content and high fat diet (18, 27), this non-linear relationship requires further investigation. Future studies with larger sample size will be needed to perform more detailed dietary assessment to further examine this relationship.

Weak or no relationship between ultrasound-measured intramuscular fat and blood lipid profile and glucose level was observed in this study. There are several potential reasons for this non-significant relationship. First, the participants recruited in this study were mostly young and healthy individuals with very similar levels of total cholesterol, HDL, triglyceride, LDL, and blood glucose. The invariability in blood lipid profile and glucose level could contribute to the weak relationship found in the study. Another explanation is the presence of intramuscular fat as a metabolic fuel source in highly physically fit individuals. Previous studies have found that increased intramuscular lipid content in both obese diabetic individuals and highly trained athletes, suggesting fat stored within skeletal muscle is not always detrimental (1, 6). In order to further investigate the relationship between blood lipid profile and intramuscular fat content, studies with larger sample size with a broader range of blood lipid profiles are needed.

In conclusion, we compared ultrasound-measured % intramuscular fat in four different muscles to some health and fitness indicators. Significant correlations were found in % intramuscular fat across different muscle groups, which are consistent with previous studies. In addition, the mean % intramuscular fat was found to be positively associated with BMI and muscle thickness. Weak to moderate associations between ultrasound-measured % intramuscular fat, physical activity level, and dietary intake were also observed while no relationship was found between % intramuscular fat and blood lipid profile and glucose level. Further studies with larger sample size are required to further examine the relationship between intramuscular lipid content and lifestyle.

#### *Acknowledgements*

The authors would like to thank all the undergraduate assistants or their help on data collection and analysis as well as all the study participants for their enthusiastic commitment to this study.

#### *Conflict of interest statement*

The authors report no conflicts of interest



## References

1. **Addison O, Marcus RL, LaStayo PC, and Ryan AS.** Intermuscular Fat: A Review of the Consequences and Causes. *International Journal of Endocrinology* 2014: 11, 2014.
2. **Bard RL, Kaminsky LA, Whaley MH, and Zajakowski S.** Evaluation of Lipid Profile Measurements Obtained From the Cholestech L.D.X Analyzer. *Journal of Cardiopulmonary Rehabilitation and Prevention* 17: 413-418, 1997.
3. **Cadore EL, Izquierdo M, Conceicao M, Radaelli Rg, Pinto RS, Baroni BM, Vaz MAI, Alberton CL, Pinto SS, Cunha G, Bottaro M, and Kruel LFM.** Echo intensity is associated with skeletal muscle power and cardiovascular performance in elderly men. *Experimental Gerontology* 47: 473-478, 2012.
4. **Carey M, Markham C, Gaffney P, Boran G, and Maher V.** Validation of a point of care lipid analyser using a hospital based reference laboratory. *Ir J Med Sci* 175: 30-35, 2006.
5. **Claus Thamer, Jürgen Machann, Oliver Bachmann, Michael Haap, Dominik Dahl, Beate Wietek, Otto Tschritter, Andreas Niess, Klaus Brechtel, Andreas Fritsche, Claus Claussen, Stephan Jacob, Fritz Schick, Hans-Ulrich Häring, and Michael Stumvoll.** Intramyocellular Lipids: Anthropometric Determinants and Relationships with Maximal Aerobic Capacity and Insulin Sensitivity. *The Journal of Clinical Endocrinology & Metabolism* 88: 1785-1791, 2003.
6. **Coen PM, and Goodpaster BH.** Role of intramyocellular lipids in human health. *Trends in Endocrinology & Metabolism* 23: 391-398.
7. **Després J.** Body Fat Distribution and Risk of Cardiovascular Disease: An Update. *Circulation* 126: 1301-1313, 2012.

8. **Després J, Moorjani S, Lupien P, Tremblay A, Nadeau A, and Bouchard C.** Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* 10: 497-511, 1990.
9. **Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, Kimura M, and Ichihashi N.** Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur J Appl Physiol* 112: 1519-1525, 2012.
10. **Goodpaster BH, Stenger VA, Boada F, McKolanis T, Davis D, Ross R, and Kelley DE.** Skeletal muscle lipid concentration quantified by magnetic resonance imaging. *The American Journal of Clinical Nutrition* 79: 748-754, 2004.
11. **Goya Wannamethee S, Gerald Shaper A, Whincup PH, and Walker M.** Overweight and obesity and the burden of disease and disability in elderly men. *International Journal Of Obesity And Related Metabolic Disorders: Journal Of The International Association For The Study Of Obesity* 28: 1374-1382, 2004.
12. **Grundy SM.** Obesity, Metabolic Syndrome, and Cardiovascular Disease. *Journal of Clinical Endocrinology & Metabolism* 89: 2595-2600, 2004.
13. **Hermsdorff HH, and Monteiro JB.** [Visceral, subcutaneous or intramuscular fat: where is the problem?]. *Arquivos brasileiros de endocrinologia e metabologia* 48: 803-811, 2004.
14. **Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, and H $\ddot{u}$ ring HU.** Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48: 1113-1119, 1999.

15. **Karelis A, St-Pierre D, Conus F, Rabasa-Lhoret R, and Poehlman E.** Metabolic and Body Composition Factors in Subgroups of Obesity: What Do We Know? *The Journal of Clinical Endocrinology & Metabolism* 89: 2569-2575, 2004.
16. **Koster A, Stenholm S, Alley DE, Kim LJ, Simonsick EM, Kanaya AM, Visser M, Houston DK, Nicklas BJ, Tylavsky FA, Satterfield S, Goodpaster BH, Ferrucci L, Harris TB, and For the Health ABCS.** Body Fat Distribution and Inflammation Among Obese Older Adults With and Without Metabolic Syndrome. *Obesity* 18: 2354-2361, 2010.
17. **Kovanlikaya A, Mittelman S, Ward A, Geffner M, Dorey F, and Gilsanz V.** Obesity and fat quantification in lean tissues using three-point Dixon MR imaging. *Pediatr Radiol* 35: 601-607, 2005.
18. **Lara-Castro C, Newcomer BR, Rowell J, Wallace P, Shaughnessy SM, Munoz AJ, Shiflett AM, Rigby DY, Lawrence JC, Bohning DE, Buchthal S, and Garvey WT.** Effects of short-term very low-calorie diet on intramyocellular lipid and insulin sensitivity in nondiabetic and type 2 diabetic subjects. *Metabolism - Clinical and Experimental* 57: 1-8, 2008.
19. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, and Storlien LH.** Skeletal Muscle Triglyceride Levels Are Inversely Related to Insulin Action. *Diabetes* 46: 983-988, 1997.
20. **Phillips DIW, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, and Taylor R.** Intramuscular triglyceride and muscle insulin sensitivity: Evidence for a relationship in nondiabetic subjects. *Metabolism - Clinical and Experimental* 45: 947-950.

21. **Pillen S, and van Alfen N.** Skeletal muscle ultrasound. *Neurological Research* 33: 1016-1024, 2011.
22. **Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, and Eckel RH.** Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss: An Update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease From the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 113: 898-918, 2006.
23. **Polgar J, Johnson MA, Weightman D, and Appleton D.** Data on fibre size in thirty-six human muscles: An autopsy study. *Journal of the neurological sciences* 19: 307-318, 1973.
24. **Rasband WS.** ImageJ. Bethesda, Maryland, USA: U.S. National Institutes of Health, 1197-2014.
25. **Ryan AS, Buscemi A, Forrester L, Hafer-Macko CE, and Ivey FM.** Atrophy and Intramuscular Fat in Specific Muscles of the Thigh: Associated Weakness and Hyperinsulinemia in Stroke Survivors. *Neurorehabilitation and Neural Repair* 25: 865-872, 2011.
26. **Shen W, Wang Z, Punyanita M, Lei J, Shnav A, Kral J, Imielinska C, Ross R, and Heymsfield S.** Adipose tissue quantification by imaging methods: a proposed classification. *Obesity Research* 11: 5-16, 2003.
27. **St-Onge M-P, Newcomer BR, Buchthal S, Aban I, Allison DB, Bosarge A, and Gower B.** Intramyocellular lipid content is lower with a low-fat diet than with high-fat diets, but that may not be relevant for health. *The American Journal of Clinical Nutrition* 86: 1316-1322, 2007.

28. **Wattjes M, Kley R, and Fischer D.** Neuromuscular imaging in inherited muscle diseases. *Eur Radiol* 20: 2447-2460, 2010.
29. **Wren TAL, Bluml S, Tseng-Ong L, and Gilsanz V.** Three-Point Technique of Fat Quantification of Muscle Tissue as a Marker of Disease Progression in Duchenne Muscular Dystrophy: Preliminary Study. *American Journal of Roentgenology* 190: W8-W12, 2008.

**Table 4.1 Physical Characteristics of Study Participants**

<b>n= 42 (16 males; 26 females)</b>	
<b>Age, yrs old</b>	24.9 ± 11.4 (19.0-68.0)
<b>Ethnicity, %</b>	
<i>Caucasian</i>	85.7
<i>White Hispanic</i>	2.4
<i>Asian</i>	7.1
<i>African American</i>	4.8
<b>BMI, kg/m<sup>2</sup></b>	23.3 ± 3.0 (18.5-33.1)
<b>Waist/Hip Ratio</b>	Males: 0.9 ± 0.1; Females: 0.8 ± 0.1 (0.69-1.15)
<b>Hypertension, %</b>	0
<b>Cardiovascular Disease, %</b>	2.4
<b>Diabetes, %</b>	2.4
<b>Current Smoker, %</b>	2.4
<b>Leg extension strength</b>	
<i>Peak TQ, N-M</i>	173.4 ± 35.4 (107.3-255.0)
<i>Average Peak TQ, N-M</i>	162.7 ± 33.7 (99.7-235.2)
<i>Average PKTQ/BW, %</i>	233.3 ± 35.4 (107.3-255.0)
<i>Average PKTQ/MA, %</i>	186.9 ± 37.7 (119.3-296.2)
<b>Leg flexion strength</b>	
<i>Peak TQ, N-M</i>	109.0 ± 25.1 (71.8-203.3)
<i>Average Peak TQ, N-M</i>	102.1 ± 23.8 (65.2-192.6)
<i>Average PKTQ/BW, %</i>	145.7 ± 27.5 (89.0-243.8)
<i>Average PKTQ/MA, %</i>	116.0 ± 26.2 (70.4-190.4)
<b>Blood profile, mg/dL</b>	
<i>Total Cholesterol</i>	173.5 ± 23.4 (100.0-228.0)
<i>HDL</i>	63.1 ± 14.5 (32.0-95.0)
<i>Triglyceride</i>	103.5 ± 46.1 (45.0-195.0)
<i>LDL</i>	91.1 ± 21.2 (36.0-132.0)
<i>Glucose</i>	89.2 ± 6.0 (79.0-104.0)

Note: Values are expressed as mean ± SD (range). BMI = body mass index, TQ = torque, PKTQ = peak torque, PKTQ/BW = peak torque normalized by body weight, PKTQ/MA = peak torque normalized by muscle area, HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol.

**Table 4.2 Comparisons between Scanning Locations****A**

	<b>Distal</b>	<b>Proximal</b>	<b>CV (%)</b>	<b>p value</b>
<b>Echo Intensity, AU</b>				
<i>Rectus Femoris</i>	54.9 ± 7.1 (37.0-68.1)	57.6 ± 8.1 (37.5-76.7)	6.2 ± 5.1	< 0.01
<i>Bicep Femoris</i>	42.1 ± 7.3 (23.1-56.9)	43.0 ± 6.8 (27.3-62.3)	5.7 ± 5.8	0.06
<i>Tibialis Anterior</i>	57.6 ± 8.0 (32.8-69.6)	56.0 ± 9.3 (27.6-71.4)	2.6 ± 2.5	0.06
<i>Medial Gastrocnemius</i>	52.4 ± 8.0 (32.2-65.9)	50.0 ± 7.5 (32.8-65.5)	4.6 ± 4.1	< 0.01
<b>Subcutaneous Fat, cm</b>				
<i>Rectus Femoris</i>	0.9 ± 0.5 (0.2-2.0)	1.0 ± 0.5 (0.2-1.9)	7.8 ± 6.4	< 0.01
<i>Bicep Femoris</i>	1.1 ± 0.8 (0.2-3.1)	0.9 ± 0.6 (0.1-2.8)	19.3 ± 11.3	< 0.01
<i>Tibialis Anterior</i>	0.3 ± 0.1 (0.1-0.7)	0.4 ± 0.2 (0.1-1.0)	18.0 ± 12.5	< 0.01
<i>Medial Gastrocnemius</i>	0.5 ± 0.3 (0.1-1.2)	0.6 ± 0.4 (0.1-1.6)	19.3 ± 10.6	< 0.01

**B**

	<b>Distal</b>	<b>Proximal</b>	<b>Difference</b>	<b>CV (%)</b>	<b>p value</b>
<b>Ultrasound Fat, %</b>					
<i>Rectus Femoris</i>	13.3 ± 2.5 (7.9-20.3)	13.7 ± 2.6 (7.9-22.7)	0.4 ± 0.7	3.6 ± 3.5	< 0.01
<i>Bicep Femoris</i>	16.9 ± 5.5 (9.4-29.8)	15.3 ± 4.4 (8.5-27.9)	1.6 ± 2.0	7.2 ± 6.6	< 0.01
<i>Tibialis Anterior</i>	14.9 ± 2.8 (8.3-19.8)	15.1 ± 3.5 (7.6-22.6)	0.3 ± 1.5	5.4 ± 4.9	0.3
<i>Medial Gastrocnemius</i>	13.7 ± 3.1 (7.8-20.5)	14.3 ± 3.7 (8.5-22.9)	0.6 ± 1.7	6.0 ± 5.3	0.02

Note: Values are expressed as mean ± SD (range). CV = coefficient of variation.

**Table 4.3 Correlations of Ultrasound % Fat between Muscle Groups**

<b>Muscle</b>	<b>Rectus Femoris (n= 42)</b>	<b>Biceps Femoris (n= 42)</b>	<b>Tibialis Anterior (n= 42)</b>	<b>Medial Gastrocnemius (n= 42)</b>
<b>Rectus Femoris</b>	1	0.67**	0.69**	0.78**
<b>Bicep Femoris</b>		1	0.79**	0.73**
<b>Tibialis Anterior</b>			1	0.71**
<b>Medial Gastrocnemius</b>				1

Note: \* = Correlation is significant at the 0.05 level (2-tailed), \*\* = Correlation is significant at the 0.01 level (2-tailed)



**Table 4.4 Correlations between Ultrasound % Fat and Other Body Composition Measures**

	BMI kg/m <sup>2</sup>	Waist/Hip Ratio	Muscle Thickness cm
<b>Ultrasound % fat</b>			
<i>Rectus Femoris</i>	0.34*	0.35*	-0.52**
<i>Bicep Femoris</i>	0.42**	-0.08	--
<i>Tibialis Anterior</i>	0.25	-0.05	-0.43**
<i>Medial Gastrocnemius</i>	0.38*	0.21	0.65**
<i>Average of All Muscle Groups</i>	0.40*	0.09	--

Note: \* = Correlation is significant at the 0.05 level (2-tailed), \*\* = Correlation is significant at the 0.01 level (2-tailed)

**Table 4.5 Correlations between Ultrasound % Fat Controlled by Age and Gender and Other Body Composition Measures**

	Control variables	BMI kg/m <sup>2</sup>	Waist/Hip Ratio	Muscle Thickness cm
<b>Ultrasound % fat</b>				
<i>Rectus Femoris</i>		0.33*	0.17*	-0.40**
<i>Bicep Femoris</i>	Age	0.61**	0.05	--
<i>Tibialis Anterior</i>	Gender	0.32*	-0.02	-0.18
<i>Medial Gastrocnemius</i>		0.37*	0.07	-0.56**
<i>Average of All Muscle Groups</i>		0.51*	0.06	--

Note: \* = Correlation is significant at the 0.05 level (2-tailed), \*\* = Correlation is significant at the 0.01 level (2-tailed)

**Table 4.6 Correlations between Ultrasound % Fat and Physical Activity Level**

	Walking MET	Total MET
<b>Ultrasound % fat</b>		
<i>Rectus Femoris</i>	-0.15	-0.34*
<i>Bicep Femoris</i>	0.02	-0.17
<i>Tibialis Anterior</i>	-0.06	-0.21
<i>Medial Gastrocnemius</i>	-0.17	-0.39*

Note: \* = Correlation is significant at the 0.05 level (2-tailed). MET = metabolic equivalent of task.

**Table 4.7 Correlations between Mean Ultrasound % Fat, Physical Activity Level and Diet**

	Walking MET	Total MET	Sugary Beverage times/wk	Fast Food times/wk
<b>Average % fat of Muscle Groups</b>	-0.09	-0.29	-0.06	0.22

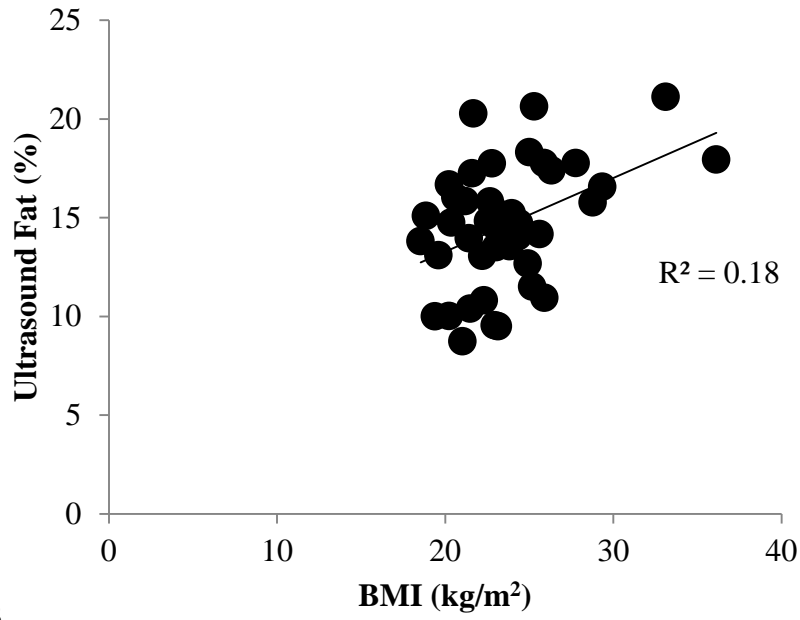
Note: \* = Correlation is significant at the 0.05 level (2-tailed). MET = metabolic equivalent of task.

**Table 4.7 Correlations between Mean Ultrasound % Fat and Blood Glucose and Lipid Profile**

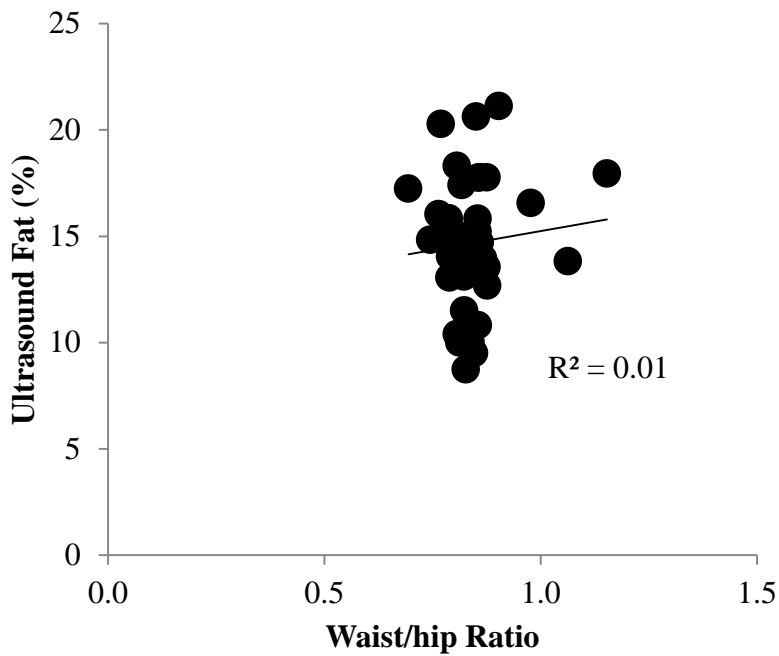
	TC	HDL	Triglyceride mg/dL	LDL	Glucose
<b>Average % fat of Muscle Groups</b>	0.04	0.16	0.10	-0.08	0.02

Note: \* = Correlation is significant at the 0.05 level (2-tailed). HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol.

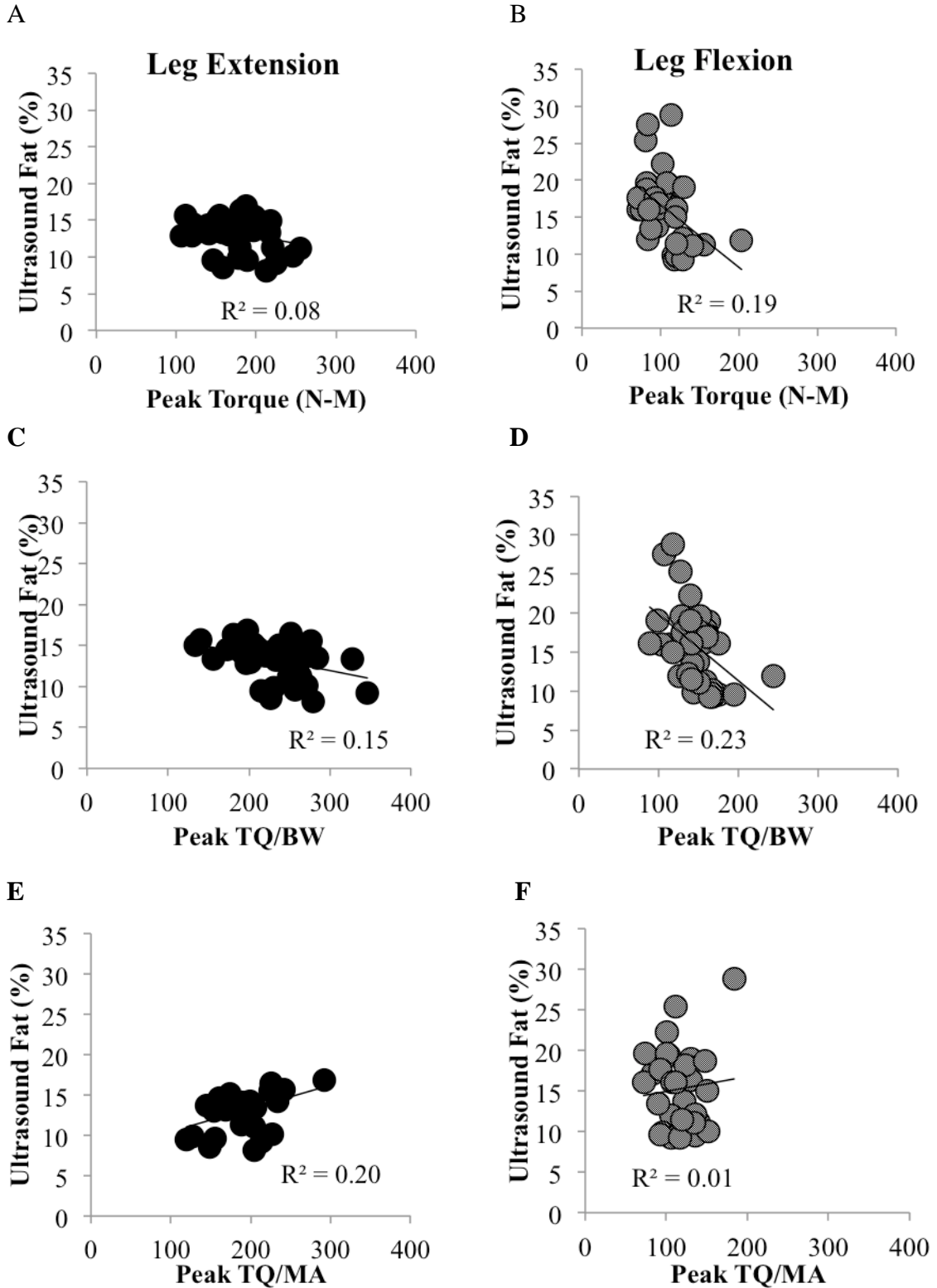
A



B

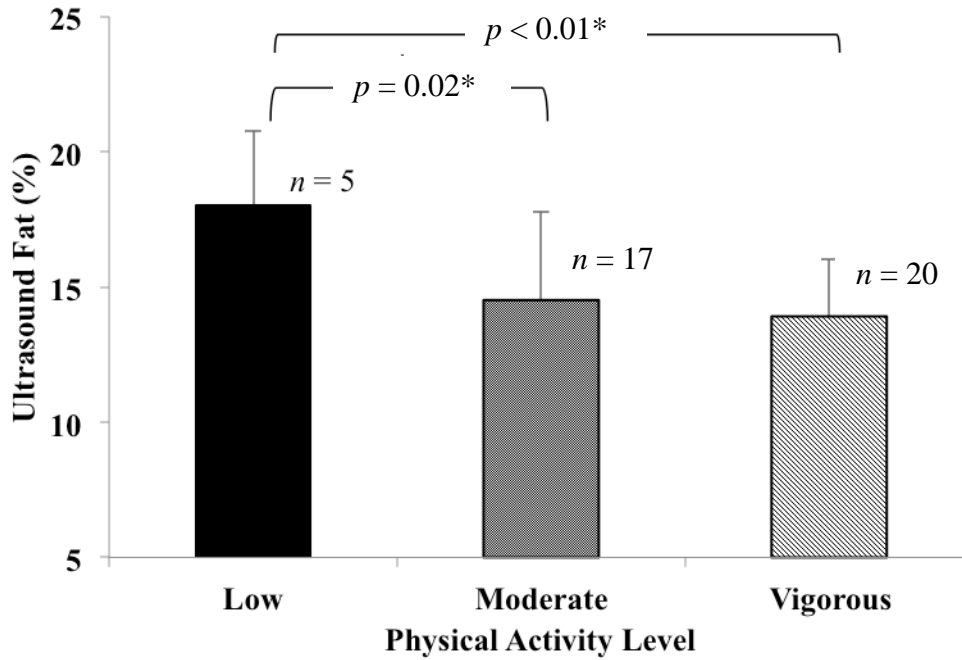


**Figure 4.1.** Correlation graphs that demonstrate the relationship between ultrasound-measured intramuscular fat and BMI (A) and between ultrasound-measured intramuscular fat and waist and hip ratio (B).

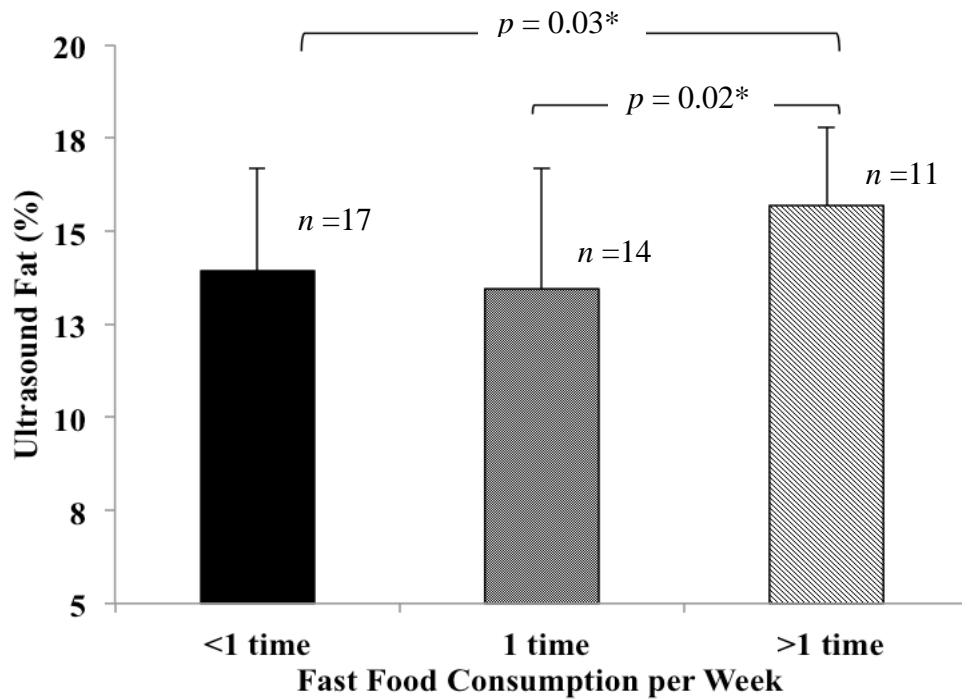


**Figure 4.2.** Correlation graphs that demonstrate the relationship between ultrasound-measured intramuscular and muscular strength.

A



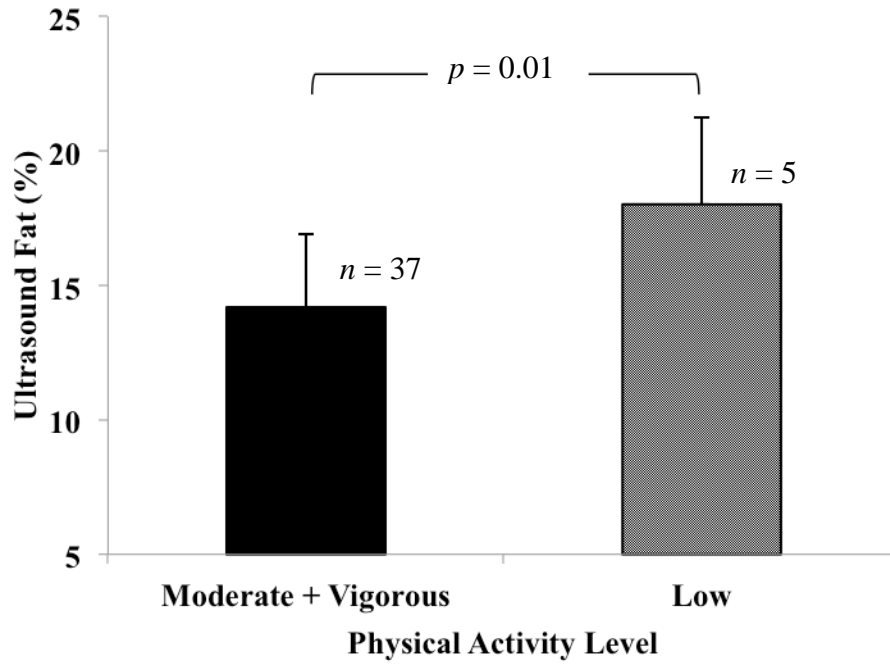
B



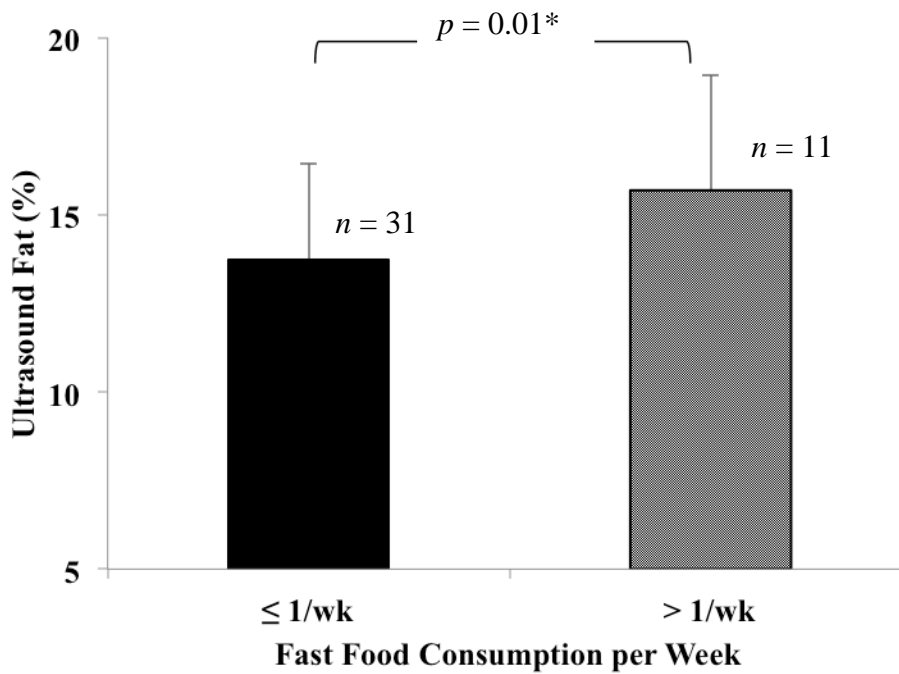
**Figure 4.3.** Comparisons between ultrasound-measured intramuscular fat of three physical activity levels: low, moderate, and vigorous, and three levels of fast food consumption: < 1 time/week, 1 time/week, and >1 time/week.



A



B



**Figure 4.4.** Comparisons between ultrasound-measured intramuscular fat of two physical activity levels: low + moderate group and vigorous, and two levels of fast food consumption frequency:  $\leq 1$  time/week and  $> 1$  time/week.

## **CHAPTER 5**

### **SUMMARY AND CONCLUSIONS**

The primary findings of the first study were the strong correlations between muscle echo intensity after correcting for subcutaneous fat thickness and the establishment of calibration equations to convert muscle echo intensity into % intramuscular fat. Prior to the beginning of this study, we were confident about the possibility of quantifying muscle echo intensity into % intramuscular fat as previous studies have established calibration methods to evaluate and grade meat quality in cows and pigs (4, 9). Currently, human studies that use the ultrasound technique report muscle quality in arbitrary units, which allows comparisons to be made between muscle echo intensity and other health and fitness measures. While using arbitrary echo intensity units is simple, it does not allow for comparisons between muscle echo intensity and other % body and muscle fat techniques. The degree of influence of intramuscular fat on human skeletal muscle echo intensity is still unknown. Although the % intramuscular fat calibration methods in animal science are available, I felt that direct application of the methods to humans was not appropriate due to the potential difference in the muscle composition between humans and animals. To expand the utilization of the ultrasound technique in humans, converting human skeletal muscle echo intensity to a unit that is consistent with other body or muscle composition techniques is necessary.

This study presented calibration equations for four different muscle groups in the lower extremities, which is an important step toward improving the ultrasound technique.

The simple application of the calibration equations is encouraging, as future studies can calculate % intramuscular fat by just obtaining subcutaneous fat thickness and echo intensity of a particular muscle. In addition, utilization of the ultrasound technique as an alternative tool for examination of muscle health has several advantages. First of all, it is relatively cheap when compared to other imaging techniques. A MR machine costs approximately \$2,000,000 while a CT machine costs around \$100,000 to \$400,000. DXA is relatively inexpensive but still costs around \$100,000 to \$150,000. An ultrasound device costs approximately \$10,000 to \$70,000 depending on the type and number of software being installed. Secondly, it is portable. An ultrasound device can be delivered to almost any place where a power outlet is available. This is a characteristic that other imaging techniques do not possess. Thirdly, the ultrasound scanning procedure can be performed in a time efficient way. Once a researcher is proficient with the ultrasound technique, it takes less than 30 seconds to perform a muscle scan. Last but not least, it can be applied to every individual. For individuals who cannot have MRI, CT, and DXA scans due to the nature of the imaging technique, ultrasound can be an option. More studies are needed to establish calibration equations for different muscle groups. Once we have the equations for all the major muscle groups, building the equations into the ultrasound device can further enhance the practicality of ultrasound in both research and clinical settings.

In the first study, we utilized  $T_1$  weighted MR images to calibrate muscle echo intensity. While Dixon MRI technique is suggested to be the ideal way to examine intramuscular lipid content, we do not have the option currently (11). With the time and funding limitations, we used our best effort to collect high resolution  $T_1$  weighted MR

images with available resources. We believe that the images we acquired work reasonably well, as the intramuscular fat content can be easily distinguished. The method we used to analyze % intramuscular fat is based on self-determined pixel intensities of pure fat, pure muscle, and connective tissue and can therefore be considered as subjective. We minimized any bias that might occur by blocking the identity of participants and randomly changing the order of images during the image analysis. The MR images were also analyzed with a commercially available program which used the binary method to color-code different tissue. While using the analysis program can be more objective, the binary method has the tendency to underestimate % fat when the fat content is relative low or overestimate when the fat content is too high. It seems difficult for the program to distinguish fat within a muscle when the majority of the muscle area is lean muscle mass or to recognize lean muscle mass when the majority of the muscle is fat. The binary method might work better when examining tissues in different compartments (i.e. intermuscular fat, muscle groups, subcutaneous fat) than identifying different tissues within a compartment. The manually-determined pixel intensity method we used to analyze the images can introduce potential errors as well. When a muscle group has high intramuscular fat content, it becomes harder to determine the area where the pure muscle is. As a result, it might underestimate the actual amount of intramuscular fat. Nevertheless, we feel that the self-determined pixel intensity method introduced less errors and the results aligned better with our qualitative observations on the images. Future studies should compare % intramuscular fat obtained using both the Dixon MRI technique and our manually-determined pixel intensity method to test the validity of our current method.

A stronger correlation and smaller variability between % intramuscular fat and echo intensity in the RF was observed. One possible explanation is that the ultrasound is able to capture the entire RF area, which makes the echo intensity more representative of the muscle health. Depending on the size of the person being tested, both TA and MG may not have been completely captured. This is especially true for MG due to its long shape in the transverse axis. BF is harder to scan because of its size and shape, which makes it difficult to be captured in both the transverse and longitudinal axis by ultrasound. The other possible explanation is that there are different compositions of muscle groups. In the first study, we did not account for the contribution of fibrous tissues to muscle echo intensity. MG is thought to contain more connective tissue when compared to RF, which can make connective tissue the primary factor contributing to increased muscle echo intensity. Future studies that focus on the texture analysis of the ultrasound technique can help to answer this question. The last explanation is the possible influence of intramuscular fat content on muscle echo intensity. It is possible that the relationship between intramuscular fat and muscle echo intensity would start to change once the amount of intramuscular fat reaches to a certain point. If this is true, this means that the ultrasound technique has a threshold and is limited to a certain range of intramuscular fat content. Establishing another correction factor to account for this potential influence could help address this issue. Besides all the issues addressed above, it is also important to understand the need for keeping all the ultrasound settings and scanning angles consistent throughout the study in order to make sure the ultrasound results are comparable across all muscle groups, as well as every study participant (10). Standardizing the ultrasound technique is critical as the results of ultrasound echo

intensity can be altered by changing just one variable, such as the probe angle being held during assessment; thus making comparisons between studies difficult. To summarize, all the calibration equations established in the first study should work reasonably well, while the ones for the RF can be more reliable.

The present studies utilized a convenient sample of healthy college-aged adults with a few exceptions. We recruited individuals with wide range of BMI and physical activity level with the intention to acquire a good range of intramuscular lipid content. While the finding is consistent with previous literature (7, 13), it is still surprising to find higher than expected % intramuscular fat in some of our younger active participants. We had very healthy and active participants who had more intramuscular fat when compared to their sedentary counterparts. The traditional notion of living in a healthy lifestyle is not always associated with good muscle health, which further supports the need of examining the distribution instead of the quantity of adipose tissue. On the other hand, evidence has shown that individuals who are highly trained have high lipid droplets as faster metabolic fuel source (1). How do we determine whether the intramuscular lipid content is good or detrimental to our health? Is it always the quantity that matters or is the quality important? In addition, if highly endurance trained individuals can have higher % of intramuscular lipid content, what would happen once they become sedentary? As several studies have suggested the association of increased intramuscular fat and mitochondrial dysfunction (5, 8), such questions can perhaps be answered by incorporating measures such as near-infrared spectroscopy to examine the mitochondrial capacity of a muscle.

In the second study, we found weak to moderate correlations between % intramuscular fat and other health and fitness indicators. The findings are comparable to previous studies that examined the relationships to other imaging techniques such as Dixon MRI, MRS and CT scan (2, 3). While consistent with the literature, the results warrant the need of further investigating the mechanism of intramuscular fat on health and functional outcomes. The simplicity of examining intramuscular fat with one other variable is good. For example, from those findings we have understood the associations of increased physical activity and low-fat diet with reduced intramuscular fat (6, 11, 12). However, future studies are needed to look at the relationships of muscle lipid with the combined effects of several variables such as exercise, diet, functional outcomes, blood lipid profile, and the presence of adipokines or adipocytokines to better understand the role of intramuscular fat. Larger sample size might also be needed for future studies to compare intramuscular lipid content with other health indicators to really test for the presence of significant relationships. This further supports the use of the ultrasound technique for such studies that require large sample sizes.

During the process of completing my dissertation, I was fortunate to be able to still teach a wellness program for individuals with disabilities. Talking to the participants and students reminded me of the need to be inclusive with our research tools and the importance to improve imaging techniques for examination of muscle health in this population. These studies provided opportunities for me to reflect on my past before I entered the field of research. We have to keep reminding ourselves that one of the goals of research is to improve the well-being of society as a whole; therefore, having research tools that can accommodate every individual with and without disabilities is essential.

The ultrasound technique proposed in the present studies can potentially achieve the goal with its low-cost and practicality. Once all the questions mentioned in the previous paragraphs are addressed, this ultrasound technique can potentially be applied in the rehabilitation field to help guide clinicians or exercise professionals during their patients' rehabilitation progress.



## Reference

1. **Addison O, Marcus RL, LaStayo PC, and Ryan AS.** Intermuscular Fat: A Review of the Consequences and Causes. *International Journal of Endocrinology* 2014: 11, 2014.
2. **Claus Thamer, Jürgen Machann, Oliver Bachmann, Michael Haap, Dominik Dahl, Beate Wietek, Otto Tschritter, Andreas Niess, Klaus Brechtel, Andreas Fritsche, Claus Claussen, Stephan Jacob, Fritz Schick, Hans-Ulrich Häring, and Michael Stumvoll.** Intramyocellular Lipids: Anthropometric Determinants and Relationships with Maximal Aerobic Capacity and Insulin Sensitivity. *The Journal of Clinical Endocrinology & Metabolism* 88: 1785-1791, 2003.
3. **Goodpaster BH, Kelley DE, Thaete FL, He J, and Ross R.** Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *Journal of Applied Physiology* 89: 104-110, 2000.
4. **Hassen A, Wilson DE, Amin VR, Rouse GH, and Hays CL.** Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science* 79: 11-18, 2001.
5. **Holloszy JO.** Skeletal muscle „Ämitochondrial deficiency,Ä does not mediate insulin resistance. *The American Journal of Clinical Nutrition* 89: 463S-466S, 2009.
6. **Horowitz JF, and Klein S.** Lipid metabolism during endurance exercise. *The American Journal of Clinical Nutrition* 72: 558s-563s, 2000.
7. **Kovanlikaya A, Mittelman S, Ward A, Geffner M, Dorey F, and Gilsanz V.** Obesity and fat quantification in lean tissues using three-point Dixon MR imaging. *Pediatr Radiol* 35: 601-607, 2005.

8. **Newsholme P, Gaudel C, and Krause M.** Mitochondria and Diabetes. An Intriguing Pathogenetic Role. In: *Advances in Mitochondrial Medicine*, edited by Scatena R, Bottoni P, and Giardina BSpringer Netherlands, 2012, p. 235-247.
9. **Parish JA, Rhinehart JD, and C. VR.** Ultrasound Scanning Beef Cattle for Body Composition Starkville, MS: Mississippi State University Extension Service, 2011.
10. **Pillen S, and van Alfen N.** Skeletal muscle ultrasound. *Neurological Research* 33: 1016-1024, 2011.
11. **Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WHM, Nicolay K, and Schrauwen P.** Intramyocellular Lipid Content and Molecular Adaptations in Response to a 1-Week High-Fat Diet. *Obesity Research* 13: 2088-2094, 2005.
12. **St-Onge M-P, Newcomer BR, Buchthal S, Aban I, Allison DB, Bosarge A, and Gower B.** Intramyocellular lipid content is lower with a low-fat diet than with high-fat diets, but that may not be relevant for health. *The American Journal of Clinical Nutrition* 86: 1316-1322, 2007.
13. **Wren TAL, Bluml S, Tseng-Ong L, and Gilsanz V.** Three-Point Technique of Fat Quantification of Muscle Tissue as a Marker of Disease Progression in Duchenne Muscular Dystrophy: Preliminary Study. *American Journal of Roentgenology* 190: W8-W12, 2008.