Characterizing site-specific body composition of older adults and their associations with strength, functional capacity, and metabolic health

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Michael Paris

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Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

External Examiner	Maria Cristina Gonzalez, MD, PhD Professor, Health and Behaviour
Supervisor	Marina Mourtzakis, PhD Associate Professor, Kinesiology
Internal Member	Jack Callaghan, PhD Professor, Kinesiology
Internal Member	Michaela Devries-Aboud, PhD Assistant Professor, Kinesiology
Internal-External Member	Alfred Yu, PhD Professor, Electrical and Computer Engineering

Authors Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

Michael Paris was the sole author for Chapters 1, 2, 3, 4, 9, and 10, which were written under the supervision of Dr. Marina Mourtzakis. This thesis consists in part of 4 manuscripts written for publications. Exceptions to sole authorship of material are as follow:

Research presented in Chapters 5, 7, and 8 – This research was conducted at the University of Waterloo by Michael Paris under the supervision of Dr. Marina Mourtzakis. Michael Paris and Dr. Mourtzakis contributed to study design. Dr. Egor Avrutin, Dr. Kirsten E. Bell, and Michael Paris contributed to participant recruitment and collection. Michael Paris performed all data processing and analysis. Michael Paris drafted initial manuscripts, which Dr. Mourtzakis provided intellectual input on.

Research presented in Chapter 6 – This research was conducted at the University of Waterloo by Michael Paris, under the supervision of Dr. Marina Mourtzakis. Michael Paris, Noah Letofsky, and Dr. Marina Mourtzakis contributed to study design. Michael Paris recruited and collected participants. Michael Paris and Noah Letofsky contributed to data processing and analysis. Michael Paris drafted the initial manuscript, which all co-authors provided intellectual input on.

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Abstract

Background: Ageing is associated with skeletal muscle atrophy and increased intramuscular adipose tissue. Emerging evidence indicates that specific muscle groups may be largely responsible for the ageing-related degradation of muscle mass and composition. However, how these site-specific measures of skeletal muscle mass and composition compare to traditional whole-body measures of body composition in relation to strength and metabolic health are not well understood. Our objectives were to: 1) characterize site-specific differences in skeletal muscle mass and composition between younger and older adults and 2) compare the associations between site-specific and traditional measures of muscle mass and composition with strength, functional capacity, and metabolic health of older adults.

Methods: Data for this thesis was derived from a prospectively recruited cohort of older males (n=32) and a secondary analysis of younger and older males and females (n=96). All participants underwent dual-energy x-ray absorptiometry analysis of regional and appendicular lean tissue and ultrasound measured muscle thickness and echo intensity (8 distinct landmarks). Older males in the prospective cohort were also evaluated for strength and a glucose tolerance test.

Younger and older adults were matched for relative muscle mass (study 1) and absolute fat thickness (study 2) to examine differences in muscle thickness and echo intensity, respectively. For studies 3 and 4, site-specific muscle thickness, echo intensity, and lean tissue were assessed in relation to muscle strength, functional capacity, and metabolic health of older males.

Results: For study 1, older adults exhibited significantly lower muscle thickness at the anterior upper leg (26% smaller) and anterior abdomen (36% smaller), but not regional lean tissue (0-

15% smaller) compared with younger adults. In study 2, older adults presented with elevated muscle echo intensity (poorer muscle composition) at the anterior upper leg (28% higher) and anterior abdomen (58% higher), but not the anterior upper arm (11% higher) compared with the younger cohort. For study 3, both DXA appendicular lean tissue and site-specific muscle thickness provided similar magnitude associations with muscle strength. In study 4, elevated muscle echo intensity at the anterior upper leg was associated with poorer glucose homeostasis in healthy older males. However, in older males with prediabetes or diabetes, elevated skeletal muscle echo intensity was associated with better glucose homeostasis.

Conclusions: The anterior upper leg and anterior abdomen display substantial reductions in muscle thickness and increases in echo intensity with advancing age. In relation to muscle strength, both appendicular lean tissue and site-specific muscle thickness provided similar magnitude of associations. Elevated muscle echo intensity is associated with poorer glucose homeostasis in healthy older males but displays divergent associations in older males with prediabetes and diabetes. These divergent associations require further clarification to better understand the validity of echo intensity as a metric of skeletal muscle composition.

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List of Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
CSA	Cross-sectional area
CT	Computed tomography
DICOM	Digital imaging and communications in medicine
DXA	Dual-energy x-ray absorptiometry
dSAT	Deep subcutaneous adipose tissue
HDL-C	High-density lipoprotein cholesterol
IMAT	Intramuscular adipose tissue
IMCL	Intramyocellular lipids
LDL-C	Low-density lipoprotein cholesterol
MRI	Magnetic resonance imaging
SD	Standard deviation
sSAT	Superficial subcutaneous adipose tissue

CHAPTER 1

INTRODUCTION

1.1 Thesis overview

The proportion of Canadians entering older age is rapidly expanding, with projections predicting that nearly one in four Canadians will be over the age of 65 by 2036 [1]. The ageing trajectory is associated with a myriad of detrimental physiological changes, including losses in skeletal muscle mass and increases or redistribution of adipose tissue depots. These deleterious shifts in skeletal muscle and adipose tissue are associated with impairments in strength, physical function, metabolic health, and mobility, which ultimately lead to reductions in quality of life [2]. In addition to the physical toll experienced by older adults as a result of ageing-related changes in body composition, there are substantial health care costs associated with these deleterious physiological changes [3].

Importantly though, ageing-related changes in body composition are not solely a factor of time, as lifestyle factors such as physical activity and adequate protein consumption can attenuate these deleterious changes [4]–[6]. Furthermore, these same lifestyle factors can improve body composition, function, and strength if performed later in life [7], [8]. Identification of older adults with features of poor body composition are needed to better tailor these lifestyle interventions aiming to improve muscle strength and functional capacity of older adults; however, detection of these deleterious body composition features is not a trivial task.

The loss of skeletal muscle mass with advancing age was originally termed sarcopenia [9], however, this term has evolved to also incorporate reduced strength or functional capacity

[10]. Most definitions and guidelines focusing on operationalizing a definition of sarcopenia suggest the use of dual-energy x-ray absorptiometry (DXA) as the primary modality for quantifying ageing-related declines in skeletal muscle mass and for identifying older adults with low muscularity [11], [12], including the influential European Working Group on Sarcopenia in Older Persons. The primary metric for evaluating skeletal muscle using DXA is appendicular lean tissue mass, which encompasses the lean soft tissue of the arms and legs [13]. Appendicular lean tissue mass is strongly associated with whole body skeletal muscle mass measured using magnetic resonance imaging (MRI) in older adults [14]. However, accumulating evidence suggests that ageing-related losses in skeletal muscle mass do not occur in a uniform manner across the body. Indeed, several studies have demonstrated that compared with younger adults, older adults demonstrate relatively less muscle mass in the lower limbs compared with the upper limbs [15], [16]. Therefore, evaluating ageing-related changes in skeletal muscle mass using whole-body indices, such as appendicular lean tissue mass, may not provide the most sensitive markers for identification of older adults experiencing declines in muscle mass.

Quantification of specific muscle groups within the lower limbs may be more advantageous for detecting early ageing-related declines in muscle mass. While the influence of advancing age on site-specific measures of skeletal muscle mass are beginning to emerge, the uniformity of ageing-related shifts in muscle composition are less well understood, as the majority of research has focused on evaluation of thigh muscle composition. Furthermore, there is a paucity of literature examining how mass and composition of specific muscle groups compare to traditional whole-body indices of body composition in relation to strength, functional capacity, and metabolic health of older adults. Understanding how site-specific and

whole-body indices of skeletal muscle tissue relate to strength, functional capacity, and metabolic health will provide key information for how to best quantify skeletal muscle tissue in aged adults for identifying older adults who may require targeted lifestyle interventions.

1.2 Overarching objectives

The overarching objectives of this thesis are to:

1. Characterize site-specific differences in skeletal muscle mass and composition between younger and older adults
2. Examine the associations between site-specific muscle mass and composition with strength, functional capacity, and metabolic health of older adults
3. Compare site-specific and traditional whole-body indices of skeletal muscle tissue in relation to strength, functional capacity, and metabolic health of older adults

1.3 Thesis structure

This thesis is organized into the following sections:

- Chapter 2 provides a general literature overview on body composition terminology, the effects of advancing age on traditional measures of skeletal muscle and adipose tissue, and site-specific changes in muscle mass and composition with advancing age.
- Chapter 3 provides an overview of the specific objectives and hypothesis for each study.
- Chapter 4 provides a general overview of the primary methods and concepts used in this thesis.

- Chapter 5 (Study 1) compares site-specific differences in skeletal muscle mass and composition between younger and older adults who are matched for relative whole body skeletal muscle mass.
- Chapter 6 (Study 2) compares site-specific differences in skeletal muscle composition in younger and older adults who matched for absolute subcutaneous adipose tissue mass.
- Chapter 7 (Study 3) compares site-specific and traditional measures of skeletal muscle tissue in relation to muscle strength and functional capacity of older males.
- Chapter 8 (Study 4) compares site-specific and traditional measures of skeletal muscle tissue in relation to metabolic health of older males.
- Chapter 9 is an integrative discussion section that elaborates on the interplay between the overall findings and implications of this thesis.
- Chapter 10 provides the references for this thesis.

CHAPTER 2

GENERAL LITERATURE REVIEW

2.1.1 Overview of body composition frameworks

Body composition can be generally defined as the lifetime accumulation of nutrients which are retained by the body [17]. However, there are several distinctions and non-trivial differences in terminology that have important implications for understanding and describing body composition. To enable effective and succinct discussions of body composition, a widely accepted framework is necessary to ensure common terminology can be utilized. Heymsfield and colleagues [18] proposed the 5 levels of body composition, which has been widely adopted and includes the atomic, molecular, cellular, tissue-organ, and whole body levels. A given level is segmented into distinct compartments (e.g. fat-free mass), which sum to equal total body weight.

The atomic level is segmented into 11 major elements; however, it is rarely quantified due to the complexity and accessibility of the necessary equipment for measurement (e.g. in-vivo neutron activation analysis). The most commonly applied approaches for body composition analysis fall within the molecular, cellular, and tissue-organ levels. The molecular level typically segments the body into five major compartments: water, lipids, proteins, carbohydrates, and minerals. While these 5 compartments are the most often assessed, they can be further refined (e.g. triacyclglycerols separated from lipids) or merged to form several different multi compartment models (e.g. 2 compartment model: fat mass and fat-free mass). The cellular level typically consists of 4 compartments: fat, body cell mass, extracellular fluid,

and extra cellular solids. Body cell mass, which consists of the intracellular fluid and intracellular solid components, is of most interest because it constitutes the metabolically active tissues of the body [17]. The tissue-organ level can be segmented into several different compartments, depending on the tissues of interests, but typically include skeletal muscle, bone, visceral organs, adipose tissue, and the brain. Tissue-organ analysis of body composition is highly sought after due to its ability to quantify distinct anatomical locations or specific compartments within a given tissue, such as intramuscular adipose tissue. Whole body analysis is generally divided into appendages, head, and trunk. Approaches typically include assessing anthropometry of a region (e.g. mid-arm circumference) or examining whole body weight or body mass index (BMI).

While several different levels, compartments, and modalities can be used for body composition quantification, two tissues that are of immense interest in research, clinical settings, and daily life, are skeletal muscle and adipose tissue. Features of skeletal muscle and adipose tissue have garnered interests because of their importance in movement, skeletal support, metabolism, and risk and prognosis for chronic illnesses [19]. Precise terminology is necessary when discussing skeletal muscle and adipose tissue features, as commonly used modalities for body composition provide indices of these tissues, rather than direct measures. For example, common indices of skeletal muscle include fat-free mass and lean soft tissue mass, which may be correlated with each other, but have distinct differences (Figure 2.1) [20]. Similarly, adipose tissue is often interchangeably used with fat mass, however, there are clear

physiological differences, which have important implications in health and disease (Figure 2.1) [21].

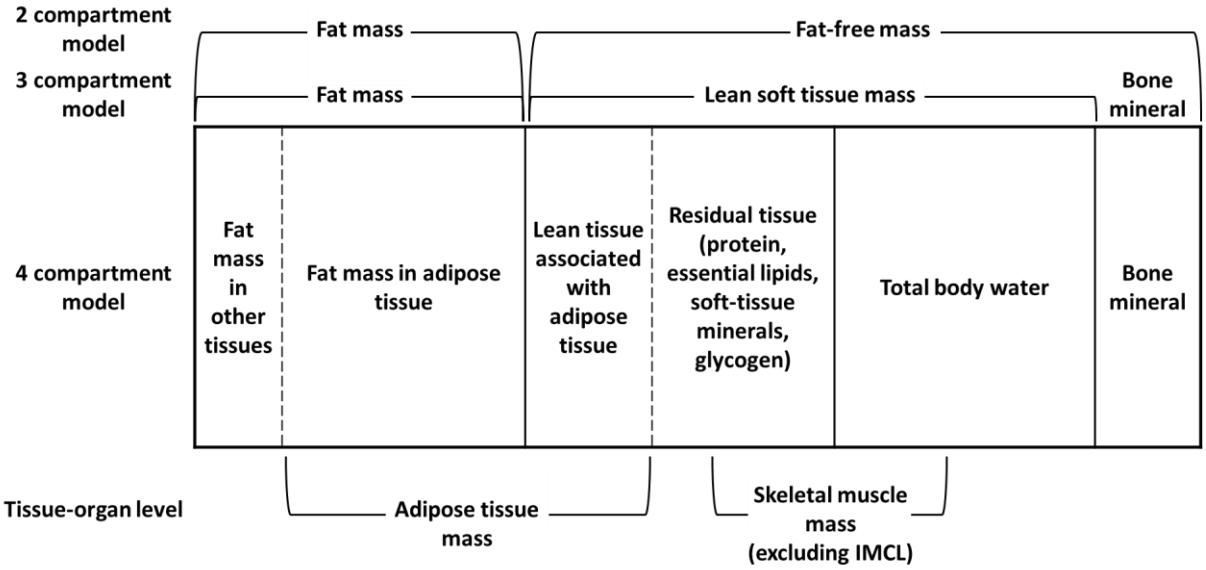


Figure 2.1. Relations between molecular and tissue-organ indices of skeletal muscle and adipose tissue mass. IMCL, intramyocellular lipids.

2.1.2 Overview of skeletal muscle mass and composition

Historically, metrics of mass (e.g. volume, cross-sectional area) were the primary feature of skeletal muscle that was examined in relation to health and disease outcomes (ageing, cancer, critical illness) [19]. Skeletal muscle mass consists of the weight of all tissues deep to the epimysium, which includes contractile proteins, cytoskeleton structure, myonuculei, glycogen, lipids (dependent upon modality used), enzymes, cellular organelles, water, soft-tissue minerals, nerves, vascular tissue, and blood. Generally, skeletal muscle mass is used as a surrogate measure of contractile protein content and has been associated with muscle force production [22], functional capacity [23], [24], and clinical outcomes (e.g. mortality) [25] in older adults. However, the composition or 'quality' of skeletal muscle can vary widely across

different individuals, which has important implications for contractile capacity and metabolic health of the muscle.

Skeletal muscle quality has emerged as a critical feature of muscle health across several aspects of metabolism, function, strength, and clinical outcomes [26], [27]. However, a single definition of skeletal muscle quality is not universally defined, making it challenging to interpret and compare across studies. The most often applied measure of muscle quality is defined as the maximal torque produced for a given movement divided by the cross-sectional area (CSA) or volume of the muscles responsible for that movement (e.g. knee extension torque/quadriceps muscle mass) [28]. An important aspect of this definition is that it is an all-encompassing measure of skeletal muscle quality (mass, composition, architecture, etc.). However, individually quantifying distinct components of muscle quality (e.g. composition) from the all-encompassing strength/mass metric, can provide additional information on the contributing factors determining muscle quality.

Skeletal muscle composition can be defined using several different metrics, but is most commonly quantified as the proportion of fat or adipose tissue comprising the total mass or area of a muscle [29]. Fat or adipose tissue deposited deep to the muscle fascia can be broadly categorized into two depots: intra-myocellular lipids (IMCL) and intra- and inter-muscular adipose tissue (IMAT). IMCL are stored as lipid droplets within the myocyte, which consists of triacylglycerols and associated regulatory proteins [30]. In contrast, IMAT are structured adipocytes containing lipid droplets, which are surrounded by muscle cells at various layers of muscular fascia (e.g. perimysium) [31]. While IMAT and IMCL may be interrelated and

correlated in some individuals, these tissues are distinct and may have different physiological characteristics and implications [29].

2.1.3 Overview of adipose tissue mass

Similar to skeletal muscle, adipose tissue or fat mass has been historically quantified as the primary metric in relation to health and disease. Fat mass is quantified at the molecular level of body composition, which encompasses triacylglycerides of the body. Adipose tissue mass is quantified at the tissue-organ level of body composition and incorporates the adipocytes, surrounding connective tissue matrix, stromal vascular fraction, immune cells, and vascular and neural tissues [21]. Adipose tissue can be broadly categorized into four groups, subcutaneous adipose tissue, visceral adipose tissue, IMAT, and bone marrow adipose tissue; however, several further distinctions are possible [21]. Subcutaneous adipose tissue is located between the dermis and muscle fascia, which is widely distributed throughout the body, whereas visceral adipose tissue is located within the abdominal cavity, surrounding the internal organs within the peritoneal fascia.

Within the trunk and gluteal region, the subcutaneous adipose tissue depot can be further classified into the superficial (sSAT) and deep (dSAT) subcutaneous adipose tissue compartments [21]. The sSAT and dSAT compartments are separated by Scarpa's fascia (Figure 2.2), with the sSAT compartment being in similar size, morphology, and metabolism to the subcutaneous adipose tissue of the appendages, whereas dSAT is more similar in size, morphology, and metabolism to the visceral adipose tissue compartment [32]–[34]. Similar to subcutaneous adipose tissue, the visceral compartment can also be further segmented into the

intrathoracic, intrabdominal, and intrapelvic compartments, all of which differentially modulate disease risk and metabolic health [21].

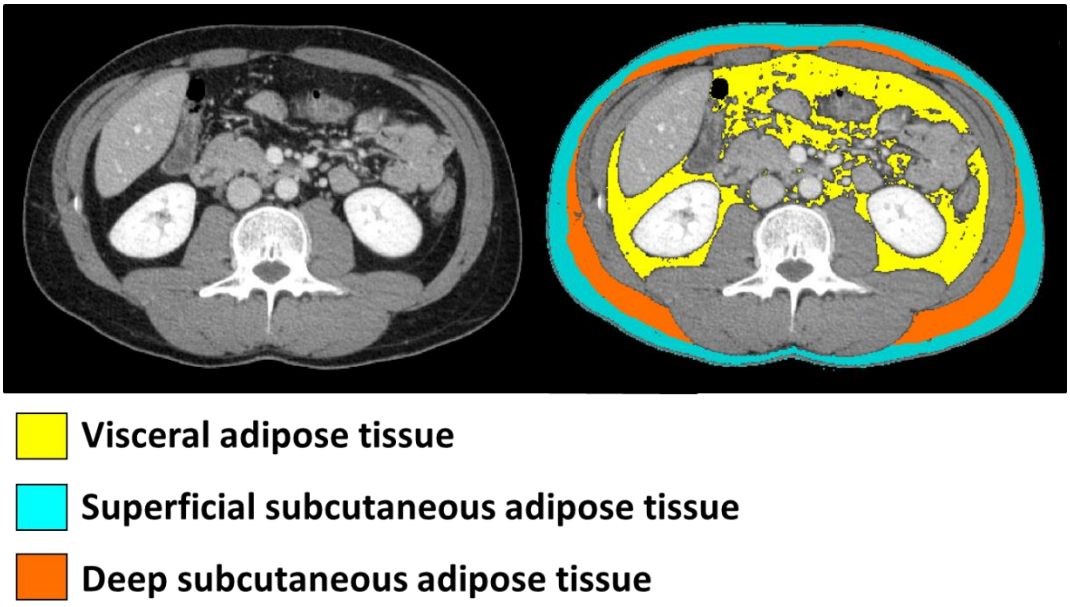


Figure 2.2. Depiction of superficial and deep subcutaneous adipose tissue on computed tomography scans

2.2.1 Ageing-related changes in skeletal muscle mass and composition

Ageing-related loss of skeletal muscle has been observed for decades [35] and was initially termed sarcopenia in 1988 by Rosenberg [9]. Skeletal muscle mass is generally stable in adulthood until the fifth decade of life, after which a gradual decline of 0.5-1% per year is often observed [36]. However, the degree of muscle loss, age of on-set, and sex and ethnic prevalence of sarcopenia are heterogeneous across studies [36]. Furthermore, the rate of muscle loss may not be consistent across the lifespan, as some studies have observed accelerated losses with advancing age [15], whereas others observe a linear loss across the entire lifespan [37], [38]. Typically, whole body approaches have been used to describe the age-associated changes in body composition, yet changes in muscle mass do not occur uniformly

across the body (Figure 2.3). Janssen et al. (2000) [15] demonstrated that the lower limbs display a rate of loss twice that of the upper limbs with increasing age using whole body MRI scans in 200 females and 268 males. These non-uniform changes in skeletal muscle tissue with ageing may have important implication for identifying and managing sarcopenia in older adults.

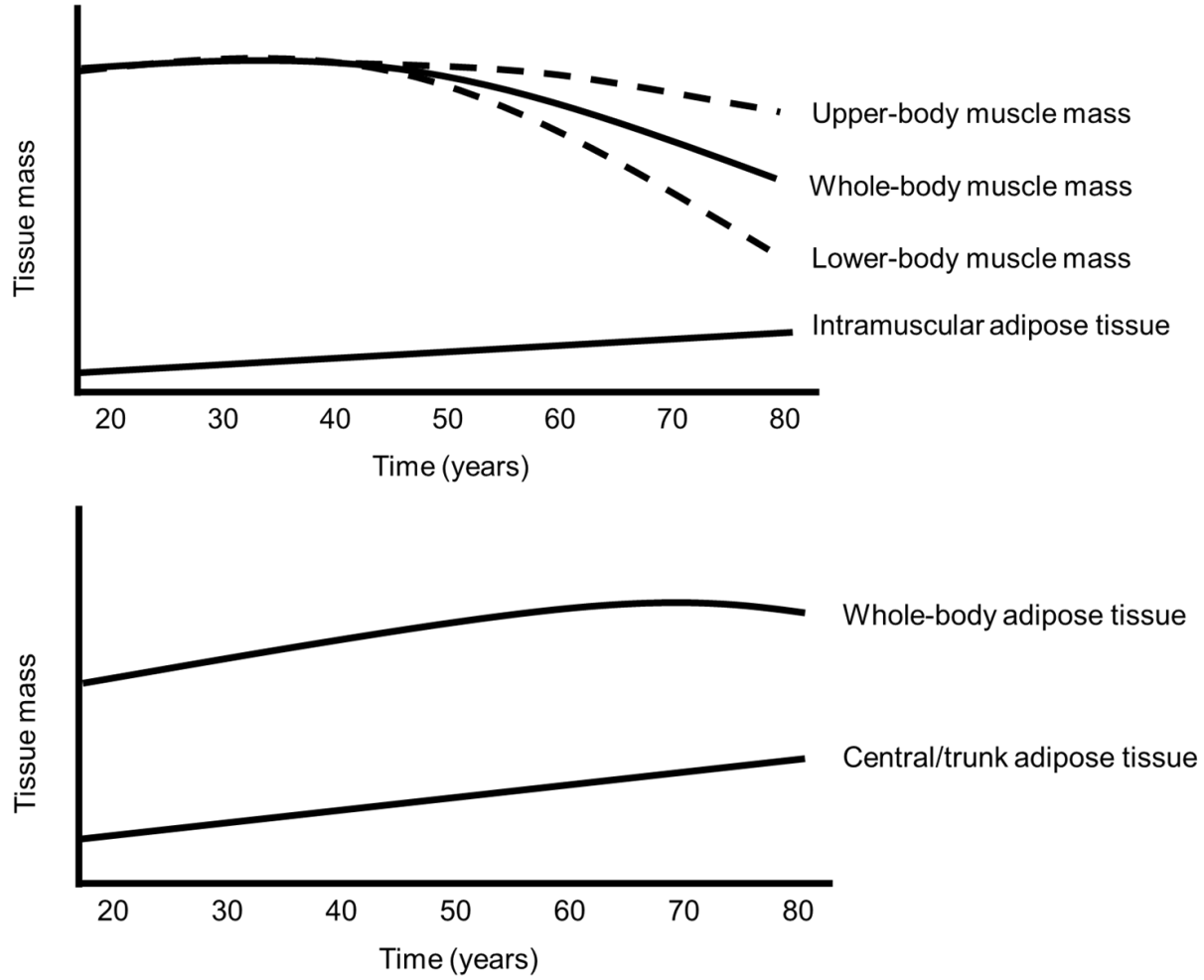


Figure 2.3. Theoretical ageing-related changes in skeletal muscle and adipose tissue

While age-associated losses in skeletal muscle mass are well-established, less focus has been placed on evaluating how age influences muscle composition. Despite receiving less attention, skeletal muscle composition has been observed to deteriorate at a more rapid rate than losses in skeletal muscle mass [39]. Over a period of 5 years, the health, ageing, and body composition cohort (longitudinal cohort of older adults, baseline 70-79 years of age)

experienced ~4 % reduction in thigh muscle area, but ~40 % increase in IMAT, which was independent of changes in body mass [39]. Similarly, increasing age is positively associated with increased IMAT within the quadriceps muscles and older adults exhibit elevated IMAT in the lower limb musculature compared with younger adults [40], [41].

2.2.2 Ageing-related changes in adipose tissue mass

Adipose tissue is the most variable compartment of body composition in adulthood, which can range from as low as 5% to more than 50% of total body weight [17]. Using DXA, Atlantis et al. (2008) [91] observed across decades of life (35-75+ years of age) that absolute fat mass was not significantly different at the whole body level, but percent body fat increased with increasing age, likely due to losses in lean tissue. Others have observed that total fat mass increases slowly with age until mid to late sixties, after which a plateau or slight decline may occur [43] (Figure 2.3). However, longitudinal data from the health, ageing, and body composition cohort indicates that total body fat mass, measured using DXA, increases over a period of 5 years in older adults [44]. While the degree of ageing-related increased in fat or adipose tissue is unclear, there is a clear redistribution of fat mass towards central trunk storage and deposition within skeletal muscle [45]; however, these alterations are highly influenced by sex and ethnicity [17]. While there is minimal data on the influence of age on dSAT and sSAT, given the positive associations between dSAT and visceral adipose tissue [32], [46], it is likely that the dSAT compartment increases with age.

2.3.1 Development of ageing-related cut-points for abnormal skeletal muscle and adipose tissue features

Body composition analysis is increasingly being recognized as an important feature of gerontological assessments, as it is useful in diagnosis of malnutrition [47], mobility impairments [24], [28], obesity, and in risk stratification for several non-communicable diseases (e.g. cardiovascular, diabetes, certain cancers, etc.) [48]–[50]. An important aspect of body composition analysis in older adults is the identification of deleterious tissue features (e.g. sarcopenia, obesity), which have important implications for metabolic disease, impaired mobility, and quality of life. Generally, a cut-point is used to classify individuals who display body composition features above or below a certain threshold as abnormal and normal. Other approaches may categorize individuals into three or more categories (e.g. normal, overweight, obese), demonstrating a general degree of abnormality [23], [51]. In general, percentile and outcome approaches have been applied in the development of these cutpoints for classification of older adults, which are usually sex-specific due to differences in various body composition compartments between males and females. A percentile-based approach involves sampling a reference cohort of healthy young adults, with 2 standard deviations (SD) above or below the mean score being considered abnormal (e.g. 2 SD below average muscle mass or 2SD above average adipose tissue mass) [10], [52]. Similarly, the lowest or highest percentile (e.g. quintiles) within a group of older adults is often used as a cutpoint for separation of the older adults into different groups [53]. In a more outcome-centric approach, cut-points have also been established for identifying older adults with poor performance (e.g. muscle strength) or clinical outcomes (e.g. mortality) [54].

Baumgartner et al. (1998) [52] first operationally defined low skeletal muscle mass as 2 SD below the mean DXA derived appendicular lean tissue mass normalized to height squared of a young adult cohort. Following this initial definition, several cutpoints have been established across a wide variety of populations using different modalities and normalization metrics (e.g. height, weight, BMI, limb length) [55]–[58]. Despite the immense interest in identification of abnormal body composition, no consensus has been established for classification of poor muscle or adipose tissue health for a given modality. The lack of widely accepted consensus on criterion for low skeletal muscle mass results in a large variability in the prevalence of sarcopenia, ranging from 9.9% to 40.4% [59] across older adult populations. The lack of agreement on the precise definition of sarcopenia has important implications in identification of older adults who may be malnourished, require pre-habilitation prior to elective surgical procedures, rehabilitation during and following hospital admission and discharge, and for the identification of risk factors associated with development of chronic illnesses. However, recent guidelines from the European Working Group on Sarcopenia in Older persons have suggested commonly used modalities, with associated cutpoints, for identification of individuals with sarcopenia [10]

2.3.2 Consequences of abnormal skeletal muscle and adipose tissue features

Older adults with low skeletal muscle mass, identified using previously established cutpoints by Baumgartner et al (1998) [52], were 3.5-fold more likely to present with more than 3 disabilities (balance abnormality, use of a cane/walker, falling within the past year) compared with older adults with normal muscle mass [52]. This seminal work has been advanced by several large epidemiological studies including: the health, ageing, and body composition

cohort [60], [61], the ageing in Reykjavik study [62], [63], the Baltimore longitudinal study on ageing [27], [64], and the National Health and Nutrition Examination Survey (NHANES) and Korean NHANES [65]–[67]. These large-scale epidemiological studies have demonstrated that older adults with low skeletal muscle or appendicular lean tissue mass display poorer physical function and strength compared to older adults with normal muscularity. However, it is important to note that these associations between low muscle mass and poor function or strength are not always present [68]. Muscle quality or composition is also an important factor for identifying older adults with mobility impairments or overt disability, as it is associated with reduced strength and functional capacity in older adults [26], [63]. Furthermore, increased IMAT in older males and females is associated with elevated rates of mortality in community dwelling older adults [69]

Skeletal muscle mass and composition have also been investigated in relation to metabolic homeostasis in older adults; as skeletal muscle tissue is responsible for ~85% of post-prandial glucose disposal [70]. However, the relationship between skeletal muscle mass and glucose homeostasis is controversial. Some investigations have observed that elevated indices of skeletal muscle mass is associated with improved glucose handling [71]–[73], however, several others have observed no or even negative associations [74]–[77]. These discrepancies are likely due to differences in how muscle mass is normalized, as a recent study demonstrated that DXA appendicular lean tissue normalized to height was not associated with glucose tolerance, but was negatively associated when normalized to body weight [77]. Interestingly, most of the publications demonstrating positive associations between muscle mass and glucose homeostasis normalized metrics of muscle to body weight, rather than height [75]. Taken

together, these studies suggest that whole body skeletal muscle or lean tissue mass may have a relatively minor role in the determination of blood glucose homeostasis. On the contrary, muscle composition has moderate to strong associations with glucose homeostasis across a wide range of healthy, older, obese, and diabetic individuals [31], [78]–[81]. Increased IMAT or IMCL have both been associated with insulin resistance and impaired glucose tolerance in older adults [82], obese individuals [83], and diabetic patients [84].

High adiposity is well established as an independent risk factor for the development of mobility impairments in older adults [85]–[87]. In 753 men and women aged 72 to 95 years of age, the highest tertile of whole-body fat mass measured using DXA had a greater than 2 fold risk for mobility limitations [87]. Longitudinally, high baseline fat mass, independent of appendicular lean tissue mass, predicted the 2-year onset of disability in older adults [53]. While total body fat mass or adiposity is associated with limitations in mobility, adipose tissue distribution may be a more important metric for identification of poor metabolic phenotypes. Visceral adipose tissue is a considerable risk factor associated with metabolic syndrome, whereas the subcutaneous adipose tissue, particularly within the gluteal and thigh region, may have a protective role against poor metabolic health [88], [89]. However, others have observed that elevated abdominal subcutaneous adipose tissue mass is associated with impaired insulin sensitivity, independent of the visceral compartment [80]. These discrepancies between abdominal and thigh subcutaneous adipose tissue may be related to the dSAT layer within the abdominal region [90]. Kelley et al. (2000) [91] demonstrated that the elevated dSAT area is associated with poor glucose disposal during a hyperinsulinemic euglycemic clamp in lean and

obese young males and females. However, the potential interplay between dSAT and sSAT and their relation to metabolic health of older adults is less clear.

2.4.1 Site-specific measures of skeletal muscle tissue

Ageing-related changes in skeletal muscle tissue are not uniform across the body (e.g. lower limb musculature is preferentially lost compared to upper limb). Site-specific measures of skeletal muscle that are preferentially lost during the ageing trajectory may provide earlier and more sensitive metrics for detecting muscle atrophy and sarcopenia compared to traditional whole-body approaches. However, a comprehensive definition of site-specific skeletal muscle mass and composition is challenging to define. The specific site of analysis refers to the anatomical region of interest for evaluating skeletal muscle mass and composition. However, the body composition modality used, post-collection analysis, and outcomes of interests can drastically alter the definition of interpretation of site-specific muscle metrics. For example, regional analysis of the upper thigh lean tissue from DXA can provide site-specific measures of skeletal muscle mass, whereas MRI, CT, and ultrasound can distinguish the quadriceps from hamstring muscles, or individual muscles within those groups.

2.4.2 Ageing-related differences in quadriceps and hamstrings muscle mass

The most commonly evaluated measure of site-specific muscle mass in older adults is between the quadriceps and hamstrings muscle groups. Overend et al. (1992) [133] observed that older men displayed a ~25 % smaller quadriceps CSA, but a non-significant ~15% smaller hamstrings CSA compared to a younger adult cohort using midthigh CT; suggesting that certain muscle groups may be more responsible for the accelerated rate of decline in the lower limbs. The quadriceps have also been observed to decline to a greater extent than the hamstrings in

older women as well [93]. Furthermore, others have observed that only the quadriceps muscle mass declines with advancing age, whereas the hamstring muscles display a similar size to that of young adults [94], [95]. These findings are further supported by correlation analysis between age and indices of muscle mass across a wide range of ages (20-80+ years of age), which have demonstrated moderate negative association (r= -0.4 to -0.5) for the quadriceps, but not the hamstring muscle groups [96], [97]. Longitudinal data also supports the site-specific quadriceps muscle loss, as over a period of 9 years, 10 community dwelling older adults displayed a 5.7% reduction in the quadriceps CSA, but a non-significant reduction of 3.2% in the hamstrings [98].

While variability exists in the degree of hamstrings muscle atrophy across different studies, a consistent observation is that the quadriceps display a proportionally greater atrophy compared to the hamstrings with advancing age [99]. An important aspect to consider when comparing these studies is the modality and metric used to evaluate the degree of muscle mass lost, such as muscle cross-sectional area or volume from CT and MRI vs. muscle thickness from ultrasound. For the quadriceps musculature, there is strong agreement between muscle cross-sectional area and thickness for the degree of muscle atrophy (20-30%) that occurs with advancing age [99]. However, despite the strong associations between ultrasound measured hamstring muscle thickness and MRI muscle volume (r=0.87) [100], muscle thickness of the hamstrings tends to exhibit attenuated atrophy compared to cross-sectional area or volume (5-10% vs. 10-20%) [99]. These discrepancies may be related to ethnicity differences in muscle distribution, as the majority of ultrasound literature has been conducted in Japanese cohorts, whereas MRI and CT data predominantly comes from Caucasian cohorts [101]. Indeed, Ogawa et al. (2012) [142] examined the quadriceps and hamstring muscle volumes in younger and

older Japanese men using MRI and observed a 20% reduction in quadriceps volume, in agreement with other literature for anterior thigh muscle loss, but a 9% reduction in hamstrings muscle volume, in agreement with ultrasound literature in Japanese older adults; suggesting that the discrepancies across study may be due to ethnicity, rather than the modality used for analysis.

2.4.3 Additional site-specific differences in muscle mass of older adults

Several other site-specific measures of muscle mass have been compared between younger and older adults. Abe et al. (2014) [143] have provided the most comprehensive comparison of ageing-related site-specific muscle thickness differences in 746 men and 813 women split across decades of life (20-29, 30-39, 40-49, 50-59, 60-69, 70-85 years of age). Comparisons of muscle thicknesses were made across 9 distinct sites representing the major muscle groups of the anterior and posterior appendages (7 sites) and the anterior and posterior torso (2 sites). When comparing the young (20-29 years of age) and older (70-85 years of age) adult cohorts, muscle thicknesses at all sites were significantly lower in older adult males, whereas 5 of the 9 sites were significantly lower in older adult females compared with their younger counterparts [103]. However, a more in-depth examination of the degree of difference reveals that within both men and women, the largest discrepancies between older and younger adults occurs within the anterior thigh (rectus femoris and vastus intermedialis, ~30% reduction) and anterior trunk (rectus abdominis, ~30% reduction) muscle thicknesses [103]. These findings have been replicated in other large populations of Japanese and German adults, in which the abdominal and quadriceps muscle thicknesses display a negative associations with advancing age, whereas the hamstrings and posterior trunk musculature displayed no

correlations with age [104]. A more comprehensive analysis of the lower trunk musculature (approximately at the level of the umbilicus) reveals that all muscle groups (erector spinae, rectus abdominus, internal and external obliques, and transverse abdominus) are largely affected by advancing aging [105]–[107].

2.4.4 Site-specific muscle mass in relation to strength and functional capacity

While there are clear ageing-related differences in site-specific muscle atrophy, there is a paucity of literature examining how these site-specific measures of musculature compare to commonly applied indices of skeletal muscle mass (e.g. DXA) in the relationship to strength, functional capacity or in identification of sarcopenia. Minetto et al. (2015) [148] established sex-specific cutpoints in 60 younger participants for several muscle thicknesses (rectus femoris, vastus lateralis, tibialis anterior, and medial gastrocnemius), which were then applied alongside previously established BIA cutpoints in 44 frail older adults for identification of low skeletal muscle mass (2 SD below mean of young group). Using a combined thigh score (vastus lateralis and rectus femoris muscle thicknesses), 86% of older adults were classified as having low skeletal muscle mass, whereas the medial gastrocnemius and tibialis anterior identified 52% and 16% as having low muscle mass [108]. In comparison, the application of previously established BIA cutpoints identified between 5 and 75% of older adults has having low skeletal muscle mass [108]. The higher prevalence of low skeletal muscle mass identified using site-specific compared to whole body metrics of muscle mass suggests that these indices may be useful as an earlier biomarker for identification of ageing-related loss of skeletal muscle.

Little data is available comparing site-specific and whole-body muscle mass indices in relation to strength, functional capacity, or metabolic health of older adults. Thiebaud et al.

(2017) [136] examined associations between either DXA-derived appendicular lean tissue mass or site-specific measures of muscle thickness (anterior and posterior upper arms and legs) and measures of upper and lower body strength in younger (n=12), middle-aged (n=13), and older adults (n=10). Despite significant differences in site-specific measures of muscle thickness (anterior thigh and posterior arm), but not appendicular lean tissue across age cohorts, similar correlations (0.4 to 0.7) were observed when comparing thickness or appendicular lean tissue mass and muscle strength [95]. However, only the ratio between the anterior and posterior muscle thicknesses were compared with muscle strength. In a more direct comparison, Tsukasaki et al. (2020) [109] determined that midthigh quadriceps cross-sectional area provided stronger associations than DXA appendicular lean tissue mass in relation to knee extensors isometrics torque in both males and females (middle-aged to older adults). Metabolic outcomes have also been examined comparing site-specific musculature and whole-body approaches. In middle-aged and older obese adults, ultrasound derived muscle thickness of the rectus abdominis was a significant predictor of metabolic syndrome and elevated HbA1c, whereas appendicular lean tissue and several other muscle thicknesses (anterior and posterior thigh, anterior and posterior upper arm, and posterior trunk) were not associated with markers of poor metabolic health [110].

2.4.5 Ageing-related differences for site-specific skeletal muscle composition

Recently, the European Working Group on Sarcopenia in Older Persons have incorporated skeletal muscle composition into their definitions of sarcopenia [10]. The majority of our current knowledge on the age-associated changes in muscle composition are derived from studies examining the quadriceps musculature (CT, MRI, muscle biopsy) [68], [111].

However, it is unclear if other muscle groups follow a similar trajectory of muscle composition deterioration with advancing age. Ultrasound-derived muscle echo intensity has been used as a surrogate of IMAT at several different locations across the whole body [112], [113]. Fukumoto et al. (2015) [145] observed that while all muscle groups display increased muscle echo intensity in older adults compared to younger adults, abdominal muscle echo intensity displayed the greatest deterioration (1.6 to 2-fold higher) compared to the biceps and quadriceps (1.25 and 1.4 respectively). With this limited evidence, it is unclear if the ageing-related losses in muscle composition are uniform across the body and if specific sites are stronger predictors of metabolic or functional impairments (e.g. abdominal vs quadriceps muscle composition in relation to metabolic syndrome).

CHAPTER 3

THESIS RATIONALE, OBJECTIVES, AND HYPOTHESES

3.1 Thesis rationale summary

Emerging evidence demonstrates that ageing-related losses in skeletal muscle mass are concentrated within certain muscle groups, specifically the anterior thigh and abdominal muscles. However, these site-specific comparisons between younger and older adults have not attempted to account for relative differences in muscle mass, making it challenging to determine if these site-specific differences are due to ageing-related muscle atrophy or simply due to differences in relative muscle mass between younger and older adults. Despite the emerging evidence of ageing-related site-specific skeletal muscle atrophy, our understanding of site-specific shifts in skeletal muscle composition due to advancing age are less well understood. Furthermore, there is a paucity of literature examining how these site-specific measures of muscle mass and composition compare with traditional whole-body measures of body composition in relation to strength, functional capacity, and metabolic health of older adults. A more thorough understanding of these site-specific body composition changes with advancing will provide critical information for determining the ideal procedures for identification of sarcopenia in older adults.

Study 1: **Older males exhibit reduced anterior upper leg and anterior abdominal muscle thickness compared to younger males matched for relative appendicular lean tissue index**

Objectives: In community dwelling older (≥65 years of age) and younger (18-44 years of age) males matched for relative appendicular lean tissue, our objectives are to:

1. Quantify site-specific differences in skeletal muscle thickness and lean tissue mass
2. Quantify site-specific differences in skeletal muscle echo intensity

Hypotheses:

1. Compared with younger adults, older adults will present with smaller muscle thicknesses of the anterior upper leg and anterior abdomen and less lean tissue in the upper leg
2. Muscle echo intensity will be elevated across all landmarks in the older males compared with younger males

Study 2: **Site-specific skeletal muscle echo intensity and thickness differences in subcutaneous adipose tissue matched older and younger adults**

Objectives: In older (≥60 years of age) and younger (18-44 years of age) males and females matched for absolute subcutaneous adipose tissue thickness, our objective is to:

1. Quantify site-specific differences in skeletal muscle echo intensity

Hypothesis:

1. Skeletal muscle echo intensity will be increased across all landmarks in the older adults compared with younger adults

Study 3: **Association of static and dynamic strength and function with muscle mass and composition in older males: a comparison of muscle thickness, echo intensity, and lean tissue mass**

Objectives: In community dwelling older (≥65 years of age) males, our objectives are to:

1. Compare the magnitude of associations between muscle thickness, muscle echo intensity, or lean tissue and isometric torque and isokinetic power
2. Evaluate differences in muscle thickness and echo intensity between individuals with low and normal appendicular lean tissue mass

Hypotheses:

1. Muscle thickness will display stronger associations with isometric torque and isokinetic power compared to lean tissue mass
2. Older males with low appendicular lean tissue will exhibit decreased muscle thickness and increased echo intensity across all landmarks

Study 4: **Skeletal muscle echo intensity displays divergent associations with glucose homeostasis in healthy and glucose-impaired older males.**

Objectives: In healthy and glucose impaired community dwelling older (≥65 years of age) males, our objectives are to:

1. Evaluate associations between skeletal muscle echo intensity or thickness and indices of glucose homeostasis
2. Evaluate associations between dSAT to sSAT thickness ratio and indices of glucose homeostasis

Hypotheses:

1. Elevated skeletal muscle echo intensity at the anterior upper leg and anterior abdomen, but not muscle thickness, will be associated with poor glucose homeostasis
2. Increased dSAT to sSAT thickness ratio will be associated with poor glucose homeostasis

CHAPTER 4

STUDY DESIGN AND METHODOLOGICAL OVERVIEW

4.1 Study design and participant cohorts

Data for this thesis was derived from two different sources, a prospectively recruited cohort of older males (n=32) and a secondary analysis of younger and older males and females (n=96) who were involved in a study validating muscle thickness against DXA for prediction of appendicular lean tissue mass [51].

The prospectively recruited cohort consisted of community dwelling older males (≥65 years of age) from the Kitchener-Waterloo community, which are included in Chapters 5, 7, and 8 (Table 4.1). Recruitment was limited to older males as sex-specific analysis is required for measures of body composition, strength, and metabolism, and it was not feasible to collect adequate sample sizes for both sexes. Preliminary analyses of site-specific differences in muscle thickness between younger and older adults revealed larger ageing-related declines in males compared to females. Therefore, as an initial first step, males were recruited due to the larger effect size. All participants attended 3-4 data collections sessions over the course of 7-14 days, which included assessment of body composition (ultrasound, DXA), muscle strength, functional capacity, and metabolic health.

The secondary data analysis cohort included younger (18-44 years of age) males (n=21) and females (n=32) from the University of Waterloo student population and older (≥60 years of age) males (n=20) and females (n=23) from the Kitchener-Waterloo community (Table 4.1). All

participants attended a single data collection session for assessment of body composition (DXA and ultrasound)

Table 4.1. Overview of participant cohorts

	General description	**Prospective older male cohort**	**Secondary data analysis cohort**
Study 1 (Chapter 5)	Older and younger adults matched for relative appendicular lean tissue mass and differences in muscle thickness and echo intensity are compared	Included	Younger males included
Study 2 (Chapter 6)	Older and younger adults matched for absolute subcutaneous adipose tissue thickness and differences in muscle echo intensity are compared	-	Younger and older males and females included
Study 3 (Chapter 7)	Appendicular lean tissue and site-specific muscle mass and composition evaluated in relation to muscle strength and functional capacity	Included	-
Study 4 (Chapter 8)	Appendicular lean tissue and site-specific muscle mass and composition evaluated in relation to glucose homeostasis	Included	-

4.2.1 General dual-energy x-ray absorptiometry overview

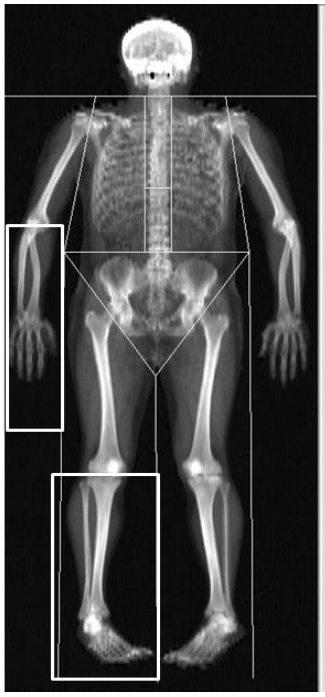
DXA is based on the principle of x-ray attenuation, which is the change in x-ray intensity (due to Compton scattering and photoelectric absorption) during passage through structures of different densities and thicknesses [116]. By utilizing two different energy x-rays (40 keV and 70 keV), a ratio between the high and low energy attenuation can be calculated and compared to previously established theoretical and experimentally derived mass attenuation coefficients for

fat, lean soft tissue and bone mineral [117]. DXA first separates the pixels from a scan into those with only soft tissue (fat and lean soft tissue) and those with soft tissue and bone mineral using a threshold value. In pixels only containing fat and lean soft tissue, the attenuation ratio between the low and high energy x-rays and previously established attenuation ratios for fat and lean tissue can be used to calculate the mass contribution of each component. With pixels containing bone, the mass of bone and soft tissue (fat and lean) can be calculated for each pixel, followed by extrapolation of adjacent pixel lean tissue fractions (e.g. 70% lean soft tissue and 30% fat) across the remaining soft tissue mass [118].

A major advantage of DXA is the ability to perform regional assessment of body composition. A DXA scan is typically segmented into the arms, legs, trunks, and head, allowing quantification of these specific regions. The most common regional assessment of body composition using DXA is appendicular lean soft tissue mass, which estimates the lean soft tissue of the appendages [13]. While DXA is unable to differentiate muscle from other lean soft tissues (e.g. visceral organs), the appendicular lean soft tissue is predominately skeletal muscle (alongside skin and connective tissues), which provides a useful metric of skeletal muscle mass in the appendages. Indeed, strong correlations are observed between MRI measures skeletal muscle mass of the limbs and appendicular lean tissue, which has led DXA to be commonly used for assessment of skeletal muscle mass deficiencies in several older adult and clinical populations [14]. Furthermore, DXA software can be used to perform region-specific analysis, such as separating the arms and legs into the upper arm, lower arm, upper leg, and lower leg.

4.2.2 Specific dual-energy x-ray absorptiometry methods

DXA scans were utilized to assess whole-body and regional indices of lean tissue mass. DXA scans were compartmentalized into the head, trunk, and left and right appendages by a single trained investigator (Apex, version 13.2) [13]. Appendicular lean tissue index was calculated as the sum of the lean soft tissue in the arms and legs, divided by the participant's height squared (kg/m^2). Further regional analyses were performed to quantify the lean tissue of the upper and lower arm and upper and lower leg (Figure 4.1).



Region	Area [cm^2]	BMC [(g)]	BMD [g/cm^2]	Fat[(g)]	Lean [(g)]
L Arm	261.05	262.67	1.006	1707.0	2897.8
R Arm	262.61	264.25	1.006	1631.9	2862.6
L Ribs	132.28	116.36	0.880		
R Ribs	126.82	117.41	0.926		
T Spine	114.72	137.69	1.200		
L Spine	74.53	103.34	1.387		
Pelvis	232.95	326.88	1.403		
Trunk		801.69		14148.2	25677.9
L Leg	443.27	600.32	1.354	3900.6	8180.4
R Leg	435.08	635.95	1.462	4399.8	8189.4
Subtotal	2083.30	2564.88	1.231	25787.5	47808.1
Head	248.56	691.82	2.783	1243.1	3555.4
Total	**2331.86**	**3256.71**	**1.397**	**27030.6**	**51363.5**

Figure 4.1. Dual-energy x-ray absorptiometry analysis. Bold white boxes indicate region specific analysis for the lower arm and lower leg.

4.3.1 General ultrasound imaging overview

Ultrasound has been used since the mid 80's for analysis of body composition and represents an accessible, non-invasive, real-time, and portable imaging tool that is rapidly

increasing in popularity for both clinical and research investigations. Ultrasound devices generate high frequency sound waves (2-20 MHz) through an electrically stimulated piezoelectrical crystal in the transducer [121]. Ultrasound images are generated based on the transmission, reflection, scattering, and absorption of the propagated sound waves through the underlying tissues. The degree of reflection or transmittance that occurs is dependent upon several factors, including differences in acoustic impedance between tissue interfaces, relative structure size, and smoothness of tissue interface [122]. Due to the differences in acoustic impedance between skin, adipose tissue, muscle, and bone, high contrast images with clear delineations between these tissues can be obtained.

Traditional 2D or B-mode ultrasound is based on the pulse echo approach, which creates a pulse of echo waves which are reflected to the transducer and detected by the piezoelectric crystals [123]. These detected echoes are coded into an electric signal based on the time of flight, estimated speed of sound in soft tissues, and intensity of the returned echo to produce a 2D contrast image. Since ultrasound waves travel through multiple different tissues (skin, adipose, muscle), the speed of sound propagation is not uniform across these tissues. In muscle, the wave propagates at 1580 m/s, whereas in adipose tissue it is 1450 m/s, with the assumed average speed of soft tissues occurring at 1540 m/s [123].

4.3.2 Ultrasound measurements of body composition

Due to the high-contrast delineations between the skin, muscles, subcutaneous adipose tissue, and bone within ultrasound images, individual muscles groups and specific adipose tissue depots can be easily visualized and analyzed for body composition. The most commonly applied metrics of muscle mass using ultrasound are muscle thickness and cross-sectional area.

Using computer-aided image processing software, the linear vertical distance of a muscle or the cross-sectional area of the muscle fascia can be quantified [124], which displays strong associations with MRI and CT measures of both thickness and area [125]. Similarly, adipose tissue thickness can be quantified from ultrasound images at specific landmarks, which relates well with regional and whole body measures of fat mass [126]. While ultrasound thickness and cross-sectional area of muscle or adipose tissue are not direct measures of the entirety of the compartment, several regression equations have been developed that predict both regional and whole-body indices based on these linear and area measures [51], [127], [128]. However, a considerable limitation of ultrasound, in comparison to other imaging modalities (e.g. MRI, CT), is the inability to distinguish intramuscular adipose tissue. This limitation may introduce additional error when applying these measures to older adults or clinical populations with a high degree of muscle degradation [122].

4.3.3 Ultrasound muscle composition

While ultrasound is unable to differentiate between intramuscular adipose tissue and functional contractile muscle tissue, a surrogate measure of muscle composition can be measured through the analysis of muscle echo intensity [129]. Skeletal muscle echo intensity is a measure of the 'greyness' or 'brightness' of the muscle of interest, which can be qualitatively described using 4 visual analog scales [130] or quantitatively measured using computer-aided histogram analysis, the latter being more commonly applied [131]–[133]. In computer-aided histogram analysis, a region of interest encompassing the skeletal muscle (excluding the fascia) is manually outlined and the average pixel intensity (8-bit image: 0 – 255) is extracted. In healthy young adults, skeletal muscle is generally hypo-echoic, due to homogenous acoustic

impedances of muscle structures [134]. However, aged muscle exhibiting a larger degree of intramuscular adipose tissue infiltration, or any factor altering the ultrasound beam transmission (e.g. hydration), will present as hyper-echoic due to the increased reflection and scattering occurring between muscle and intramuscular adipose tissue interfaces (Figure 4.2). These additional reflections and scattering increase the overall brightness and will be represented by an elevated muscle echo intensity. Increased muscle echo intensity in older adults has been associated with reduced strength, power, and VO2 max in older adult cohorts [132], [133], [135], [136].

While muscle echo intensity is a useful surrogate of muscle composition, there are several limitations associated with its measurement and interpretation. First, comparisons across different ultrasound equipment or image settings are challenging, as these factors will significantly alter the pixel intensity of the image [122]. While some have attempted to mitigate these factors by developing prediction equations between different ultrasound equipment [137], this is not a feasible approach across different sites due to a lack of a standard reference phantom. Muscle echo intensity is also confounded by participant factors, such as the thickness of the subcutaneous adipose tissue. Subcutaneous adipose tissue overlying the skeletal muscle will attenuate the ultrasound beam [138], [139], which will artificially shift muscle echo intensity to lower values (i.e. less intramuscular adipose tissue infiltration). Young et al. (2015) [138] developed a correction factor to account for the influence of adipose tissue thickness on muscle echo intensity, which improved associations with MRI-derived intramuscular adipose tissue. While some groups have applied this correction factor [140], [141], it is unclear if this correction factor is applicable across all ultrasound equipment and settings and all populations.

Furthermore, given the importance of subcutaneous adipose tissue on functional capacity and metabolic health [2], caution is needed when interpreting corrected muscle echo intensities, as the subcutaneous adipose tissue can largely impact the final output (i.e. 40.5 units per cm of adipose thickness). Therefore, it is difficult to disentangle the influences of the adipose tissue thickness from skeletal muscle echo intensity.

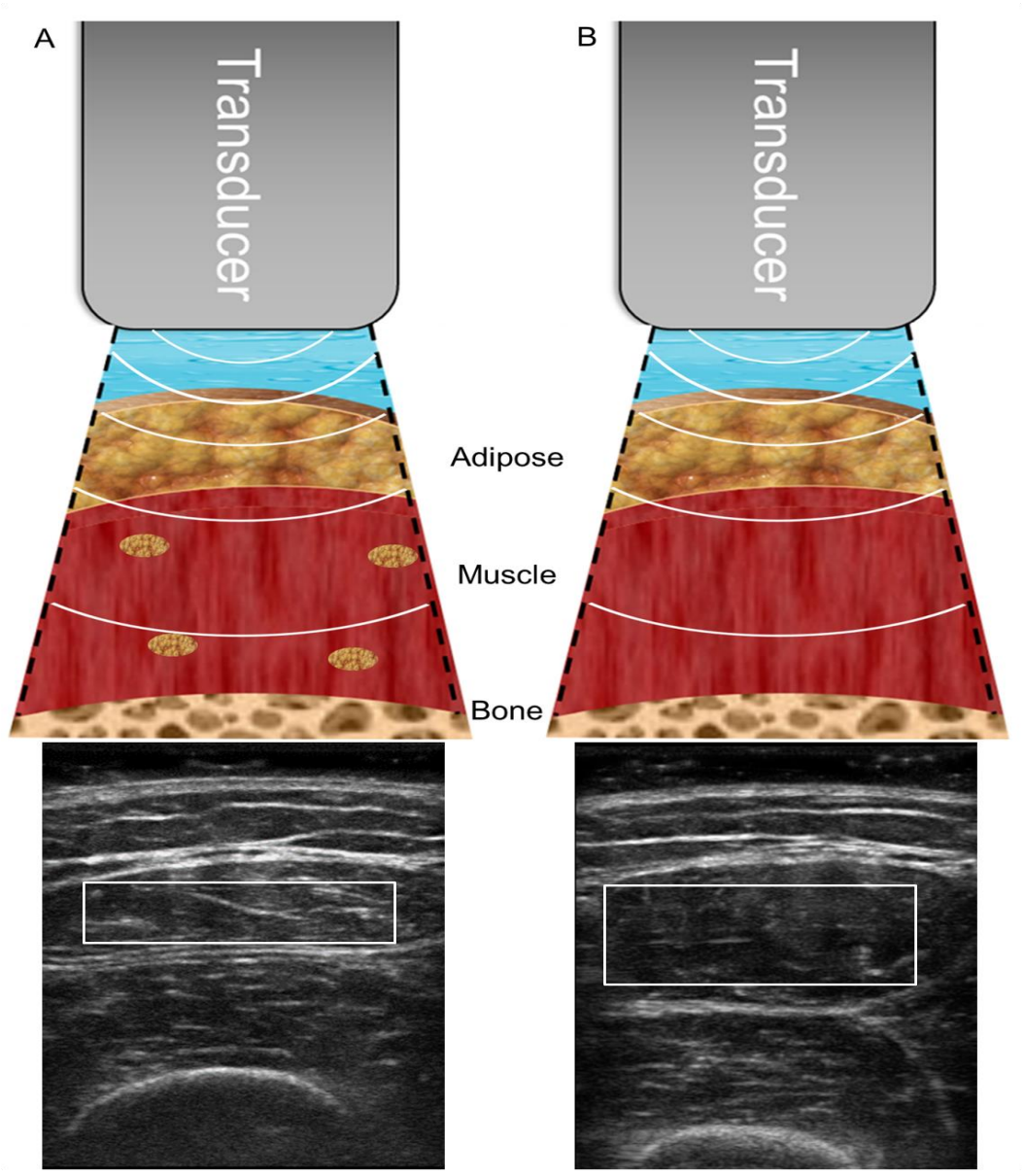


Figure 4.2. Depiction of older (A) and younger (B) rectus femoris muscle echo intensity.

4.3.4 Specific ultrasound imaging methods

Specific sites to be imaged for assessment of muscle and adipose tissue characteristics included the: anterior and posterior upper arm, anterior forearm, anterior abdomen, anterior and posterior upper leg, and anterior and posterior lower leg. A real-time B-mode ultrasound imaging device (SonoSite M-Turbo) equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz) was used to obtain transverse ultrasound images. Minimal compression of the ultrasound probe was applied against the skin to minimize alterations in muscle tissue.

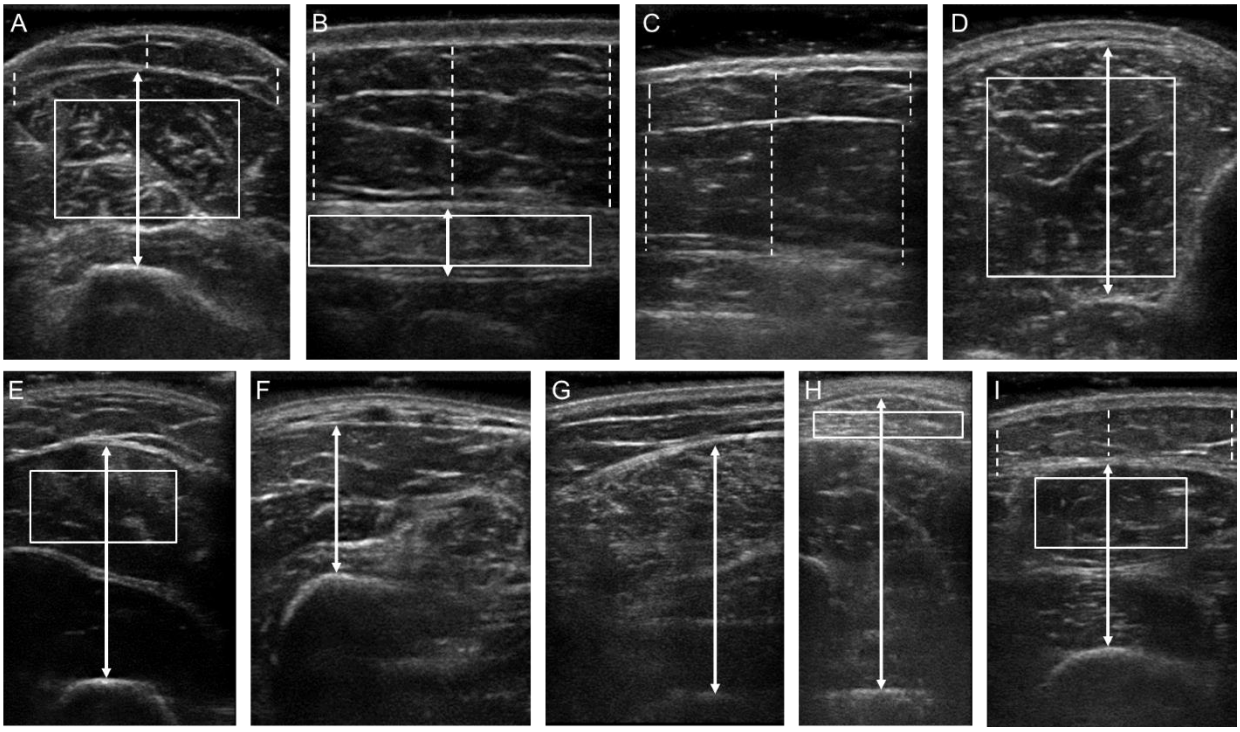


Figure 4.3. Depiction of muscle thickness, muscle echo intensity, and adipose tissue thickness for the A) anterior upper arm, B) anterior abdomen (3 cm), C) anterior abdomen (5 cm), D) anterior lower leg, E) posterior upper arm, F) anterior forearm, G) posterior upper leg, H) posterior lower leg, I) anterior upper leg. Double arrowed lines indicate muscle thickness, solid boxes indicate muscle echo intensity region of interest, and dashed lines indicate adipose tissue thickness.

Muscle thickness was analyzed for all imaged sites (Figure 4.3). Muscle echo intensity was analyzed for the rectus abdominis (anterior abdomen), tibialis anterior (anterior lower leg), biceps brachii (anterior upper arm), rectus femoris (anterior upper leg), gastrocnemius (posterior lower leg), and triceps brachii (posterior upper arm). Subcutaneous adipose was analyzed for the anterior upper arm, anterior upper leg, and anterior abdomen (Figure 4.3). The thickness of sSAT and dSAT were measured on the anterior abdomen (Figure 4.3).

4.4.1 General muscle strength and functional capacity overview

Assessment of muscle strength and functional capacity of older adults is an essential aspect in recent guidelines on diagnosis of sarcopenia [10]. Grip strength is frequently used for assessing muscle strength in older adults due to its simplicity of measurement, wealth of normative data, and generally accepted standardized protocol [142].

To quantify static and dynamic muscle strength of the limbs, isokinetic dynamometers can be used to isolate several different joints for both research and clinical applications. Isokinetic dynamometers provide objective measures of muscle function, including average or maximal torque and power. These dynamometers typically measure position, torque, and velocity, which can be used to calculate additional metrics (e.g. power). While there may be some measurement errors (e.g. lever arm velocity), in general these dynamometers are considered to be valid metrics of static, and to a lesser extent, dynamic muscle strength [143].

The 6-minute walk test is a simple, low technological evaluation of functional exercise capacity, which is well tolerated for most older adults and clinical population [144]. The 6-minute walk test is a global measure of the pulmonary, cardiovascular, circulatory, and neuromuscular systems. Importantly, it may be more reflective of activities of daily living than

other exercise tests [144]. However, because intensity is self-selected by participants, the results do not reflect maximal exercise capacity for most individuals.

The 30-second sit to stand evaluates the relative strength and endurance of the lower body muscles. A major advantage of the 30-second sit to stand test is the low floor (unable to complete a single chair stand) and the high ceiling (>20 successful chair stands) of the results, which make an excellent test for separating low and high functioning older adult. The 30-second sit to stand has been shown to have good test-retest reliability and provides valid indications of lower muscle strength in generally active, community dwelling older adults [133].

4.4.2 Specific muscle strength and functional capacity methods

Maximal grip strength of the right hand was assessed according to a standardized protocol [142]. Maximal isometric torque and isokinetic power were assessed using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York). Isometric torque of the knee extensors, knee flexors, elbow flexors, and elbow extensors and isokinetic power (60 °/s and 180 °/s) of the knee extensors were evaluated. Participants performed one familiarization session prior to their testing session (2-7 days apart). Functional capacity was assessed using a six-minute walk and 30-second sit to stand test.

4.5.1 General blood glucose and lipid metabolism overview

The oral glucose tolerance test is a widely employed procedure to evaluate glucose tolerance and homeostasis. The oral glucose tolerance test involves biochemical analysis of blood for glucose, and other regulatory proteins, before and after consumption of a standard glucose load (usually 75 or 100 g). Regulation of plasma glucose reflects both the ability of the

pancreas to secrete insulin and the sensitivity of peripheral tissues to insulin, thus the oral glucose tolerance test has been used to evaluate β-cell function and insulin sensitivity [145]. Results from an oral glucose tolerance test provide strong associations with reference measures of β-cell function and insulin sensitivity (e.g. hyperinsulinemic euglycemic clamp), offering a clinically feasible approach for analyzing glucose homeostasis [146]. From the combination of glucose and insulin values throughout the oral glucose tolerance test (e.g. 0, 30, 60, 90, and 120 minutes), several indices of peripheral insulin sensitivity can be derived [146].

A lipid panel involves assessment of fasting triacylglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, which is often used as part of a risk assessment for cardiovascular disease [147].

4.5.2 Specific blood sampling and metabolic characterization methods

After an overnight fasting (minimum 10 hours with no food or drink, except water), participants arrived for a 75 g 2-hour oral glucose tolerance test and fasted lipid panel. Following collection of the fasting blood sample (0 minute), participants consumed a 75 g glucose drink within 5 minutes, and blood samples were subsequently drawn at 15, 30, 45, 60, 90. and 120 minutes. For the fasted and postprandial blood samples, glucose, insulin, and c-peptide were analyzed. Fasted blood was also analyzed for total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triacylglycerides.

CHAPTER 5

STUDY #1: OLDER MALES EXHIBIT REDUCED ANTERIOR UPPER LEG AND ANTERIOR ABDOMINAL MUSCLE THICKNESS COMPARED TO YOUNGER MALES MATCHED FOR RELATIVE APPENDICULAR LEAN TISSUE INDEX

Michael T. Paris[1]

Kirsten E. Bell[1]

Egor Avrutin[1]

Marina Mourtzakis[1]

[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Corresponding author: Marina Mourtzakis, PhD, Department of Kinesiology, University of Waterloo, 200 University Ave W, Waterloo, ON, N2L 3G1, Canada. Email: mmourtzakis@uwaterloo.ca. Phone: 519-888-4567, Ext 38459.

5.1 Introduction

Adult ageing is associated with losses in skeletal muscle mass [148] and deleterious shifts in muscle composition [16], [22], which is characterized by deposition of intramuscular adipose tissue. Ageing-related muscle atrophy is often estimated using appendicular lean tissue mass measured by dual-energy x-ray absorptiometry (DXA), which consists of the lean soft tissue of the upper and lower limbs [10], [13]. However, accumulating evidence indicates that muscle atrophy due to advancing age does not occur uniformly across the body [15], [16], [149], [150]. Indeed, advancing age is associated with greater declines in skeletal muscle mass of the lower limbs compared to upper limbs [15]. More specifically within the lower limbs, the quadriceps muscles appear to be the most susceptible to ageing-related skeletal muscle atrophy [16], [149], [151], [152]. Similarly, the rectus abdominus muscle group also exhibits a significant loss of muscle mass with advancing age [16], [150]. However, these comparisons are generally cross-sectional and have not attempted to account for differences in relative muscularity between older and younger groups, limiting the ability to draw conclusions on whether these differences in muscle size are due to older age or differences in the relative muscle mass between groups.

The uniformity of muscle composition changes with advancing age is less well understood. Several publications have observed that the intramuscular adipose tissue of the thigh musculature increases with age [40], [153], [154]. However, the infiltration of intramuscular adipose tissue of other muscle groups is less well characterized. While several modalities can provide indices of muscle composition, ultrasound represents a versatile tool that can be easily applied across multiple muscle groups to quantify echo intensity [155], a

surrogate measure of intramuscular adipose and connective tissue [138], [156], [157]. While some groups have observed increased echo intensity in the trunk, upper limb, and lower limb muscles of older adults [16], [105], [158], [159], comprehensive comparisons across multiple muscle groups within the same older adult participants are lacking.

These site-specific changes in muscle mass and composition throughout adult ageing have important implications for the identification of older adults with poor muscle health. Muscle groups that are particularly susceptible to ageing-related muscle atrophy or deleterious composition shifts (e.g. quadriceps), may provide earlier and more sensitive markers of muscle dysfunction compared to traditional whole-body approaches (e.g. appendicular lean tissue) [160].

Here, our primary objective was to evaluate differences in muscle thickness and echo intensity across multiple landmarks (upper limbs, lower limbs, and trunk) between older (>65 years of age) and younger (18-44 years of age) males who are matched for relative appendicular lean tissue mass. Matching participants for relative appendicular lean tissue, using z-scores from the NHANES reference data [65], will minimize differences in muscle size due to absolute muscularity, enabling more equitable comparisons between younger and older adults.

5.2 Methods

5.2.1 Study design and participants

We conducted a prospective cross-sectional study which evaluated 32 community dwelling older (≥ 65 years of age) males from the Kitchener-Waterloo community. Older males were matched on an individual basis for relative appendicular lean tissue index with younger

males (18-44 years of age) from a previously collected cohort [51]. Younger males (n=21) were recruited from the University of Waterloo student population and surrounding Kitchener-Waterloo community. Participants were excluded if they: 1) had a previous history of neuromuscular disorders, 2) had undergone administration of oral or intra-venous contrast for nuclear medicine scans within the past 3 weeks, 3) had a prosthetic joint replacement, or 4) had a history of diabetes, cancer, or cerebrovascular disease. Participants were instructed to refrain from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to laboratory visits. All studies were approved by a human clinical research ethics committee at the University of Waterloo. Written informed consent was obtained from all participants in accordance with established protocols for human research.

5.2.2 Dual-energy x-ray absorptiometry

Height and weight were obtained in lightweight clothing or cloth hospital gowns using a balance beam and stadiometer, respectively. One to two Whole body DXA scans (Hologic discovery QDR4500, Hologic, Toronto, ON) were performed by certified medical radiation therapists for each participant. Quality control and calibration procedures were performed as outlined by manufacturer specifications prior to all scans. Participants were placed supine on the scanning bed, with their legs fully extended and toes internally rotated (held in place with masking tape). Hologic software (version 13.2) was used to segment the body into the head, torso, left and right arms, and left and right legs, as previously described [13]. If a participant required two scans (i.e. did not fit within the lateral limits of a single scan), scans were analyzed by excluding the missing limb(s) and averaging all other segments across the two scans.

Appendicular lean tissue index (kg/m^2) was calculated by summing the lean soft tissue in the arms and legs (kg) and dividing by the participant's height (m) squared. Further regional analyses were performed to evaluate limb-specific lean soft tissue of the upper arm, lower arm, upper leg, and lower leg. A custom region of interest was placed around the lower arm and lower leg to measure lean tissue. Upper arm and upper leg lean tissue was calculated by subtracting the lower arm or leg lean tissue from the total arm or leg lean tissue. The lower arm region of interest was placed horizontally across the medial epicondyle of humerus and encompassed all tissues of the lower arm and hand. The lower leg region of interest was placed horizontally across the tibial plateau, encompassing the tissues of the lower leg and foot.

5.2.3 Participant matching

To minimize relative differences in muscularity between younger and older males, participants were matched for relative appendicular lean tissue index using age and sex specific NHANES DXA normative data [65]. Participants were matched on an individual basis using a maximum difference of 0.5 z-score units. From the original sample of younger (n=21) and older (n=32) males, 19 matches were made within 0.5 z-score units.

5.2.4 Landmarking for ultrasound imaging

During landmarking, participants lay supine or prone on a padded table, with their feet secured in neutral rotation using a foot strap to prevent internal or external hip rotation. A flexible tape measure and pen were used to mark sites for ultrasound imaging. Sites for ultrasound imaging included the anterior and posterior upper arm, anterior forearm, anterior abdomen, anterior and poster upper leg, and anterior and posterior lower leg. Upper arm

images were taken on the anterior and posterior surface, 60% distal from the acromial process to the lateral epicondyle of the humerus. Anterior forearm images were taken on the anterior surface, 30% distal from the radial head to the styloid process of the radius. Anterior abdomen images were taken 3 cm right of the umbilicus. Anterior thigh images were taken two-thirds the distance from the anterior superior iliac spine to the superior pole of the patella. The posterior upper leg image was taken on the posterior surface, 50% between the greater trochanter and lateral epicondyle of the femur. The anterior and posterior lower leg images were taken on the anterior and posterior surface, 30% distal from the head of the fibula to the lateral malleolus. All landmarking was performed on the right side of the body. Landmarking took approximately 20 minutes, during which participants remained supine or prone to mitigate shifts in fluid distribution during ultrasound imaging.

5.2.5 Ultrasound image acquisition

Transverse images were taken using a real time B-mode ultrasound imaging device (M-turbo, Sonosite, Markham, ON), equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz). During image acquisition, imaging mode was set to “resolution”; adjustable parameters gain, time-gain-compensation, and dynamic range (50%) were held constant. The ultrasound transducer was generously coated with water-soluble transmission gel. To ensure the landmark aligned with the middle of the muscle bulk, medial-lateral movement was allowed for all landmarks to centre the muscle within the field of view. Cranial-caudal tilt of the probe was performed to obtain the brightest echo of the underlying bone and skeletal muscle fascia to ensure a consistent probe angle. Minimal compression of the ultrasound probe was applied against the skin to minimize alterations in muscle tissue, which we have previously shown to be

superior to maximal compression for estimating lean tissue mass [51]. Minimal compression was defined by: 1) maintaining a visible layer of ultrasound gel between the skin and transducer, and 2) ensuring the natural curvature of the skin, subcutaneous adipose tissue, and skeletal muscle was maintained, as previously described [51]. Imaging depth was adjusted to the minimum depth required to obtain a complete view of the muscles being analyzed. All ultrasound images were saved in the Digital Imaging and Communications in Medicine (DICOM) format and transferred to a personal computer for analysis.

5.2.6 Muscle thickness analysis

Muscle thickness was defined for each muscle group by measuring the vertical distance between the superior muscle fascia (lower boundary) and either the upper margin of the underlying bone or the inferior muscle fascia (anterior abdomen and anterior lower leg) (Figure 5.1). Muscle thickness was measured a single time for each landmark by a single trained investigator. All measurements were performed using ImageJ analysis software (NIH, Bethesda, MD, version 1.52e) and converted to a linear distance using manufacturer provide image resolutions.

5.2.7 Echo intensity analysis

Muscle echo intensity was evaluated by selecting the largest rectangular area within the muscle fascia borders (ImageJ, NIH, Bethesda, MD, version 1.52e), as previously described [161]. Specific muscles analyzed were the rectus abdominis (anterior abdomen), tibialis anterior (anterior lower leg), biceps brachii (anterior upper arm), rectus femoris (anterior upper leg), lateral gastrocnemius (posterior lower leg), and lateral triceps brachii (posterior upper arm)

(Figure 5.1). Echo intensity is expressed as an arbitrary unit (A.U.) between 0 (black) and 255 (white).

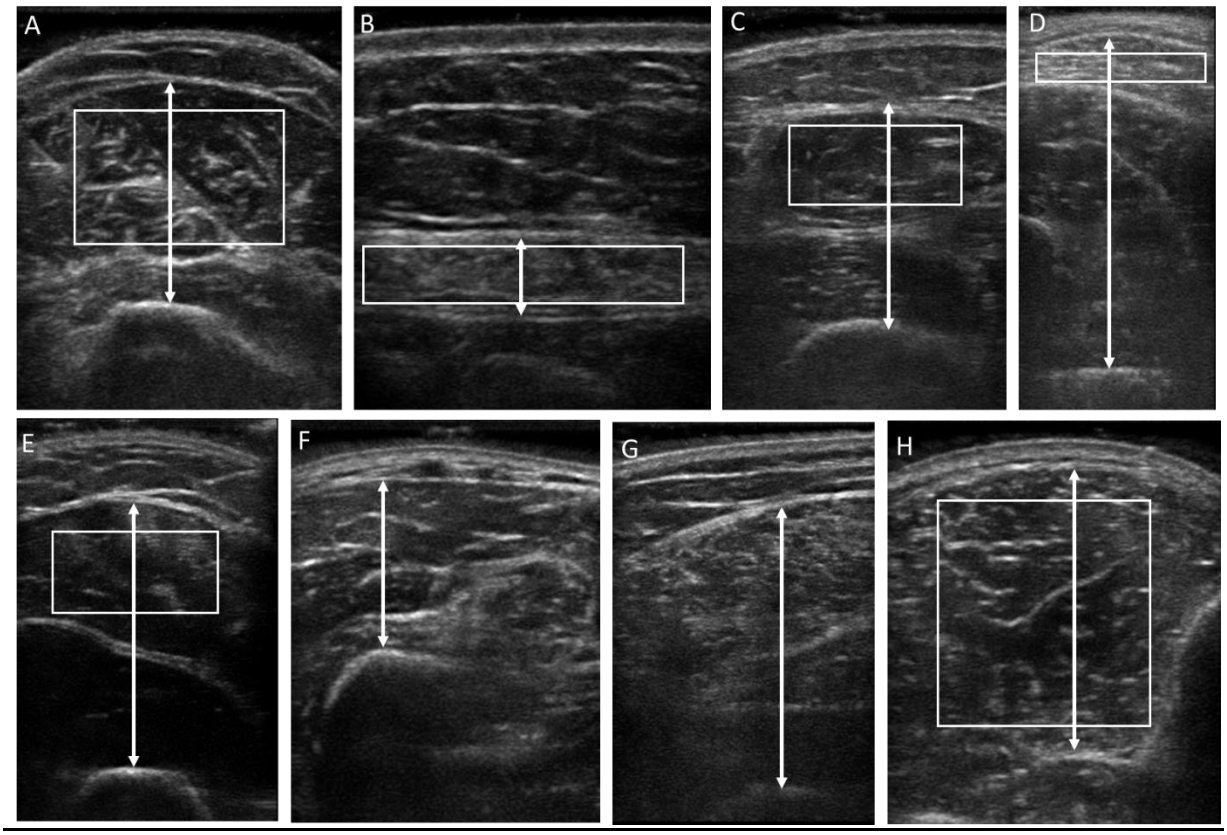


Figure 5.1. Muscle thickness and echo intensity analysis for A) anterior upper arm, B) anterior abdominal, C) anterior upper leg, D) posterior lower leg, E) posterior upper arm, F) anterior forearm, G) posterior upper leg, H) anterior lower leg. White lines indicate muscle thickness assessment and white boxes indicate area selected for analysis of echo intensity.

5.2.8 Statistical analysis

Normality of continuous variables was confirmed using Shapiro-Wilk test. Differences between younger and older males were evaluated using paired sample t-tests. Statistical significance was set as $p<0.05$. To maintain a familywise error rate of $\alpha=0.05$, adjustment for

multiple comparisons was performed separately for measures of DXA lean tissue (n=5), ultrasound muscle thickness (n=8), and ultrasound echo intensity (n=6) using a Holm-Bonferroni correction. All analyses were performed using SPSS (version 24, IBM, USA).

5.3 Results

Older males (n=19) were well matched with younger males (n=19) for appendicular lean tissue index z-score (p=0.927). The older male cohort had a higher BMI (p=0.011), percent body fat (p<0.001), and age (p<0.001) compared with younger adults (Table 5.1). After correction for multiple comparisons, there were no differences in appendicular or limb-specific lean tissue between older and younger males (Table 5.2).

Table 5.1. Participant characteristics.

	Young males (n=19)	Older males (n=19)	Unadjusted p-value
Age, y	27.3 (5.8)	72.2 (6.8)	<0.001
Height, m	1.76 (0.06)	1.74 (0.06)	0.336
Weight, kg	77.3 (11.6)	88.2 (15.4)	0.032
BMI, kg/m^2	25.1 (3.4)	29.2 (4.5)	0.011
DXA z score	-0.45 (0.75)	-0.43 (0.66)	0.927
DXA body fat, %	21.9 (4.8)	30.8 (4.9)	<0.001

Data are presented as mean (SD). BMI, body mass index; DXA, dual-energy x-ray absorptiometry.

Table 5.2. Dual-energy x-ray absorptiometry lean tissue characteristics.

	Younger males (n=19)	Older males (n=19)	Unadjusted p-value	Adjusted p-value
Appendicular lean tissue index, kg/m^2	8.37 (1.00)	7.87 (0.72)	0.171	0.513
Lower arm lean tissue, kg	1.35 (0.20)	1.34 (0.18)	0.899	1.00
Upper arm lean tissue, kg	2.10 (0.40)	1.80 (0.34)	0.038	0.188
Lower leg lean tissue, kg	2.88 (0.43)	2.95 (0.36)	0.588	1.00
Upper leg lean tissue, kg	6.71 (1.02)	6.00 (0.76)	0.047	0.190

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction.

Compared with younger males, older males displayed significantly lower muscle thickness for the anterior abdomen ($p<0.001$) and anterior upper leg ($p<0.001$) landmarks after correcting for multiple comparisons (Table 5.3). The posterior upper leg trended towards a lower muscle thickness in the old males compared with younger males ($p=0.055$); however, no differences existed for the anterior forearm, anterior upper arm, posterior lower leg, or posterior upper arm. Surprisingly, older males displayed a larger muscle thickness for the anterior lower leg ($p=0.001$) compared to the younger male cohort (Table 5.3). Figure 5.2 depicts differences in muscle thickness or lean tissue for the upper (A) and lower (B) limbs.

Table 5.3. Muscle thickness characteristics.

	Younger males (n=19)	Older males (n=19)	Unadjusted p-value	Adjusted p-value
Anterior upper arm, cm	3.59 (0.44)	3.46 (0.50)	0.391	1.00
Posterior upper arm, cm	3.45 (0.52)	3.11 (0.64)	0.120	0.481
Anterior forearm, cm	1.93 (0.33)	1.96 (0.42)	0.850	1.00
Anterior abdomen, cm	1.46 (0.33)	0.93 (0.20)	<0.001	<0.001
Anterior upper leg, cm	4.17 (0.60)	3.09 (0.61)	<0.001	<0.001
Posterior upper leg, cm	5.72 (0.56)	4.99 (0.88)	0.011	0.055
Anterior lower leg, cm	2.75 (0.29)	3.06 (0.23)	<0.001	0.001
Posterior lower leg, cm	6.40 (0.69)	6.61 (0.62)	0.411	1.00

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction.

All muscle groups, except for the posterior upper arm (p=0.377), displayed higher muscle echo intensity in the older males compared to the younger males (p<0.05, Table 5.4).

Table 5.4. Muscle echo intensity characteristics.

	Younger males (n=19)	Older males (n=19)	Unadjusted p-value	Adjusted p-value
Anterior upper arm, AU	34.9 (9.7)	53.1 (14.0)	<0.001	0.002
Posterior upper arm, AU	24.5 (9.4)	27.6 (11.6)	0.377	0.377
Anterior abdomen, AU	18.3 (16.3)	58.8 (19.5)	<0.001	<0.001
Anterior upper leg, AU	33.3 (9.8)	44.6 (13.0)	0.01	0.021
Anterior lower leg, AU	29.5 (9.5)	47.7 (12.5)	<0.001	<0.001
Posterior lower leg, AU	48.4 (12.5)	66.9 (17.5)	0.002	0.005

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction. AU, arbitrary values.

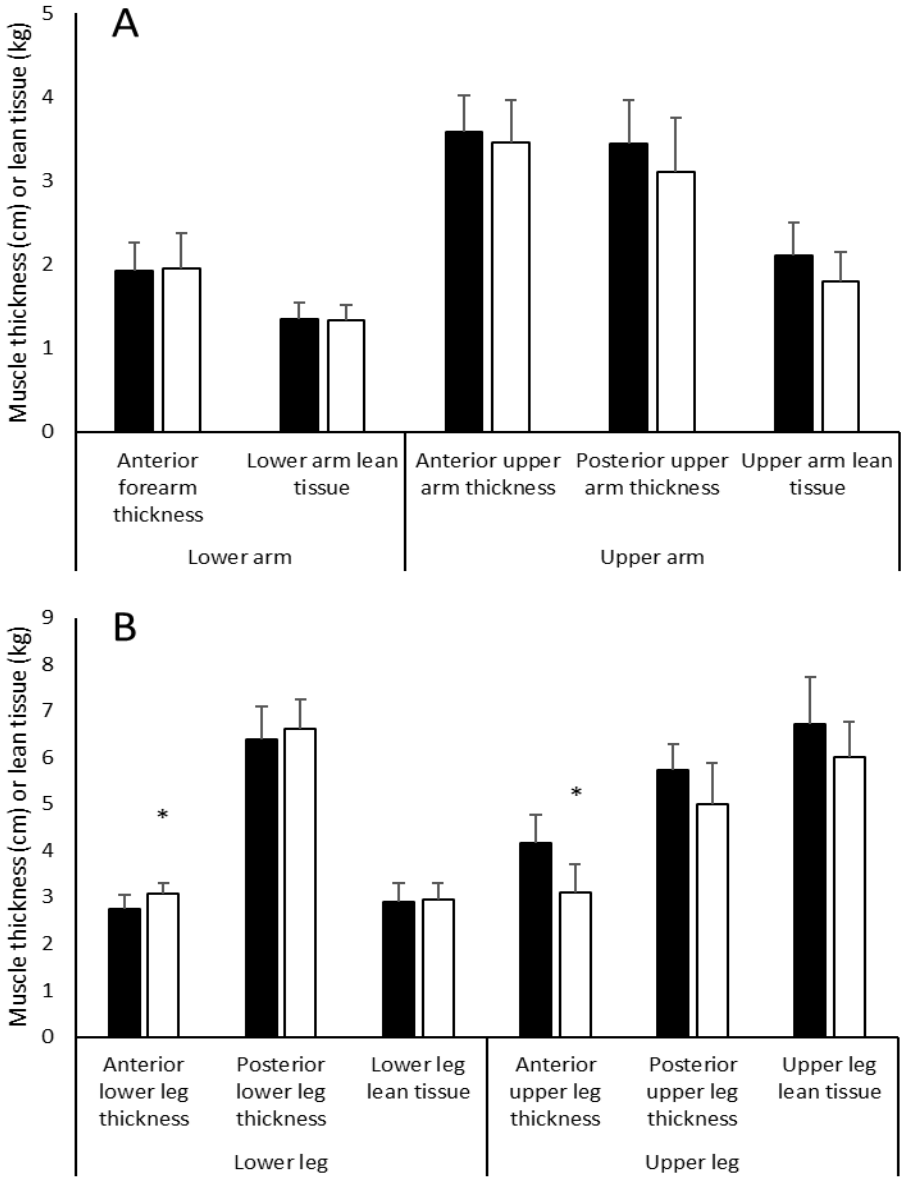


Figure 5.2. Muscle thickness and regional lean tissue differences between older and younger males for the A) upper limbs and B) lower limbs. Closed bars indicate younger males, open bars indicate older males. * denotes a significance difference from younger males, adjusted using Holm-Bonferroni correction.

5.4.1 Discussion

When matched for relative appendicular lean tissue index, we observed that differences in muscle thickness between older and younger males are not uniformly distributed across the

body. Older males presented with significantly smaller muscle thicknesses for the anterior upper leg and anterior abdomen, with a trend for the posterior upper leg; however, no differences were observed for the anterior or posterior upper arm, anterior forearm, or the posterior lower leg. Surprisingly, the anterior lower leg muscle thickness was larger in older males compared to younger males. Despite the non-uniform differences in muscle thickness, muscle echo intensity was consistently elevated in older males across all landmarks, except for the posterior upper arm, when compared with younger males.

Ageing-related skeletal muscle atrophy is typically cited to begin around the 5th decade of life and proceed at a rate of 0.5 – 1% per year; however, the extent of atrophy and age at which it begins is variable across studies [148]. These discrepancies in ageing-related muscle atrophy may be associated with several factors such as cohort differences, differences in body composition techniques, or, due to the muscle groups being used to characterize muscle loss.

5.4.2 Advancing age is associated with skeletal muscle atrophy of the anterior upper leg

Several cross-sectional comparisons have demonstrated that, with advancing age, the lower limb musculature experiences a proportionally greater degree of muscle atrophy than the upper limbs [15], [149], [162]. More specific analyses indicate that the quadriceps muscles may account for a larger proportion of ageing-related muscle atrophy than other lower limb muscles [99], [103], [150], [163]. A review by Abe et al. [24] indicates across 8 independent publications, that compared with the younger adults (n=584), older adults (n=466) consistently presented with relatively lower cross-sectional area and thickness of the quadriceps (on average, ~28% lower than young adults) compared with the hamstrings muscle groups (on average ~8% lower

than young adults). In the present study, we observed that the anterior thigh muscle thickness is significantly lower (~26%, p adjusted <0.001) in the older compared with younger adult, but only a tendency for lower hamstring thickness (~13% smaller, p adjusted = 0.055), even when relative differences in muscularity are controlled. Our work supports the idea that the hamstring muscles may be more preserved than the quadriceps muscles in terms of mass with advancing age. These findings are further substantiated in several larger cohort studies demonstrating negative correlations between anterior thigh thickness and age, but weak or lack of correlations between age and the posterior upper leg thickness [97], [152]. However, Frontera et al. [9] observed similar reductions in quadriceps (-16.1%) and hamstrings (-14.9%) cross-sectional area over a 12 year follow-up in older men (n=9, baseline age: 65.4 years). Furthermore, while there is a disconnect between muscle mass and strength with advancing age, several studies are in concordance with Frontera et al. [9], which have observed approximately similar reductions in knee extensors and flexors isometric and isokinetic torque production [92], [98], [101], [164], suggesting that muscle function of the quadriceps and hamstring muscles decrease to a similar extent with advancing age. Taken together, it is clear there is an ageing-related decline in muscle function of both the quadricep and hamstring muscle groups; however, the aetiologies of these impairments may not be similar, as the declines in specific strength (i.e. strength/mass) of the quadriceps may be more related to reductions in muscle mass [101] whereas the hamstring impairments may be more related to neuromuscular degradation [165].

5.4.3 Site-specific muscle thickness may have important implications for the identification of ageing-related muscle atrophy

In agreement with our results, others have observed no differences in muscle thicknesses of the upper limbs and lower leg [159], [160], [166], however, these lack of differences are not always observed [103], [150]. Similarly, we observed that the anterior abdominal muscles (rectus abdominus) demonstrated the greatest decline in older males compared with the younger males, which has also been observed by several other groups [103], [105], [150], [152], [167], indicating the trunk musculature may be particularly prone to ageing-related muscle atrophy. However, it should be noted that the trunk musculature was not considered when matching for appendicular lean tissue, which may add additional confounding factors when comparing muscle thicknesses between our older and younger males.

The emerging findings that ageing-related skeletal muscle atrophy is limited to specific muscle groups, such as the anterior upper leg and anterior abdomen, has important implications for identification of older adults with low skeletal muscle mass. DXA measured appendicular lean tissue mass is the most common metric of ageing-related muscle atrophy, which include all lean tissue of the limbs. However, given the relative preservation of mass for certain muscle groups, appendicular lean tissue may limit the sensitivity to detect ageing-related skeletal muscle atrophy. Even the use of the upper thigh lean tissue, which encompasses the entirety of the quadriceps and hamstrings (with additional lean tissue from other muscles and non-muscle tissue), may not be the ideal approach to assess ageing-related muscle loss, as we only observed an ~10% difference between older and younger males. Whereas the use of site-specific measures of the anterior thigh (~26%) or anterior abdominal (~36%) muscle thickness may provide more sensitive markers for measuring muscle atrophy. However, the ease of access, cost, and training required to perform these measurements

should be taken into consideration. Furthermore, there is a wealth of normative data available for DXA measures of lean tissue [65], whereas ultrasound normal values are lacking (due in large part to differences in acquisition protocols) [168].

5.4.3 Anterior upper leg and anterior abdomen muscle groups display largest ageing-related shifts in muscle echo intensity

While we and others have demonstrated that site-specific changes in muscle size do not occur uniformly across the body, less is known about changes in muscle composition with ageing. Several publications have observed increased intramuscular adipose tissue in the quadriceps and hamstring muscles with advancing age [29], [61], [153]. Increased echo intensity with advancing age has also been observed in biceps brachii [159], all of the quadriceps muscles [16], [105], [135], [169], hamstrings muscle groups [170], and several trunk muscle groups, including rectus abdominus, internal and external obliques, and transverse abdominus [16], [105], [106]. The rectus abdominus typically displays the largest ageing-related increase in echo intensity [16], [105], which we also observed (~3 fold greater in older compared with younger adults). However, given the substantial differences in body fat between the older and younger adults, the differences in subcutaneous adipose tissue, which confound the analysis of echo intensity [138], should be taken into consideration when interpreting differences in aged and/or obese individuals. While several publications, including the present one, demonstrate that certain muscles appear to be relatively “spared” from ageing-related decline in mass, it is likely that many of these muscles exhibit impairments in muscle composition or function, highlighting that caution may be necessary in discussions around the

relative importance of different muscles when assessing ageing-related degradation of the skeletal muscle system.

5.4.4 Limitations

An important limitation of the present study is the use of ultrasound for quantifying site-specific skeletal muscle thickness. Skeletal muscles are complex 3-dimensional structures, which may not be adequately represented by a single linear dimension of thickness. While the thickness of several limb muscles has been demonstrated to strongly correlate with MRI measured muscle volume [171], these reductions to linear distances could either contribute to an inability to detect differences (e.g. posterior upper leg) or perhaps even overestimate ageing-related declines (e.g. rectus abdominus). Furthermore, ultrasound muscle thickness or cross-sectional area is unable to differentiate between intramuscular adipose or connective tissue and functional contractile tissue, which would mask the true degree of muscle atrophy; whereas the use of CT or MRI, which can partially differentiate these tissues, may provide a more accurate assessment of muscle mass and composition. Lastly, while we successfully matched for appendicular lean tissue to minimize the differences in relative muscularity, the older adult cohort included here were not of low muscularity, as only one participant was below recent guidelines of <7.0 kg/m^2 for low appendicular lean tissue. Older adults with low muscularity may demonstrate differences in the specific sites that exhibit skeletal muscle atrophy or intramuscular adipose tissue deposition in comparison to those older adults with normal muscle mass [111].

5.4.5 Conclusions

In the present study, we showed that when matched for relative appendicular lean tissue index, older males display reduced muscle thickness at the anterior upper leg and anterior abdominal muscle groups compared with younger males. Whereas no differences, or even larger thicknesses, were observed for several other landmarks across the body. These results highlight the need to better understand these differences in muscle mass across the ageing lifespan to identify how best to characterize skeletal muscle mass in aged adults. We further observed that muscle echo intensity is elevated in most landmarks evaluated, suggesting that a more uniform degradation of muscle composition across the body may occur than impairments in muscle mass.

CHAPTER 6

STUDY #2: SITE-SPECIFIC SKELETAL MUSCLE ECHO INTENSITY AND THICKNESS DIFFERENCES IN SUBCUTANEOUS ADIPOSE TISSUE MATCHED OLDER AND YOUNGER ADULTS

Michael T. Paris, MSc[1]

Noah Letofsky, BSc[1]

Marina Mourtzakis, PhD[1]

[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Corresponding author: Marina Mourtzakis, PhD, Department of Kinesiology, University of Waterloo, 200 University Ave W, Waterloo, ON, N2L 3G1, Canada. Email: mmourtzakis@uwaterloo.ca. Phone: 519-888-4567, Ext 38459.

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6.1 Introduction

Ageing is associated with skeletal muscle atrophy and deterioration of muscle composition (e.g. increased intramuscular adipose tissue), which have implications for the health and disease of older adults (e.g. insulin resistance) [29], [62]. While computed tomography (CT) and magnetic resonance imaging (MRI) are considered reference standards for assessing muscle mass and composition, these modalities are expensive, expose the patient to ionizing radiation in the case of CT, and are generally not available for prospective body composition assessments [115]. Ultrasound has emerged as a portable, non-invasive, and cost-effective modality for measuring the quantity and composition of skeletal muscle [155], [168].

Ultrasound indices of muscle quantity (e.g. muscle thickness or cross-sectional area) are strongly associated with regional and whole-body skeletal muscle mass measured using MRI [128] and dual-energy x-ray absorptiometry (DXA) [51], [127]; however, ultrasound measures of skeletal muscle composition, such as echo intensity, are less well established. Echo intensity is the mean pixel intensity from a region of interest outlined within the muscle fascia of the ultrasound scan [122]. Skeletal muscle echo intensity of the quadriceps increases with age [135], [136], and has been associated with elevated adipose and connective tissue infiltration [138], [156], [157], [172]. While increased adiposity within skeletal muscle has been identified in the quadriceps of aged individuals [40], there is a lack of literature evaluating the influence of age on other muscle groups across the body; limiting our understanding of the age-associated changes in muscle composition. Furthermore, there may be sex-specific differences in the age-related degradation of muscle composition across the body, however, this has not been adequately explored.

While echo intensity provides an accessible and non-invasive surrogate of skeletal muscle composition, interpretation can be challenging. Since the ultrasound beam travels through the subcutaneous adipose tissue (SAT) before reaching the skeletal muscle layer, the sound waves will be attenuated (absorbed, scattered, or reflected); which may artificially reduce the mean muscle echo intensity, and thus may imply less adipose tissue infiltration [138], [139], [173]. However, in the evaluation of muscle echo intensity, SAT thickness is rarely accounted for when interpreting the results in obese and/or aged individuals.

The primary objective of this study was to evaluate differences in muscle echo intensity and thickness between older and younger adults by minimizing the confounding effects of SAT thickness across multiple distinct muscle groups. Our secondary objective was to evaluate the influence of age on muscle thickness across these same muscle groups. Here, we matched older and younger adults for absolute SAT thickness and examined the differences in skeletal muscle echo intensity and thickness at three landmarks representing the upper limb, lower limb, and trunk musculature.

6.2 Methods

6.2.1 Study design and participants

We performed a secondary data analysis of ultrasound images collected from 96 participants who were involved in a study validating muscle thicknesses against DXA [51]. The muscle size (thickness and DXA) and composition (echo intensity) features presented here have been previously published [51]; the primary purpose for this publication is to understand muscle composition differences between younger and older adults who are matched for SAT

thickness. Participants were ≥ 18 years of age and refrained from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to data collection. For the secondary data analysis, we matched younger and older adults for absolute SAT thickness (see below for details) and evaluated differences in thickness and echo intensity of the anterior upper arm, abdomen, and anterior upper leg muscles. This study was reviewed and cleared by a University of Waterloo Clinical Research Ethics Committee.

6.2.2. Dual-energy x-ray absorptiometry analysis

Certified Medical Radiation Technologists performed whole body DXA scans (Hologic Discovery QDR 4500, Hologic, Toronto, ON), as previously described [51]. Scans were segmented into the head, trunk, left and right upper limbs, and left and right lower limbs by a single trained investigator according to a standardized protocol [13]. Total body fat (fat mass/ body weight, %) and appendicular lean tissue index (soft lean tissue in arms and legs/height^2, kg/m^2) were calculated for all participants. Appendicular lean tissue index and body fat measures are used for descriptive purposes.

6.2.3 Ultrasound analysis

Ultrasound images of the anterior abdomen (rectus abdominis), anterior upper arm (biceps and brachialis), and anterior upper leg (rectus femoris and vastus intermedialis) were utilized to represent the trunk, upper limbs, and lower limbs for body composition analysis, respectively (Figure 6.1). Images were obtained as previously described [51]. Briefly, a B-mode ultrasound imaging (M-Turbo SonoSite, Markham, ON) device equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz) was used for capturing transverse ultrasound images.

The resolution mode was used for all images and gain, time-gain-compensation, and dynamic range (set to 0) were in the default setting. Images were taken on the right side of the body at the anterior upper arm (anterior surface, 60% distal from acromion to lateral epicondyle of the humerus), abdominal (3 cm right of the umbilicus), and anterior upper leg (anterior surface, midpoint between the greater trochanter and lateral epicondyle of the femur). Participants were supine for 20 minutes prior to imaging to mitigate potential influence of fluid shifts on muscle measures.

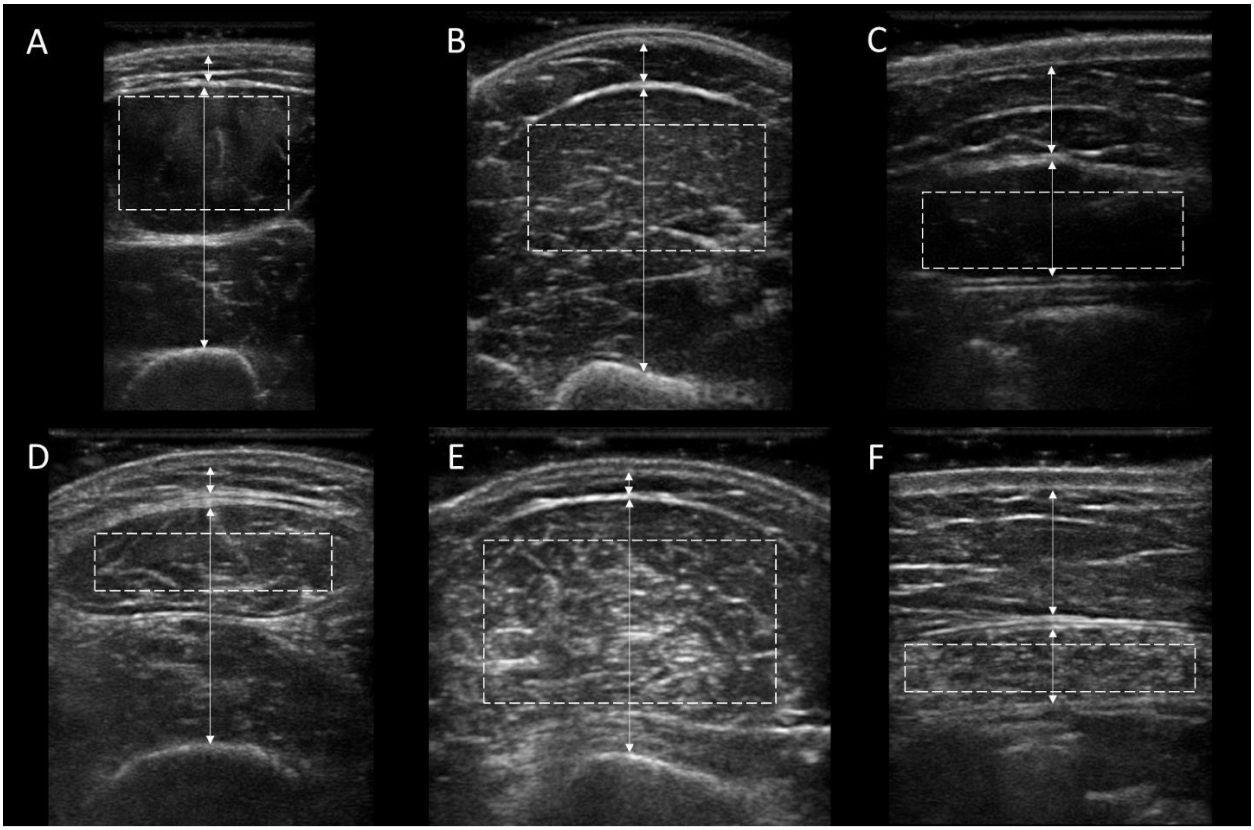

Figure 6.1. Representative images depicting muscle thickness, adipose tissue thickness, and muscle echo intensity analysis of younger (upper panel) and older (lower panel) for the A/D) anterior thigh, B/E) anterior upper arm, and C/F) abdominal landmarks. Vertical lines indicate assessment of muscle and adipose tissue thickness. Dashed boxes indicate area analyzed for muscle echo intensity.

Muscle and subcutaneous adipose tissue thickness were measured using image analysis software (ImageJ: Version 1.51, National Institutes of Health, Bethesda, MD). Muscle thickness was quantified as the linear distance between the inferior border of the superior muscle fascia and the superior border of the underlying bone (for the upper and lower limbs) or deep muscle fascia (for the abdominal landmark) (Figure 6.1). Adipose tissue thickness was quantified as the linear distance between the deep border of the skin and the superior border of the superficial muscle fascia. All thicknesses were measured twice by a single investigator (in pixels using ImageJ), averaged, and converted to a linear distance using manufacturer conversion factors.

We quantified skeletal muscle echo intensity using image analysis software (ImageJ: Version 1.51, National Institutes of Health, Bethesda, MD). Echo intensity was quantified by manually placing a rectangular region of interest within the muscle (rectus femoris, rectus abdominus, and biceps brachii), that included as much of the muscle area as possible, excluding any fascia [161]. Mean muscle echo intensity (arbitrary units (A.U.)) was measured twice on the same image (same ROI placement criteria) by a single investigator and averaged.

6.2.4 Participant matching

Participants were first stratified by sex and age (young: <45 years of age and old: ≥60 years of age), and then matched for SAT thickness (older and younger SAT thickness was within 0.5 cm). SAT thickness may confound measures of skeletal muscle echo intensity due to beam attenuation in deeper tissues. For example, increased SAT thickness may result in reduced echo intensity due to beam scattering and reflection. By matching participants for SAT thickness at each landmark (anterior upper arm, anterior upper leg, and abdominal region), the confounding

effects of the adipose tissue layer on muscle echo intensity would be normalized between younger and older groups. Matching occurred independently at each landmark; in other words, each cohort of participants for a given landmark may or may not contain the same participants. Participants who were not matched for absolute SAT thickness at given landmark were excluded from muscle thickness and echo intensity analysis.

6.2.5 Statistical analyses

Normality of continuous variables was confirmed using quantile-quantile plots. A two-way analysis of variance (ANOVA) was used to evaluate the effects of sex, age group (younger and older groups), and sex by age group interactions on demographic, physical, and body composition metrics. To adjust for multiple two-way ANOVA comparisons (age group, sex, sex by age group interaction) on ultrasound features of muscle (thickness and echo intensity), we applied a Hold-Bonferroni correction to maintain a familywise error rate of $\alpha=0.05$. Bland-Altman plots were used to evaluate the degree of SAT thickness matching between younger and older participants for each landmark. All statistics were performed using SPSS (version 24, IBM, USA). Statistical significance was set as $p<0.05$.

6.3 Results

At the anterior upper arm, abdominal, and anterior upper leg landmarks, 58 (24 males and 34 females), 52 (22 males and 30 females), and 60 (30 males and 30 females) younger and older participants were matched for SAT thickness, respectively (Table 6.1). The anterior upper arm and abdomen had 69% overlap of participants, the anterior upper arm and anterior upper leg had 67% overlap of participants, and the abdomen and anterior upper leg had 55% overlap

of participants. SAT thickness was successfully matched between younger and older males, as no differences were observed for the anterior upper arm (younger: 0.35 ±0.23 cm, older: 0.34 ±0.20 cm, p=0.867), abdomen (younger: 1.96 ±0.90 cm, older: 2.06 ±0.86 cm, p=0.740), and anterior upper leg (younger: 0.67 ±0.40 cm, older: 0.71 ±0.39 cm, p=0.851) landmarks (Table 6.1). Similarly, no differences in SAT thickness were observed between younger and older females for the anterior upper arm (younger: 0.63 ±0.30 cm, older: 0.65 ±0.30 cm, p=0.882), abdomen (younger: 2.61 ±0.90 cm, older: 2.62 ±0.85 cm, p=0.975), and anterior upper leg (younger: 1.27 ±0.63 cm, older: 1.27 ±0.60 cm, p=0.997) (Table 6.1). Bland-Altman plots for the anterior upper arm, anterior upper leg, and abdominal landmarks depict the degree SAT thickness matching between older and young adults at the individual and group level (Figure 6.2).

Table 6.1. Demographic and physical characteristics

	Males		Females			
	Younger (<45 y)	Older (≥60 y)	Younger (<45 y)	Older (≥60 y)	p-value age	p-value sex
Anterior upper arm, n	12	12	17	17	-	-
Age, years	27.4 (5.0)	71.6 (4.3)	28.8 (8.4)	71.8 (6.1)	<0.01	0.56
Height, m	1.74 (0.05)	1.78 (0.06)	1.68 (0.07)	1.60 (0.05)	0.04	<0.01
Weight, kg	80.0 (13.0)	87.3 (6.2)	68.7 (13.8)	67.7 (9.8)	0.10	0.01
BMI, kg/m^2	26.1 (3.1)	27.3 (2.1)	24.5 (5.3)	26.5 (3.6)	0.45	0.26
Body fat, %	22.9 (3.8)	29.3 (4.1)	34.0 (5.4)	40.8 (4.8)	<0.01	<0.01
ALTI, kg/m^2	8.75 (1.18)	7.57 (0.63)	6.28 (1.03)	5.69 (0.66)	<0.01	<0.01
SAT thickness, cm	0.35 (0.23)	0.34 (0.20)	0.63 (0.30)	0.65 (0.30)	0.89	<0.01

Anterior upper leg, n	11	11	15	15	-	-
Age, years	29.0 (6.8)	72.8 (5.5)	29.0 (6.8)	72.8 (5.5)	<0.01	0.85
Height, m	1.74 (0.07)	1.78 (0.07)	1.65 (0.08)	1.60 (0.05)	0.21	<0.01
Weight, kg	75.6 (10.2)	83 (10.2)	68.1 (15.6)	65.8 (10.2)	0.15	0.12
BMI, kg/m^2	24.9 (2.2)	26.3 (3.0)	24.9 (2.2)	26.3 (3.0)	0.41	0.91
Body fat, %	22.4 (4.1)	28.0 (5.5)	22.4 (4.1)	28.0 (5.5)	0.02	<0.01
ALTI, kg/m^2	8.16 (0.82)	7.36 (0.65)	6.54 (1.07)	5.71 (0.59)	0.03	<0.01
SAT thickness, cm	0.67 (0.40)	0.71 (0.39)	1.27 (0.63)	1.27 (0.60)	0.88	<0.01
Abdomen, n	15	15	15	15	-	-
Age, years	27.6 (6.5)	74.7 (6.3)	29.5 (8.7)	71.9 (5.9)	<0.01	0.46
Height, m	1.77 (0.06)	1.78 (0.06)	1.67 (0.06)	1.60 (0.04)	0.43	<0.01
Weight, kg	81.0 (10.3)	84.1 (12.2)	69.9 (13.7)	63.4 (7.2)	0.45	<0.01
BMI, kg/m^2	25.9 (3.0)	26.3 (2.8)	25.1 (5.4)	24.9 (2.8)	0.78	0.56
Body fat, %	21.7 (4.7)	28.4 (4.7)	34.1 (6.0)	39.2 (5.1)	<0.01	<0.01
ALTI, kg/m^2	8.83 (1.14)	7.32 (0.80)	6.47 (1.09)	5.42 (0.51)	<0.01	<0.01
SAT thickness, cm	1.96 (0.90)	2.06 (0.86)	2.61 (0.90)	2.62 (0.85)	0.73	0.05

ALTI, appendicular lean tissue index; BMI, body mass index; SAT, subcutaneous adipose tissue.

Following SAT thickness matching, significant differences in age and body fat percent, but not BMI, were observed between younger and older adults across each landmark (Table 6.1). Across each landmark, older adults exhibited lower appendicular lean tissue index ($p<0.05$) compared with younger adults (Table 6.1). Importantly for evaluation of potential sex by age group interactions on muscle features, age was similar between males and females ($p>0.05$) for each landmark evaluated (Table 6.1).

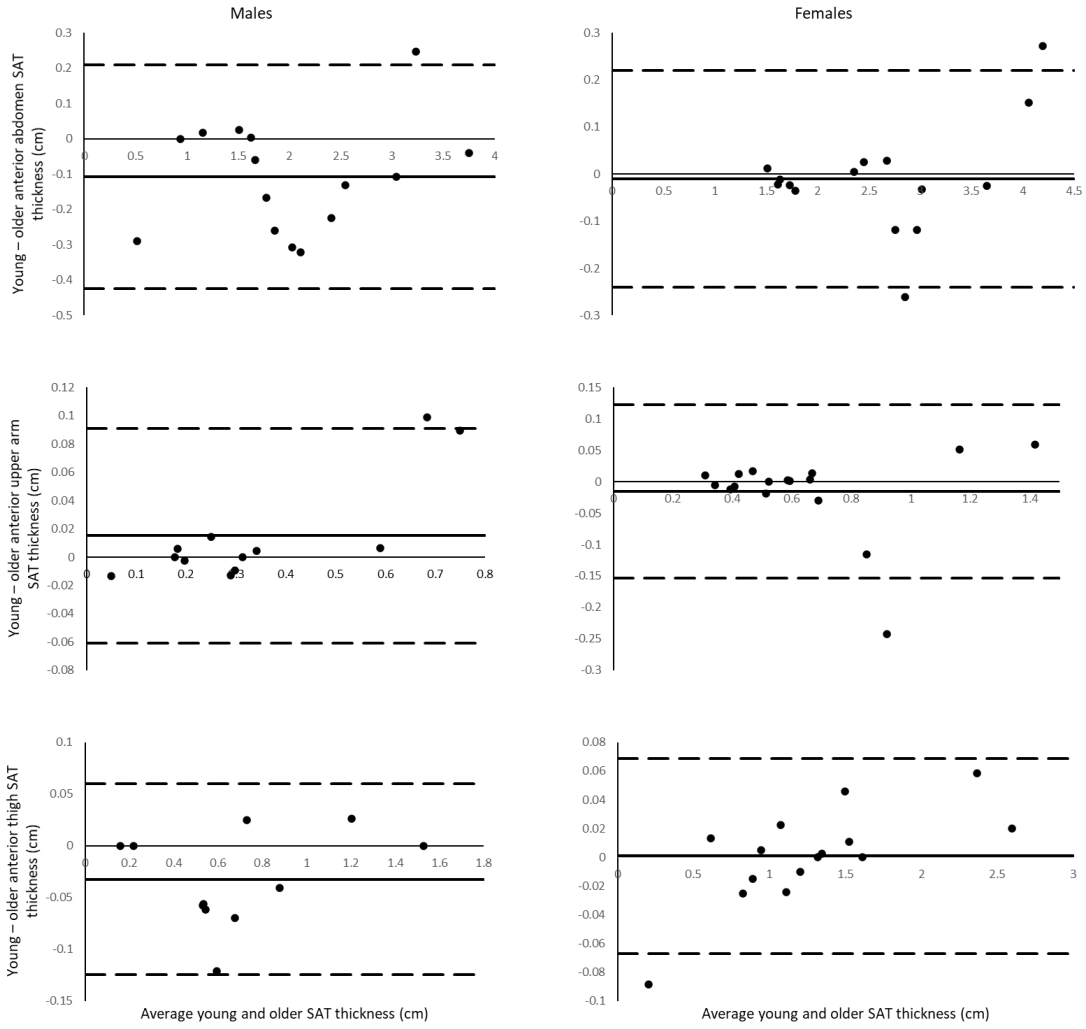

Figure 6.2. Bland-Altman plots comparing subcutaneous adipose tissue thickness between older and younger adults for the anterior upper arm, abdominal, and anterior upper leg landmarks. Solid black line indicates average difference between older and younger adults. Dashed black lines indicate upper and lower limits of agreement (mean ± 2*standard deviation of the differences).

Muscle echo intensity at the abdomen was higher for older males and females ($p<0.01$) compared with younger males (younger: 18.7 ±15.2 A.U., older: 60.9 ±23.4 A.U.) and females (younger: 38.3 ±27.7 A.U., older: 73.4 ±31.0 A.U.) (Figure 6.3). Similarly, muscle echo intensity at the anterior upper leg was higher for older males and females ($p<0.01$) compared with younger males (younger: 36.0 ±8.03 A.U., older: 54.3 ±9.79 A.U.) and females (younger: 40.3 ±6.75 A.U., older: 52.4 ±7.60 A.U.) (Figure 6.3). However, muscle echo intensity at the anterior upper arm was not different between older males and females ($p=0.18$) compared with younger males (younger: 43.4 ±8.92 A.U., older: 48.9 ±10.1 A.U.) and females (younger: 47.0 ±6.55 A.U., older: 53.2 ±13.1 A.U.) (Figure 6.3).

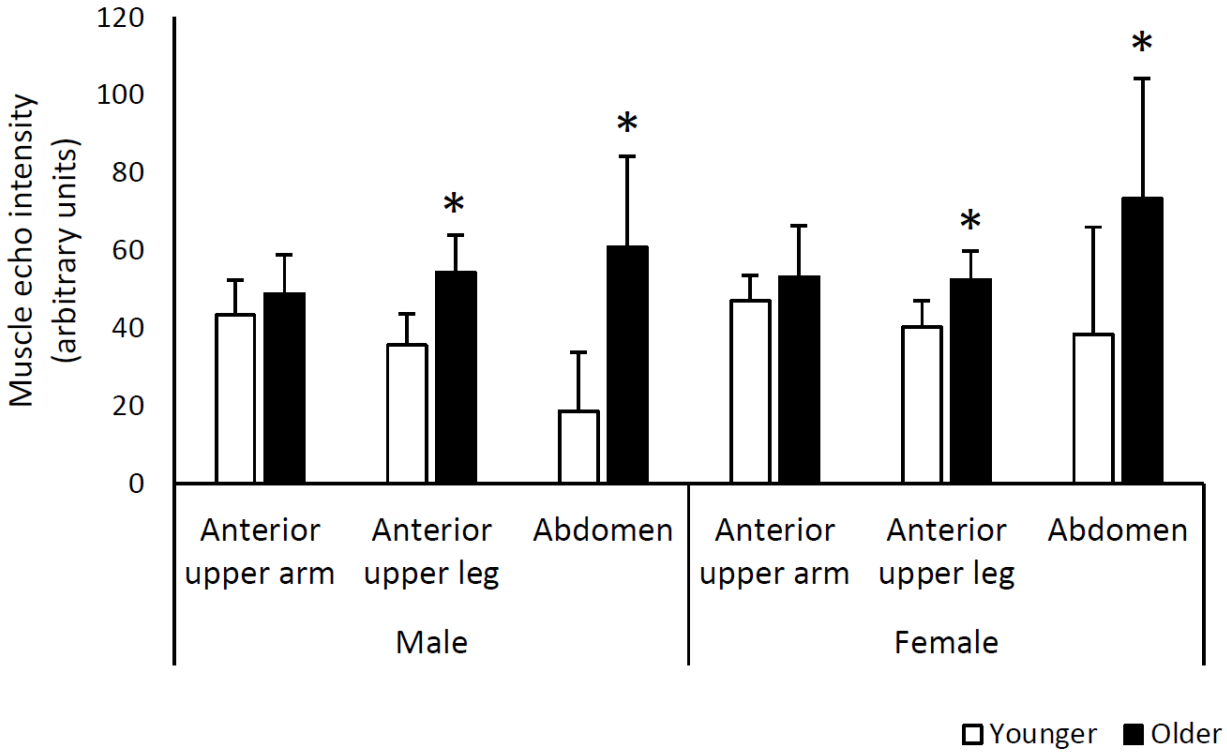

Figure 6.3. Muscle echo intensity of older adults and younger adults. Data and error bars are presented as mean and standard deviation. *main effects compared with younger adults. #main effects compared with females.

Older males and females had significantly smaller muscle thicknesses ($p<0.01$) compared with their corresponding younger male and female counterparts at the abdomen (younger males: 1.50 ±0.29 cm, older males: 0.84 ±0.24 cm; younger females: 1.08 ±0.24 cm, older females: 0.77 ±0.16 cm) and anterior upper leg (younger males: 4.31 ±0.65 cm, older males: 3.03 ±0.45 cm; younger females: 3.49 ±0.68 cm, older females: 2.44 ±0.49 cm) (Figure 6.4). However, mean muscle thickness of the anterior upper arm was not different between older males and females ($p=0.13$) compared to younger males (younger: 3.72 ±0.54 cm, older: 3.44 ±0.44 cm) and females (younger: 2.52 ±0.48 cm, older: 2.38 ±0.33 cm) (Figure 6.4).

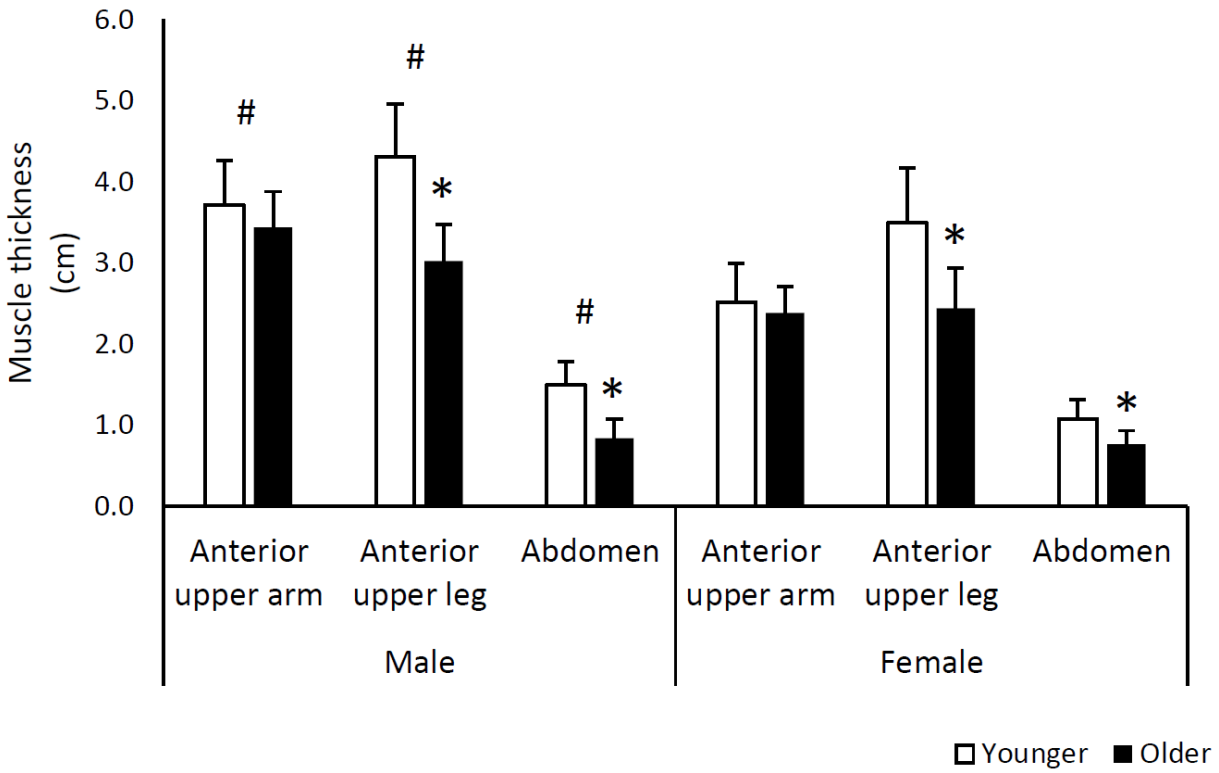

Figure 6.4. Muscle thickness of older adults and younger adults. Data and error bars are presented as mean and standard deviation. *main effects compared with younger adults. #main effects compared with females.

6.4.1 Discussion

We observed that older adults, when compared with younger adults, exhibited elevated skeletal muscle echo intensity of the anterior upper leg and abdomen, but not the anterior upper arm muscle groups. Similarly, the anterior upper leg and abdominal muscle thickness, but not the anterior upper arm, were significantly smaller in the older adult cohort compared with younger adults. Interestingly, these results were consistently observed between males and females, as no there were no age by sex interactions when comparing muscle echo intensity or thickness for any evaluated landmark. These findings suggest that age-associated degradation of skeletal muscle quantity and composition may not be uniformly distributed across the body.

Ageing is associated with increased adipose tissue infiltration of the thigh musculature [40], [156], [174]; which occurs regardless of changes in body weight [39]. This age-associated deterioration of thigh muscle composition has implications for the development of insulin resistance and impaired functional capacity of older adults [175]. However, muscle composition shifts of other muscle groups due to advancing age is less commonly evaluated. Yoshiko et al. (2017) observed that the hamstrings muscle groups of older adults displayed a significantly higher intramuscular adipose tissue cross-sectional area compared to young adults, however, no differences were present in the quadriceps intramuscular adipose tissue between young and old adults. The lower trunk musculature (level of the 3rd lumbar vertebrae) exhibited a 67% reduction in CT muscle attenuation from 40 to 90 years of age [177]. This is important, given that CT attenuation of abdominal muscles is associated with metabolic impairments [178]. Fukumoto et al. (2015) observed increased skeletal muscle echo intensity across the biceps, quadriceps, rectus abdominis, internal and external obliques, and the transverse abdominis in middle-aged (50-64 y), young-old (65-74 y), and old-old (≥75 y) females, compared to younger (19-30 y) females. While all these muscle groups demonstrated elevated echo intensity in older adults, the trunk muscle groups had the largest discrepancies (2-fold higher) in the old-old compared to the younger cohort [105]; suggesting that the trunk muscles may display the largest shifts in composition with age. However, the evaluation of muscle echo intensity across different age groups may be influenced by differences in SAT thicknesses between younger and older adults.

The effects of SAT thickness on muscle echo intensity are rarely accounted for. Young et al. (2015) demonstrated that the associations between muscle echo intensity and MRI

measured intramuscular adipose tissue is confounded by the SAT layer thickness. By matching our older and younger adults for absolute SAT thickness at each landmark, the confounding effect of the SAT layer on muscle echo intensity is normalized between the younger and older adult cohorts. Similar to Fukumoto et al. (2015), we observed significantly higher echo intensity in the anterior upper leg (~1.5 fold) and abdominal muscles (2-3 fold higher) of the older adult cohort compared with the younger adults across both sexes; however, we did not observe an age-associated difference in the echo intensity of the anterior upper arm muscles.

Ageing is also accompanied by a deterioration of skeletal muscle quantity. Skeletal muscle atrophy is typically cited to occur at a rate of 0.5 – 1.0 % per year beginning in the 5th decade of life [36]. However, Janssen et al. (2000) used MRI to evaluate muscle volume across different age ranges and observed that the lower limb musculature exhibits a larger relative reduction compared with the upper limbs with increasing age, indicating that ageing may be associated with regional skeletal muscle loss. Muscle group specific analyses have demonstrated that even within the trunk or an appendage, certain muscle groups may contribute more to age-related muscle atrophy [92], [96], [97], [106]. Abe et al. (2014) used ultrasound to compare 9 distinct muscle groups in older adults (70-79 years of age, n=139) with those of a younger adult cohort (20-29 years of age, n=227). Compared to the younger cohort, the older adult cohort had lower muscle thickness in the anterior abdominal region (rectus abdominis, ~70% of younger cohort) and anterior upper leg (rectus femoris and vastus intermedialis, ~70% of younger cohort); however, there were only marginal differences in the anterior upper arm (bicep brachii and brachialis, ~98% of younger cohort) [103]. Our results (averaged across males and females) align well with the degree of muscle atrophy observed in

the anterior upper leg (~70%), abdomen (~60%), and anterior upper arm (~92%) in our older cohort compared to our younger cohort.

Taken together, these results indicate that age-associated muscle impairments of the anterior abdomen and anterior thigh may occur to a greater extent than the anterior upper arm. These observed differences in muscle composition and thickness across the body agree with observations that muscle strength also appears to decrease earlier in the lower body compared to upper body [179]. While the reasons for these differences in age-related muscle impairments between the upper and lower body are not entirely understood, they may in part be related to reductions in physical activity. Reductions in physical activity would presumably impact the lower limb musculature to a greater extent compared with the upper limbs, due to their involvement in common movement activities (e.g. climbing stairs). Future work accounting for differences in physical activity are needed to better differentiate the influence of age and activity (or lack thereof). While these differences may exist, it will be critical to examine if these differences have implications for metabolic health or functional capacity of older adults. For example, Ido et al. (2015) observed that abdominal muscle thickness is a stronger indicator of metabolic syndrome in obese adults compared to DXA appendicular lean tissue index; suggesting that site-specific measures of muscle size may be advantageous over traditional measures of lean tissue (DXA) for metabolic impairments. Given the ~2-3 fold higher echo intensity and ~0.6-fold lower thickness, the anterior abdominal muscle group may be a critical site to identify older adults with metabolic and functional impairments. However, these impairments will need to be evaluated against measures of muscle thickness and echo intensity at other common landmarks (i.e. quadriceps musculature), to determine if specific sites are

more important than others for metabolic or functional impairments. Furthermore, the lack of observed sex by age interactions on muscle echo intensity or thickness need to be further explored, as a recent analysis of aggregated data demonstrated that age-related decline in dynamic muscle function of the knee extensors occurs earlier and to a greater extent in females, compared with males [180].

There are several limitations within this secondary data analysis. The matching of absolute SAT thicknesses between older and younger adults at the three distinct landmarks resulted in overlapping participants across the groups, preventing statistical comparisons of muscle thickness and composition differences across each landmark. Furthermore, by matching participants on absolute SAT thickness, older adults on the upper spectrum of SAT thickness and younger adults on the lower end will have been excluded, due to fewer matches occurring in these opposite extremes. This may be particularly important for the abdominal landmark, as there will likely be differences between younger and older adults for both the deep and superficial SAT compartments [32]; which may confound echo intensity analysis of the rectus abdominus muscle. Lastly, the upper limb, trunk, and lower limbs are being represented by a single muscle group; however, emerging evidence is demonstrating that even within an appendage, age-associated differences exist in specific muscle groups (i.e. quadriceps display more muscle atrophy than the hamstrings) [103].

6.4.2 Conclusions

In conclusion, we observed age-associated differences in skeletal muscle thickness and echo intensity of the abdomen and anterior upper leg, but not the anterior upper arm of older adults compared to younger adults matched for SAT thickness. These non-uniform

deteriorations across the upper limb, trunk, and lower muscles with age highlight the importance of quantifying these muscle groups separately when evaluating age-associated changes in body composition and their implications on strength, function, and metabolism. Future work accounting for potential differences in physical activity between age groups are needed to better understand why these site-specific differences in muscles tissue may be occurring with advancing age.

CHAPTER 7

STUDY #3: ASSOCIATION OF STATIC AND DYNAMIC STRENGTH AND FUNCTION WITH MUSCLE MASS AND COMPOSITION IN OLDER MALES: A COMPARISON OF MUSCLE THICKNESS, ECHO INTENSITY, AND LEAN TISSUE MASS

Michael T. Paris[1]

Egor Avrutin[1]

Kirsten E. Bell[1]

Marina Mourtzakis[1]

[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Corresponding author: Marina Mourtzakis, PhD, Department of Kinesiology, University of Waterloo, 200 University Ave W, Waterloo, ON, N2L 3G1, Canada. Email: mmourtzakis@uwaterloo.ca. Phone: 519-888-4567, Ext 48459.

7.1 Introduction

Sarcopenia is a progressive ageing-related condition that is characterized by a loss of skeletal muscle strength and mass, as well as deleterious shifts in muscle composition towards increased intramuscular adipose tissue [10], [181]. Although the precise definition of sarcopenia is disputed [182], several recent working groups, including the European Working Group on Sarcopenia [10], [11], recommend appendicular lean tissue mass as the primary metric to characterize losses in skeletal muscle mass with advancing age and for identifying older adults with low muscle mass. While appendicular lean tissue provides an accurate representation of the muscle mass contained within the upper and lower limbs [14], we, and others have demonstrated that ageing-related loss of skeletal muscle mass does not occur uniformly across the body [16], [160], [166]. Indeed, advancing age is associated with a greater degree of skeletal muscle atrophy at the rectus abdominis and quadricep muscles in comparison to other muscle groups [16], [101], [103], [152]. Focusing the assessment of ageing-related impairments in muscle mass and function to those muscle groups that present with earlier declines, may provide more sensitive biomarkers to identify older adults with sarcopenia. However, these site-specific or regional measures of muscle mass (and composition) have not been compared to traditional ageing-related metrics of muscle (e.g. appendicular lean tissue) in relation to strength and physical function. Thus, addressing these gaps is important to advancing our understanding of the role of atrophy in these specific muscle groups in the context of physical function and, consequently, assessment of sarcopenia.

Ultrasound has emerged as a portable, non-invasive, and cost-effective tool to quantify site-specific muscle mass and composition [168], [183]. Ultrasound-measured muscle thickness

is strongly associated with volume and whole-body indices of muscle mass measured using magnetic resonance imaging [128] and dual-energy x-ray absorptiometry (DXA) [51], [127], [184]. Muscle echo intensity, a surrogate measure of muscle composition, is associated with increased intramuscular adipose and connective tissue infiltration [138], [156], [185] and increases with advancing age for several muscle groups across the body [16], [61], [105], [135]. Therefore, ultrasound represents a promising tool to assess site-specific muscle mass and composition in the context of sarcopenia progression.

The primary objective of this study was to compare the magnitude of associations between either muscle mass or composition indices derived using DXA and ultrasound with skeletal muscle function in older males. Skeletal muscle function was evaluated through analysis of muscle strength and functional capacity (e.g. 30-second sit to stand). DXA derived appendicular and regional lean tissue and ultrasound measured muscle thickness and echo intensity were compared. As a secondary objective, we evaluated muscle thickness, echo intensity, and muscle function differences between older males with normal versus low appendicular lean tissue mass.

7.2 Methods

7.2.1 Study design and participants

We conducted a prospective cross-sectional study, which evaluated 32 community dwelling older (≥ 65 years of age) males from the Kitchener-Waterloo community. All participants attended 3-4 data collection sessions over of the course of 7-14 days. Body composition (DXA, ultrasound) was assessed on a single day after an overnight fast (minimum

10 hours). Functional performance measurements were performed on a single day, which included a six-minute walk and 30-second sit to stand test. All participants performed 1 familiarization session for maximal isometric torque and isokinetic power assessments prior to the testing session. Familiarization and testing sessions were performed between 2 and 7 days apart. Participants were excluded if they: 1) had a previous history of neuromuscular disorders, 2) had undergone administration of oral or intra-venous contrast for nuclear medicine scans within the past 3 weeks, 3) had a prosthetic joint replacement, 4) had a history of cancer or cerebrovascular disease, or 5) participated in structed exercise sessions within the past 6 months. Participants were instructed to refrain from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to all laboratory visits. All studies were approved by a human research ethics committee at the University of Waterloo. Written informed consent was obtained from all participants in accordance with established protocols for human research.

7.2.2 Anthropometry

Height and weight were obtained in a cloth hospital gown using a stadiometer (to the nearest 0.5 cm) and balance beam scale (to the nearest 0.1 kg), respectively. Waist circumference was measured to the nearest 0.1 cm by placing the inferior border of a flexible tape measure at the top of the iliac crest. For waist circumference, two measurements were taken and averaged.

7.2.3 Ultrasound Landmarking

Landmarking, in preparation for ultrasound measurements, was performed on participants in supine or prone on a padded massage table, with their feet secured in neutral rotation using a foot strap to prevent internal or external hip rotation. A flexible tape measure and pen were used to mark the sites for ultrasound imaging. Sites for ultrasound imaging included the anterior and posterior upper arm, anterior forearm, anterior abdomen, anterior and poster upper leg, and anterior and posterior lower leg. The upper arm was imaged on the anterior and posterior surface, 60% distal from the acromial process to the lateral epicondyle of the humerus. Anterior forearm images were captured on the anterior surface, 30% distal from the radial head to the styloid process of the radius. Anterior trunk images were taken 3 cm right of the umbilicus. Anterior thigh images were taken two-thirds the distance from the anterior superior iliac spine to the superior pole of the patella. The posterior upper leg image was taken on the posterior surface, 50% between the greater trochanter and lateral epicondyle of the femur. The anterior and posterior lower leg images were taken on the anterior and posterior surface, 30% distal from the head of the fibula to the lateral malleolus. Limb lengths were measured to the nearest 0.5 cm and sites for image acquisition were marked to the nearest 0.1 cm. During landmarking, participants remained supine or prone for 20 minutes to mitigate shifts in fluid distribution [186].

7.2.4 Ultrasound image acquisition

All images were taken on the right side of the body. Images were taken in the transverse plane using a real time B-mode ultrasound imaging device (M-turbo, Sonosite, Markham, ON), which was equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz). Imaging mode was set to “resolution” and adjustable parameters gain, time-gain-compensation, and

dynamic range (50%) were held constant throughout acquisition. To minimize tissue compression, the ultrasound transducer was generously coated with water-soluble transmission gel. Minimal compression was defined by: 1) ensuring the natural curvature of the muscle, adipose tissue, and skin was maintained, and 2) a visible layer of ultrasound gel is maintained between the probe and skin, as previously described [51]. To ensure the landmark aligned with the middle of the muscle bulk, medial-lateral movement was allowed for all landmarks to centre the muscle within the field of view. Imaging depth was adjusted to the minimum depth required to visualize the muscle being analyzed. All landmarks were imaged twice. Images were saved using the Digital Imaging and Communications in Medicine (DICOM) format and transferred to a personal computer for analysis.

7.2.5 Muscle thickness and echo intensity

Muscle thickness was analyzed for each landmark by measuring the vertical distance between either the upper margin of the underlying bone or the inferior muscle fascia (anterior abdomen and anterior lower leg) and the superior muscle fascia (lower boundary). Muscle thickness was measured once per image (two images per landmark) by a single trained investigator and averaged for each landmark.

Muscle echo intensity was evaluated by selecting the largest rectangular area within the muscle fascia borders, as previously described [161]. Specific muscles analyzed were the rectus abdominis (anterior abdomen), tibialis anterior (anterior lower leg), biceps brachii (anterior upper arm), rectus femoris (anterior upper leg), lateral gastrocnemius (posterior lower leg), and triceps brachii (posterior upper arm). Echo intensity is expressed as an arbitrary value (AU)

between the values of 0 (black) and 255 (white). All ultrasound equipment settings were maintained as described above. Muscle thickness and echo intensity measurements were performed using ImageJ (NIH, Bethesda, MD, version 1.53e).

7.2.6 Dual-energy x-ray absorptiometry

Certified medical radiation technologists performed whole body DXA scans (Hologic discovery QDR4500, Hologic, Toronto, ON) for each participant. Prior to each scan, quality control and calibration procedures were performed using manufacturer specifications. Participants lay supine on the scanning bed, with their legs fully extended and toes held internally rotated using masking tape, limiting movement during scanning. Hologic software (version 13.2) was used to segment the body into the head, torso, left and right arms, and left and right legs, as previously described [13]. Participants required two scans if they did not fit within the lateral limits of the scanning table. Participants with two scans were analyzed by excluding the missing limb(s) and averaging all other segments across the two scans.

Appendicular lean tissue index was calculated by dividing the lean soft tissue in the arms and legs (kg) by the participants height (m) squared (kg/m^2). Participants were classified as low appendicular lean tissue (<7.0 kg/m^2) according to recent guidelines from the European Working Group on Sarcopenia [10]. Region specific lean tissue analysis was evaluated for the upper arm, lower arm, upper leg, and lower leg. Using the Hologic software, custom region of interest boxes were placed around: 1) the lower arm, horizontally across the medical epicondyle of the humerous, and 2) the lower leg, horizontally across the tibial plateau. Upper

arm and upper leg lean tissue was calculated by subtracting the lower arm or leg lean tissue from the total arm or leg lean tissue.

7.2.7 Isometric torque and isokinetic power

Isometric torque and isokinetic power were assessed on the right side of the body using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York). Isometric torque of the elbow extensors and flexors and knee extensors and flexors was evaluated. Isokinetic power of the knee extensors was also evaluated. Participants were seated against the backrest with a hip angle of 85° and securing straps placed tightly across the participants waist and chest. Participants were provided visual feedback on their torque in real time on a screen at a 1 m distance. Verbal encouragement was provided throughout testing.

For elbow flexion and extension, an upper limb support was positioned approximately 15° laterally from the sagittal plane and a securing strap was placed across the upper arm to limit movement to the elbow axis of rotation. The height of the upper limb support was adjusted to maintain a 30° angle between the shoulder and trunk and the Biodex dynamometer rotation knob was aligned with the lateral epicondyle of the humerus. The dynamometer was rotated 15° to align with the participant's upper arm orientation. The dynamometer arm was positioned parallel with the participants humerus and set to 0° (full extension). Maximal voluntary isometric torque of the elbow extensors and flexors was performed at 90° of flexion (confirmed using a goniometer). Alternating between flexion and extension, 6 contractions were performed with 30 seconds of rest between trials (~1 minute of rest between successive

extension or flexion trials). Each trial lasted 5 seconds, during which the participant was instructed to extend or flex their elbow as hard as possible.

For knee extension and flexion, a securing strap was placed around the upper thigh to limit movement to the knee axis of rotation. Chair height and position were adjusted to align the knee epicondyle with the Biodex dynamometer rotation knob. The knee dynamometer attachment was positioned and secured 5 cm above the calcaneus. The knee was positioned into 90° of flexion (confirmed using a goniometer) and the 0 position was set. Torque measures were corrected for the influence of gravity (corrected at 30° of flexion). Maximal voluntary isometric torque of the knee extensors and flexors was performed at 60° of flexion. Alternating between flexion and extension, 6 contractions were performed with 30 seconds of rest between trials (~1 minute of rest between successive extension or flexion trials). Each trial lasted 5 seconds, during which the participant was instructed to extend or flex their knee as hard as possible. Maximal voluntary isokinetic power for the knee extensors was evaluated for 3 contractions at 1.05 rad/s (60°/s) and 3 contractions at 3.14 rad/s (180°/s), with one minute of rest between trials. For isokinetic power, participants began with their knee at 90° and extended as rapidly and forcefully as possible until 30° deg of flexion.

All trials were sampled at 100 Hz and processed offline using custom Python (Python Software Foundation, Beaverton, Oregon, version 3.7) scripts. For all trials, maximal voluntary torque (isometric) or peak power (isokinetic) was extracted. The highest value for maximal torque or peak power across all trials was used for further analysis.

7.2.8 Handgrip strength

Grip strength of the right hand was assessed using a Jamar hand-held dynamometer according to a standardized protocol [142]. Briefly, participants were seated upright in a chair with their right arm rested on the armrest, with their elbow in 90° of flexion. Participants squeezed with as much force as possible for 3 seconds. Three trials were performed with 1 minute of rest between contractions. Maximal force across the 3 trials was taken as the peak force produced.

7.2.8 Functional performance

The six-minute walk test was performed using a 20 m path in a hallway corridor. Participants were instructed to walk as far as possible within 6 minutes, but without running or jogging, according to a standardized protocol [144]. Participants walked around two cones spaced 20 m apart. A 30 second sit to stand was evaluated to test lower body endurance. With the arms placed across the chest to prevent assistance during standing, participants were instructed to fully stand up and sit down as many times as possible within 30 seconds. The chair's seated surface was approximately 46 cm from the ground.

7.2.9 Statistical analysis

Normality of continuous variables was confirmed using Shapiro-Wilk test. Differences between older adults presenting with low or normal appendicular lean tissue were evaluated using independent sample t-tests. Pearson correlation coefficients were used to evaluate associations between muscle metrics (muscle thickness, echo intensity, or lean tissue) and isometric torque, isokinetic power, or functional performance. The magnitude of correlation coefficients between muscle thickness or regional lean tissue mass and isometric torque or

isokinetic power, was evaluated using the Pearson and Filson's z evaluation in the cocor software package [187]. Multiple linear regression was used to evaluate the combined effects of muscle thickness and echo intensity on associations with isometric torque or isokinetic power.

Statistical significance was set as $p<0.05$. To correct for multiple comparisons between older adults with low or normal appendicular lean tissue index, a Holm-Bonferroni correction was used to maintain a family wise error rate of 0.05 for torque and power (n=9), muscle thickness (n=8), and echo intensity (n=6), independently. Similarly, to correct for multiple correlation comparisons, a Holm-Bonferroni correction was used to maintain a family wise error rate of 0.05 for isokinetic torque or power (n=26) and physical function (n=16). All statistical analyses were performed using SPSS (version 26, IBM, USA), unless previously noted.

7.3 Results

Males with low appendicular lean tissue (n=12) were older ($p<0.001$) and had a lower BMI ($p=0.004$) compared with those with normal appendicular lean tissue (n=20); however, there were no differences for height ($p=0.789$), waist circumference ($p=0.079$), or percent body fat ($p=0.704$) (Table 7.1). Appendicular and all region-specific lean tissue mass were significantly lower ($p<0.05$) in the low compared with normal appendicular lean tissue group (Table 7.1).

Table 7.1. Participant characteristics

	All (n=32)	Low appendicular lean tissue (n=12)	Normal appendicular lean tissue (n=20)	Unadjusted p-value
Age, y	75.4 (7.9)	81.9 (6.6)	71.5 (5.8)	<0.001
Height, m	1.73 (0.08)	1.74 (0.07)	1.73 (0.08)	0.789
Weight, kg	80.0 (13.3)	72.9 (8.2)	84.2 (14.1)	0.017
BMI, kg/m^2	26.5 (4.0)	24.1 (1.8)	28.1 (4.3)	0.004
Waist circumference, cm	99.3 (9.5)	95.5 (6.2)	101.5 (10.5)	0.079
Appendicular lean tissue index, kg/m^2	7.28 (0.87)	6.48 (0.48)	7.76 (0.68)	<0.001
Upper arm lean tissue, kg	1.65 (0.36)	1.36 (0.22)	1.76 (0.35)	0.001
Lower arm lean tissue, kg	1.26 (0.19)	1.16 (0.17)	1.33 (0.17)	0.009
Upper leg lean tissue, kg	5.50 (0.89)	4.90 (0.68)	5.86 (0.81)	0.002
Lower leg lean tissue, kg	2.73 (0.38)	2.50 (0.34)	2.87 (0.34)	0.006
Body fat, %	29.6 (4.2)	29.3 (3.3)	29.9 (4.7)	0.704

Data are presented as mean (SD). BMI, body mass index.

Compared with the normal appendicular lean tissue group, maximal isometric torque of the knee extensors (p=0.048), knee flexors (p=0.009), and elbow flexors (p=0.008) and peak isokinetic power for knee extensors at 60 °/s (p=0.049) were significantly lower in the low appendicular lean tissue group (Table 7.2). However, no differences existed for maximal isometric torque of the elbow extensors (p=0.140), peak isokinetic power of the knee extensors at 180 °/s (p=0.105), grip strength (p=0.165), six-minute walk distance (p=0.282), or 30-second sit to stand (p=0.310) (Table 7.2).

Table 7.2. Strength and physical function characteristics

	All (n=32)	Low appendicular lean tissue (n=12)	Normal appendicular lean tissue (n=20)	Unadjusted p-value	Adjusted p-value
Knee extensors isometric torque, Nm	168.5 (45.7)	141.9 (39.5)	184.5 (42.4)	0.008	0.048
Knee extensors 60 °/sec isokinetic power, W	138.6 (42.1)	113.7 (35.8)	153.6 (39.0)	0.007	0.049
Knee extensors 180 °/sec isokinetic power, W	288.8 (87.9)	243.4 (72.6)	316.0 (86.4)	0.021	0.105
Knee flexors isometric, Nm	88.6 (23.0)	72.5 (13.0)	98.3 (22.4)	0.001	0.009
Grip strength, kg	38.4 (8.9)	34.5 (8.7)	40.7 (8.4)	0.055	0.165
Elbow extensors isometric torque, Nm	62.9 (17.1)	54.8 (14.5)	67.8 (17.1)	0.035	0.140
Elbow flexors isometric torque, Nm	51.2 (15.4)	40.3 (11.1)	57.7 (14.1)	0.001	0.008
Six-minute walk distance, m	540.9 (87.8)	519.1 (103.0)	554.1 (77.1)	0.282	0.282
30-second sit to stand, repetitions	15.3 (4.9)	13.6 (4.6)	16.3 (4.9)	0.155	0.310

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction.

All muscle thicknesses, derived by ultrasound, were smaller ($p<0.05$) in the low compared with normal appendicular lean tissue group except for the anterior lower leg

(p<0.05), which was larger in the low appendicular lean tissue group (Table 7.3). The low appendicular lean tissue group displayed elevated echo intensity of the anterior upper leg (p=0.003) and anterior lower leg (p=0.016) compared to the normal appendicular lean tissue group (Table 7.4).

Table 7.3. Muscle thickness characteristics

	All (n=32)	Low appendicular lean tissue (n=12)	Normal appendicular lean tissue (n=20)	Unadjusted p-value	Adjusted p-value
Anterior upper arm, cm	3.15 (0.50)	2.78 (0.28)	3.37 (0.47)	<0.001	0.002
Posterior upper arm, cm	2.81 (0.74)	2.39 (0.52)	3.06 (0.75)	0.011	0.032
Anterior forearm, cm	4.60 (0.50)	4.21 (0.45)	4.84 (0.38)	<0.001	0.002
Anterior abdomen, cm	0.87 (0.19)	0.76 (0.18)	0.93 (0.17)	0.012	0.023
Anterior upper leg, cm	2.68 (0.67)	2.26 (0.38)	2.94 (0.67)	0.003	0.007
Posterior upper leg, cm	4.42 (0.92)	3.79 (0.85)	4.79 (0.76)	0.002	0.006
Anterior lower leg, cm	2.97 (0.28)	3.06 (0.22)	2.82 (0.32)	0.021	0.021
Posterior lower leg, cm	6.16 (0.79)	5.56 (0.71)	6.54 (0.58)	<0.001	0.001

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction.

Table 7.4. Muscle echo intensity characteristics

	All (n=32)	Low appendicular lean tissue (n=12)	Normal appendicular lean tissue (n=20)	Unadjusted p-value	Adjusted p-value
Anterior upper arm, AU	63.5 (11.9)	66.0 (11.8)	62.0 (12.0)	0.371	0.741
Posterior upper arm, AU	35.3 (9.7)	40.2 (10.9)	32.3 (7.8)	0.023	0.092
Anterior abdomen, AU	58.4 (18.9)	67.5 (21.4)	53.0 (15.3)	0.034	0.101
Anterior upper leg, AU	52.5 (13.9)	62.8 (11.4)	46.3 (11.6)	<0.001	0.003
Anterior lower leg, AU	55.6 (12.2)	63.4 (10.7)	50.9 (10.6)	0.003	0.016
Posterior lower leg, AU	80.1 (17.6)	81.4 (21.9)	79.4 (14.9)	0.761	0.761

Data are presented as mean (SD). Adjusted p-values were derived using a Holm-Bonferroni correction. AU, arbitrary units.

Maximal isometric knee extensors torque was significantly associated with anterior upper leg muscle thickness (r=0.5, p=0.023) and echo intensity (r=-0.41, p=0.038) and appendicular (r=0.52, p=0.017) and upper leg lean tissue (r=0.70, p<0.001) (Table 7.5). Knee extensors peak isokinetic power at 60 and 180 °/s were significantly associated with anterior upper leg muscle thickness (r=0.55, p=0.010; r=0.62, p=0.002) and echo intensity (r=-0.47, p=0.025; r=-0.48, p=0.028) and appendicular (r=0.57, p=0.007; r=0.57, p=0.007) and upper leg lean tissue (r=0.71, p<0.001; r=0.63, p=0.002). Maximal isometric torque of the knee flexors was significantly associated with the posterior upper leg muscle thickness (r=0.39, p=0.025) and appendicular (r=0.69, p<0.001) and upper leg lean tissue (r=0.71, p<0.001) (Table 7.5).

Maximal grip strength was significantly associated with anterior forearm thickness (r=0.53, p=0.015) and appendicular (r=0.63, p=0.002) and lower arm lean tissue (r=0.65, p=0.001) (Table 7.5). Maximal isometric torque of the elbow extensors was significantly associated with posterior upper arm muscle thickness (r=0.71, p<0.001) and echo intensity (r=-0.59, p=0.005) and appendicular (r=0.66, p<0.001) and upper arm lean tissue (r=0.83, p<0.001). Maximal isometric torque of the elbow flexors was significantly associated with anterior upper arm muscle thickness (r=0.58, p=0.006) and echo intensity (r=-0.43, p=0.040) and appendicular (r=0.58, p=0.006) and upper arm lean tissue (r=0.65, p=0.001) (Table 7.5).

Apart from maximal isometric torque of the knee flexors (p=0.010), no significant differences (p>0.05) existed for the magnitude of the correlation coefficient between muscle thickness or region-specific lean tissue (strongest associations for ultrasound and DXA respective) and isometric torque or isokinetic power (Table 7.5).

Table 7.5. Correlation coefficients between muscle thickness, muscle echo intensity, appendicular lean tissue index, or region-specific lean tissue and maximal muscle torque or power.

	Muscle thickness	Echo intensity	Appendicular lean tissue index	Region-specific lean tissue
Knee extensors isometric torque, Nm	**0.5**	**-0.41**	**0.52**	**0.70**
Knee extensors 60 °/sec isokinetic power, W	**0.55**	**-0.47**	**0.57**	**0.71**
Knee extensors 180 °/sec isokinetic power, W	**0.62**	**-0.48**	**0.57**	**0.63**
Knee flexors isometric, Nm	**0.39**	-	**0.69**	**0.71***
Grip strength, kg	**0.53**	-	**0.63**	**0.65**
Elbow extensors isometric torque, Nm	**0.71**	**-0.59**	**0.66**	**0.83**
Elbow flexors isometric torque, Nm	**0.58**	**-0.43**	**0.58**	**0.65**

Data are presented are correlation coefficient. Bold value indicates significant correlation after Holm-Bonferroni correction. * indicates magnitude of correlation with isometric torque or isokinetic power is significantly greater than magnitude of muscle thickness correlation with isometric torque or isokinetic power. Correlation coefficients for muscle thickness and echo intensity were derived between: anterior upper leg and knee extensors (torque and power), posterior upper leg and knee flexors (torque), anterior forearm and grip strength, posterior upper arm and elbow extensors (torque), anterior upper arm and elbow flexors (torque). Correlation coefficients for region-specific lean tissue were derived between: upper leg lean tissue and knee extensors (torque and power) and flexors (torque), lower arm lean tissue and grip strength, upper arm lean tissue and elbow extensors (torque) and flexors (torque).

The addition of echo intensity did not change the magnitude of association between muscle thickness and maximal isometric torque or peak isokinetic power for any evaluated muscle group (Table 7.6).

Table 7.6. Multiple linear regression analysis of muscle thickness and echo intensity compared to maximal muscle torque or power.

	Muscle thickness standardized B coefficient	Muscle thickness p-value	Echo intensity coefficient	Echo intensity p-value	Regression coefficient
Knee extensors isometric torque, Nm	0.395	0.06	-0.158	0.45	0.51
Knee extensors 60 °/sec isokinetic power, W	0.420	0.04	-0.203	0.31	0.57
Knee extensors 180 °/sec isokinetic power, W	0.531	0.009	-0.136	0.47	0.63
Elbow extensors isometric torque, Nm	0.566	0.002	-0.231	0.16	0.73
Elbow flexors isometric torque, Nm	0.488	0.006	-0.204	0.23	0.61

Data are presented as standardized B-coefficients. Regressions for muscle thickness and echo intensity were derived between: anterior upper leg and knee extensors (torque and power), posterior upper arm and elbow extensors (torque), anterior upper arm and elbow flexors (torque).

After correction for multiple comparisons, neither muscle thickness, echo intensity, appendicular lean tissue, or lower body lean tissue was significantly ($p>0.05$) associated with six-minute walk or 30-second sit to stand tests (Table 7.7).

Table 7.7. Correlation coefficients between muscle thickness, muscle echo intensity, appendicular lean tissue index, or region-specific lean tissue and six-minute walk distance or 30-second sit to stand.

		six-minute walk	30-second sit to stand
Anterior abdominal	thickness, cm	0.12	0.09
	echo intensity, AU	-0.30	-0.47
Anterior upper leg	thickness, cm	0.04	0.1
	echo intensity, AU	-0.17	-0.11
Posterior lower leg	thickness, cm	0.25	-0.06
	echo intensity, AU	-0.03	-0.25
Lean tissue	Appendicular lean tissue index, kg/m^2	0.12	0.03
	Lower body lean tissue, kg	0.29	-0.05

Data are presented are correlation coefficient. Bold value indicates significant correlation after Holm-Bonferroni correction. AU, arbitrary units.

7.4.1 Discussion

Here, we demonstrated that both DXA lean tissue (appendicular and region specific) and ultrasound site-specific muscle thickness provide similar magnitude associations with indices of muscle strength in older males. However, upper leg lean tissue mass provided stronger associations with isometric knee flexors than posterior upper leg thickness. While both muscle thickness and echo intensity were significantly associated with isometric torque and isokinetic power, the combination through multiple linear regression did not improve the associations. Furthermore, neither muscle thickness, muscle echo intensity, or DXA lean tissue measures were associated with six-minute walk or 30-second sit to stand tests. Compared with older males who had normal appendicular lean tissue mass, all muscle thicknesses were thinner and muscle echo intensity of the rectus femoris and tibialis anterior were greater in the low appendicular lean tissue group.

Ageing-related losses in skeletal muscle mass and composition are thought to begin around the 5th decade of life and accelerate with advancing age (>80 years of age) [148]. Even amongst apparently healthy, highly functioning community dwelling older adults, these ageing-related impairments in muscle tissue progress; however, there is significant variability in the severity and rate of progression of sarcopenia amongst individuals [188]. Due to this significant variability and the deleterious impact on mobility [189], independence [180], health care costs [3], and rates of mortality [115], operationalizing a definition of sarcopenia is prudent to enable identification of older adults with these deleterious muscle features in order to provide targeted therapies to mitigate further deterioration (e.g. nutrition interventions). While these operational definitions of sarcopenia have evolved to include measures of muscle strength and

function [181], assessment of muscle mass is still widely regarded as an important construct for diagnosis.

7.4.2 Older adults with low appendicular lean tissue mass display lower muscle thickness across several landmarks in comparison with older adults with normal appendicular lean tissue

Appendicular lean tissue mass, which is strongly correlated with whole body muscle mass measured using MRI [14], is typically suggested as the primary metric to evaluate ageing-related changes in muscle mass [10]. However, as we and others have previously shown, advancing age is associated with regional skeletal muscle atrophy, particularly within the rectus abdominis and quadriceps muscles [16], [97], [169]. However, how site-specific skeletal muscle atrophy presents in individuals with low skeletal muscle mass is less well understood. Here, we observed that older males with low appendicular lean tissue mass presented with smaller muscle thickness across all sites except for the anterior lower leg, which was larger. Interestingly, we have also observed increased muscle thickness of the anterior lower leg in older adults compared with younger adults who are matched for relative appendicular lean tissue mass. These increases in the anterior lower leg muscle thickness may be related to increased intramuscular adipose tissue or disturbances in fluid balance, as increases in echo intensity of the tibialis anterior is also observed in the low compared to normal appendicular lean tissue males.

Several other publications have also observed smaller muscle thicknesses for the biceps, quadriceps, and gastrocnemius muscles amongst frail [190], pre-sarcopenic [191], sarcopenic

[191], [192], functionally-dependent [190], and weak [111], [191] older males and females when compared to older adults with normal muscle mass or strength. Similarly, increased muscle echo intensity has also been observed in the quadriceps [190], [191], but not the biceps [111] or gastrocnemius [111] muscles in these same cohorts of older adults, which is in agreement with our observations. Furthermore, in frail nursing home residents (n=16, age=84.8 years), Takeshima et al. (2015) [193] observed an 11-33% reduction in muscle thickness from 9 distinct sites across the body over the course of 1 year, suggesting global losses in muscle mass in frail older adults. However, at baseline, 100% of the patients displayed low quadriceps muscle thickness, whereas the other muscle groups displayed low muscle thickness in 13-75% of patients [193]. Taken together, these data suggest that while typical ageing-related losses of skeletal muscle mass occurs primarily within the quadriceps and rectus abdominis muscles, the progression towards a state of impairment (e.g. sarcopenic) involves deterioration of several muscle groups across the body. Therefore, appendicular lean tissue may be a useful surrogate for identification of those older adults with progressed impairments in muscle mass, but regional or site-specific muscle mass assessments may be more sensitive to early ageing-related losses.

7.4.3 Appendicular lean tissue and site-specific muscle thickness demonstrate similar magnitude associations with muscle strength

In comparison to isometric torque or isokinetic power, both ultrasound muscle thickness and appendicular or regional lean tissue provided similar magnitude of associations (excluding posterior upper leg), indicating both metrics may be useful in the identification of sarcopenia. However, given a more robust sample size, regional lean tissue mass would likely emerge as a

stronger indicator of muscle strength than muscle thickness. Although, the use of site-specific muscle cross-sectional area may bridge this difference, which can be readily achieved through the use of panoramic ultrasound. In line with our findings, Thiebaud et al. (2017) [95] observed similar magnitude of associations between 1 repetition maximum knee extension and flexion strength with either anterior to posterior thigh muscle thickness ratio or appendicular lean tissue mass. Furthermore, the anterior to posterior upper arm muscle thickness ratio and appendicular lean tissue mass displayed similar magnitude of associations with 1 repetition maximum for chest press and lat-pull down [95]. However, these associations were evaluated across young, middle-aged, and older males; whereas our comparisons are limited to older males. In a more direct comparison, Tsukaski et al. (2020) [109] compared quadriceps cross-sectional area (computed tomography) and DXA lean tissue mass in relation to isometric knee extensors torque in 1818 adults (40-89 years of age). Although quadriceps cross-sectional area provided statistically stronger associations with muscle strength than DXA appendicular or thigh lean tissue, the correlation coefficient were of similar magnitude (males: cross-sectional area - 0.50, DXA - 0.47; females: cross-sectional area - 0.49, DXA - 0.41) [109].

7.4.4 Addition of echo intensity with skeletal muscle thickness does not improve associations with muscle strength

For skeletal muscle composition, Harris-Love et al. 2018 [185] observed that both appendicular lean tissue mass and rectus femoris echo intensity provided similar magnitude associations with isokinetic knee extension torque at 60 and 180 °/s; however, after adjusting knee extension torque by bodyweight, echo intensity provided stronger associations (although no formal statistical tests were performed). While we observed associations that were similar in

magnitude between echo intensity and isometric torque or power as Harris-love et al. 2018 [185], regional lean tissue mass provided stronger associations with muscle strength. While it is not entirely clear why these differences emerged, they could be related to age differences in the cohorts (62.5 ±9.2 vs. 75.4 ±7.9 years).

A potential advantage that ultrasound (and other imaging-based modalities) provides over DXA measured appendicular lean tissue in the assessment of sarcopenia, is the ability to quantify a surrogate of muscle composition (echo intensity) in addition to muscle thickness, which may provide complementary metrics for evaluating ageing-related muscle degradation. While we observed that echo intensity was independently correlated isometric torque and isokinetic power, when combined with muscle thickness using multiple linear regression, echo intensity did not improve the association with muscle strength compared to muscle thickness alone. However, Watanabe et al. (2013) [194] observed that both muscle thickness and echo intensity of the rectus femoris were significantly associated with maximal isometric knee extensor torque in multiple-linear regression (B-coefficients 0.381 and -0.294). These discrepancies are potentially due to the moderate correlation we observed between muscle thickness and echo intensity (data not shown), whereas Watanabe et al. (2013) [194] did not observe any association.

Others have also observed negative association between muscle thickness and echo intensity [195], which may be related to the confounding influence of image resolution on skeletal muscle echo intensity [196]. It is not entirely apparent why these differences exist with Watanabe et al. (2013) [194], as we recruited males of a similar age range (this study: 65-92, Watanabe et al. (2013) [194] 65- 91), but could potentially be due to ethnic differences

(Japanese vs. Caucasian) or protocol differences (e.g. standing vs. supine ultrasound imaging). However, data from the Healthy Ageing and Body Composition study observed that midthigh cross-sectional area explained a much larger proportion of the variance (R^2 = 0.304 men and R^2 = 0.248 women) in isometric knee extension torque than thigh muscle intramuscular adipose tissue (R^2 = 0.016 men and R^2 = 0.030 women) [22].

7.4.5 Muscle thickness and echo intensity may not be associated with functional capacity of older males

In relation to functional performance, after correcting for multiple comparisons, no metrics of muscle mass or composition were associated with the six-minute walk or 30-second sit to stand test; however, rectus abdominis echo intensity displayed moderate (r= -47) associations with 30-second sit to stand. Others have observed that the ratio between the anterior and posterior thigh muscle thickness is associated with zig-zag walking time, but not gait speed [166], [197]. Marcus et al. (2012) [198] observed moderate associations between six-minute walk distance and thigh intramuscular adipose tissue (r= -0.33) and muscle cross-sectional area (r= 0.38) in 109 older adults. While we did not observe any association with thigh thickness or echo intensity, the older adults in Marcus et al. (2012) [198] recruited were likely of poorer muscle function, as they had at least 2 comorbidities, were at risk of falling or had experienced a fall in the past 12 months, and had a shorter six-minute walk distance (409.9 ±120.3 vs. 540.9 ±87.8 m).

7.4.6 Limitations

A critical limitation to this study is the lack of participants presenting with sarcopenia (according to recent guidelines of low muscle strength and mass) [10]. While several of the participants presented with low appendicular lean tissue mass, only 2 participants had a low grip strength (<27 kg), which limit the generalizability of these findings to individuals with sarcopenia. Furthermore, the individuals with low appendicular lean tissue are considerably older (81.9 vs. 71.5 years) than those with normal appendicular lean tissue, making it challenging to disentangle the effects of advancing age and progression of sarcopenia.

7.4.7 Conclusions

In older males, site-specific measures of muscle thickness and DXA lean tissue provided similar magnitude of associations with static and dynamic muscle strength. While muscle echo intensity was independently associated with muscle strength, when combined with muscle thickness, echo intensity did not improve the association with muscle strength. These data highlight that both appendicular lean tissue and site-specific measures of muscle mass (thickness, regional lean tissue), are useful tools for assessing muscle mass of older adults in the identification of sarcopenia.

CHAPTER 8

STUDY #4: SKELETAL MUSCLE ECHO INTENSITY DISPLAYS DIVERGENT ASSOCIATIONS WITH GLUCOSE HOMEOSTASIS IN HEALTHY AND GLUCOSE IMPAIRED OLDER MALES.

Michael T. Paris[1]

Kirsten E. Bell[1]

Egor Avrutin[1]

Marina Mourtzakis[1]

[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Corresponding author: Marina Mourtzakis, PhD, Department of Kinesiology, University of Waterloo, 200 University Ave W, Waterloo, ON, N2L 3G1, Canada. Email: mmourtzakis@uwaterloo.ca. Phone: 519-888-4567, Ext 38459.

8.1 Introduction

Ageing-related losses in skeletal muscle mass and deleterious shifts in muscle composition towards increased intramuscular adipose tissue predispose older adults to substantial health risks, including increased risk of insulin resistance and development of diabetes [29], [199]. These negative consequences of ageing-related changes in muscle tissue are further compounded by shifts in adiposity towards increased storage within the abdominal region [45]. The subcutaneous adipose tissue within the lower abdominal region is composed of the superficial and deep subcutaneous adipose tissue (sSAT and dSAT, respectively) [33]. The dSAT compartment, which is similar in morphology and adipokine profile to visceral adipose tissue [32], increases with advancing age and is associated with impaired glucose homeostasis [32], [33], [90].

Ultrasound has emerged as a useful modality for measuring skeletal muscle mass and composition, as well as dSAT and sSAT thickness [46], [155], [196]. Ultrasound measures of muscle thickness or cross-sectional area are strongly associated with regional and whole-body indices of muscle mass derived using magnetic resonance imaging (MRI) [128] and dual-energy x-ray absorptiometry (DXA) [51], [127], [184]. Similarly, ultrasound echo intensity, which is the average pixel intensity from a region of interest within a skeletal muscle [200], is associated with intramuscular adipose tissue [138], [185], extramyocellular lipids [156], and muscle attenuation derived from computed tomography [201]. While most work has focused on the quadriceps muscles, Young et al. (2016) [138] demonstrated that after correcting for subcutaneous adipose tissue thickness, skeletal muscle echo intensity is strongly associated with MRI measured intramuscular adipose tissue for the rectus femoris (r=0.91), biceps femoris

(r=0.80), tibialis anterior (r=0.80), and the gastrocnemius (r=0.76). The dSAT and sSAT thickness measured using ultrasound demonstrates moderate associations with computed tomography derived dSAT (r=0.49) and sSAT (r=0.48) cross-sectional area [46]. While ultrasound measures of muscle mass, composition, and dSAT and sSAT are associated with measures of these tissues using reference modalities, their relation to glucose homeostasis or metabolic health is not well established, particularly within older adults.

Here, we determined how ultrasound measurements of muscle thickness, muscle echo intensity, and dSAT and sSAT thickness associate with measures of glucose homeostasis (oral glucose tolerance test) in older males. We evaluated muscle thickness and echo intensity across 3 distinct muscle groups (anterior upper arm, anterior abdomen, and anterior upper leg) and the dSAT and sSAT at the anterior abdomen, as well as traditional measures of body composition (DXA, waist circumference). We examined these associations separately for individuals with normal and impaired glucose control (prediabetes and diabetes) to examine if metabolic health alters the relationships between measures of ultrasound body composition and glucose homeostasis.

8.2 Methods

8.2.1 Study design and participants

A prospective cross-sectional study was conducted on 32 community-dwelling older males (≥65 years) recruited from the Kitchener-Waterloo community. Participants attended 2 fasted (minimum 10 hours) data collection sessions for assessment of body composition and blood metabolic markers. DXA and ultrasound were performed on a single day. Metabolic blood analysis included assessment of a 75 g 2-hour oral glucose tolerance test (glucose, insulin, and

c-peptide), and fasting total cholesterol, triacylglycerides, high-density lipoprotein cholesterol (HDL-C) and, low-density lipoprotein cholesterol (LDL-C). Participants were excluded if they: 1) had a previous history of neuromuscular disease, 2) had undergone administration of oral or intra-venous contrast for nuclear medicine scans within the past 3 weeks, 3) had a prosthetic joint replacement, 4) had a history of cancer or cerebrovascular disease, 5) participated in structed exercise sessions within the past 6 months, or 6) had a previous history or known diagnosis of diabetes. While diabetic participants were excluded during initial screening, several incidental findings of poor glucose homeostasis were observed following metabolic analysis, which were included within a distinct group for sub-analysis (see below for additional details). Participants were instructed to refrain from participating in moderate to vigorous physical activity for at least 48 hours and from consuming alcohol for 24 hours prior to any data collection session. All studies were approved by a human research ethics committee at the University of Waterloo. Written informed consent was obtained from all participants in accordance with established protocols for human research.

8.2.2 Anthropometry and blood pressure

Participant's height and weight were obtained in cloth hospital gowns to the nearest 0.5 cm and 0.1 kg using a balance beam and stadiometer, respectively. Waist circumference was assessed by placing the inferior border of a flexible tape measure at the top of the iliac crest and measuring to the nearest 0.1 cm at the end of a normal expiration. Waist circumference measures were performed twice and averaged. After 15 minutes of sitting, resting blood pressure was averaged across two measurements using a manual sphygmomanometer.

8.2.3 Ultrasound landmarking

Participants were landmarked in supine position using a flexible tape measure and pen. A foot strap was placed around the ankles (~15 cm gap between the left and right medial malleolus) to minimize hip rotation during landmarking and imaging. Sites to be imaged were the anterior upper arm (biceps brachii and brachialis), anterior upper leg (rectus femoris and vastus intermedialis), and the anterior abdomen (rectus abdominis). The upper arm was imaged on the anterior, 60% distal from the acromial process to the lateral epicondyle of the humerus. Anterior abdomen images were taken 3 and 5cm right of the umbilicus for analysis of muscle thickness and dSAT and sSAT thickness, respectively. Anterior upper leg images were taken two-thirds the distance from the anterior superior iliac spine to the superior pole of the patella. Limb lengths were measured to the nearest 0.5 cm and sites for image acquisition were marked to the nearest 0.1 cm. All images were taken on the right side of the body. Landmarking took ~20 minutes, for which all participants remained supine to mitigate shifts in fluid distribution during imaging [186].

8.2.4 Ultrasound image acquisition

Ultrasound images were captured in the transverse plane using a multi-frequency linear array transducer (L38xi: 5-10 MHz) on a real time B-mode ultrasound device (M-turbo, Sonosite, Markham, ON). Adjustable parameters gain, time-gain-compensation, and dynamic range (50%) were held constant throughout imaging and the mode was set to resolution. Minimal compression of the ultrasound probe against the underlying tissue was achieved by generously coating the transducer with water-soluble transmission gel and: 1) ensuring the

natural curvature of the skin, adipose tissue, and muscle was maintained on the screen, and 2) a visible layer of ultrasound gel was present between the transducer and skin, as previously described [51]. The transducer was placed at the landmarked site and moved in a medial-lateral direction to centre the muscle belly within the field of view. Image depth was adjusted as needed to visualize the muscle being analyzed. Each landmark was imaged twice with removal of the transducer between images. Images were saved in the Digital Imaging and Communications in Medicine format and transferred to a personal computer for further analysis.

8.2.5 Muscle thickness, muscle echo intensity, and adipose tissue thickness

All ultrasound image analyses were performed using ImageJ (NIH, Bethesda, MD, version 1.53e). Muscle thickness was measured as the vertical distance between the upper margin of the underlying bone (anterior upper arm and leg) or inferior muscle fascia (anterior abdomen) and the superior muscle fascia (Figure 8.1). Each image was analyzed once (two images per landmark) by a single investigator and averaged for each landmark. Muscle thickness was measured in pixels and converted to a linear distance using the provided manufacturer image resolutions.

Muscle echo intensity was measured by placing the largest rectangular region of interest within the muscle fascia borders, as previously described [161]. The rectus abdominis (anterior abdomen), biceps brachii (anterior upper arm), and rectus femoris (anterior upper leg) were used for echo intensity analysis (Figure 8.1). Echo intensity was expressed as an arbitrary value (AU) between 0 and 255 (8-bit image).

For the anterior upper arm, anterior abdomen (3 cm image), and anterior upper leg, subcutaneous adipose tissue thickness was measured as the vertical distance between the lower boundary of the skin and the upper boundary of the underlying muscle fascia. For each image, subcutaneous adipose thickness was measured at 3 locations: the left, middle, and right side of the image (Figure 8.1). Adipose tissue thickness was averaged across the image and then across each landmark (2 images per landmark). For the deep and superficial subcutaneous adipose tissue (5 cm anterior abdomen image), the central fascia (Scarpa's fascia) with the brightest and most continuity across the image was used for analysis. The dSAT was measured from the inferior aspect of the central fascia to the superior muscle fascia and sSAT was measured from the superior aspect of the central fascia to the inferior border of the skin (Figure 8.1). Adipose thickness was measured in pixels and converted to a linear distance using the provided manufacturer image resolutions.

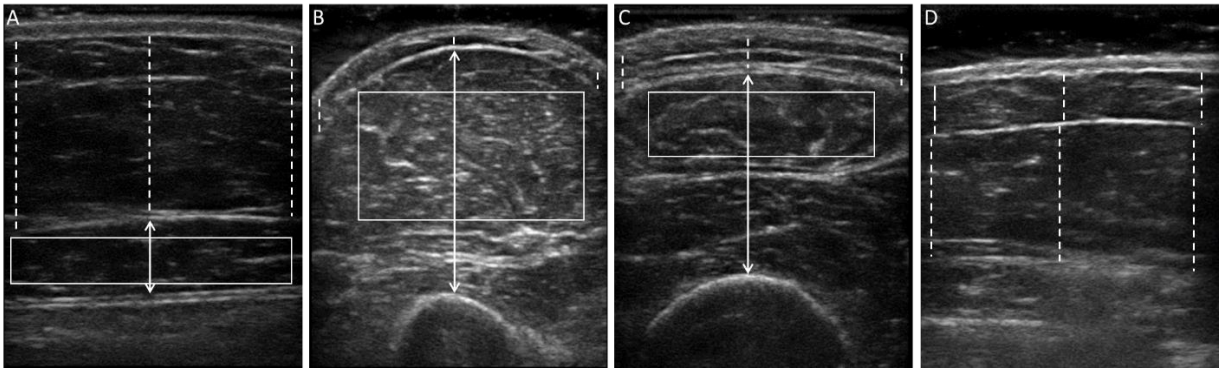

Figure 8.1. Analysis of skeletal muscle thickness, skeletal muscle echo intensity, and adipose tissue thickness for A) anterior abdomen (3 cm), B) anterior upper arm, C) anterior upper leg, and D) anterior abdomen (5 cm). Rectangular boxes depict area used for analysis of skeletal muscle echo intensity. Solid double arrowed lines depict vertical analysis for skeletal muscle thickness. Dashed lines depict analysis of 1) subcutaneous adipose tissue thickness (A, B, C) or 2) deep and superficial subcutaneous adipose tissue (D).

8.2.6 Dual-energy x-ray absorptiometry

Participants received 1-2 whole body DXA scans (Hologic discovery QDR4500, Hologic, Toronto, ON). Prior to each scan, certified medical radiation technologists performed quality control and calibration procedures as outlined by the manufacturer. Participants lay supine on the scanning bed with their arms and legs extended, with their toes internally rotated and held in place using masking tape. Hologic software (version 13.2) was used by a single trained investigator to segment the body into the head, torso, left and right arms, and left and right legs, as previously described [13]. One participant required two scans, which was analyzed by excluding the missing limb(s) and averaging the included segments across the two scans. Appendicular lean tissue index was calculated by dividing the lean soft tissue in the arms and legs (kg) by the participants height (m) squared (kg/m^2).

8.2.7 Blood sampling

Following an overnight fast (minimum 10 hours with no food or drink, except for water), participants arrived for fasted blood samples and a 75 g 2-hour oral glucose tolerance test. A sterile catheter was inserted into an antecubital vein and 24 mL of blood was drawn into serum (18 mL) and K_2 EDTA plasma (6 mL) vacutainers (time: 0 min). Immediately following collection of the first blood sample, participants consumed a 75 g glucose drink (Trutol Glucose Tolerance Beverage, Thermo Fisher Scientific, East Providence, RI) within 5 minutes. Following completion of the glucose drink, plasma samples (6 mL) were drawn at 15, 30, 45, 60, 90, and 120 minutes. Serum samples were left to clot at room temperature whereas plasma samples were immediately placed on ice. Following collection of the final 2-hour timepoint, serum and plasma

samples were centrifuged at 2500 g for 15 minutes at 4° C. Serum and plasma samples were aliquoted and stored at -80° C until biochemical analysis.

8.2.8 Biochemical analysis

Fasted serum collected at the 0 min timepoint was analyzed for triacylglycerides, total cholesterol, and HDL-C. LDL-C was indirectly calculated according to a recently developed equation by Sampson et al. (2020) [147] (equation 1), which better predicts LDL-C in participants with elevated triacylglycerides compared with the traditional Friedewald equation.

$$LDLC = \frac{TC}{0.948} - \frac{HDLC}{0.971} - \left(\frac{TG}{8.56} + \frac{TG\ x\ nonHDLC}{2140} - \frac{TG^2}{16100}\right) - 9.44 \quad (1)$$

TC, total cholesterol, TG, triacylglycerides. All concentrations are in mg/dL. Triacylglycerides, total cholesterol, and HDL-C were analyzed in duplicate using commercially available triacylglyceride-GPO, cholesterol, and HDL-C precipitating reagent set (Pointe Scientific, Canton, MI) and the absorbances were read at 500 nm (Cytation 5, BioTek, Winooski, VT) (see Appendix A1 for further details).

Plasma glucose was analyzed for the 0, 15, 30, 45, 60, 90, and 120 min timepoints. Plasma insulin and c-peptide were analyzed for the 0, 30, 60, 90, and 120 min timepoint. Glucose was analyzed in triplicate using a peroxidase-glucose oxidase enzymatic reaction (see Appendix A1 for further details). Briefly, 2.5 mL of peroxidase-glucose oxidase and o-dianisidine dihydrochloride mixture (Thermo Fisher Scientific, East Providence, RI) was added to 10 µL of plasma and incubated for 30 minutes at 37°C. Absorbance was read at 450 nm (Cytation 5, BioTek, Winooski, VT). Insulin and c-peptide were analyzed in duplicates using commercially

available radio-immunoassay kits for human insulin and c-peptide, respectively (MilliporeSigma, Billerica, MA).

8.2.9 Metabolic characterization

Participants were classified as pre-diabetic or diabetic if they displayed elevated fasting glucose or 2-hour glucose according to the American Diabetes Association (pre-diabetes: fasting 5.6-6.9 mmol/L, 2-hour 7.8-11.0 mmol/L; diabetes: fasting ≥7.0 mmol/L, 2-hour ≥11.1 mmol/L). Pre-diabetic and diabetic participants were grouped together (denoted as 'Glucose impaired') and compared to individuals with normal fasting glucose and glucose tolerance (denoted as 'Healthy').

To further characterize glucose homeostasis, we evaluated the Matsuda index [146], area under the curve (AUC) for glucose (mmol-min/L), insulin (pmol-min/L), and c-peptide (pmol-min/L) for the 2-hour glucose tolerance test, and the product of the glucose and insulin AUC ($mmol^2$-min^2/L^2). Matsuda index was calculated according to equation 2 [146].

$$Matsuda\ index = \frac{10\ 000}{\sqrt{fasting\ glucose\ x\ fasting\ insulin\ x\ mean\ glucose\ x\ mean\ insulin}} \quad (2)$$

Glucose and insulin concentrations are in mg/dL and µU/mL, respectively. AUC for the 2-hour glucose, insulin, and c-peptide curves were approximated for absolute values using the trapezoid rule. The product of the glucose (mmol-min/L) and insulin (mmol-min/L) AUC was calculated to evaluate glucose and insulin homeostasis in response to a glucose load.

8.2.10 Statistical analysis

Normality of continuous variables was confirmed using Shapiro-Wilk test. Differences

between older adults in the healthy and glucose impaired groups were evaluated using independent sample t-tests. Pearson correlation coefficients were used to evaluate associations between glucose homeostasis metrics and body composition features. Correlations coefficients were interpreted as weak (r≤ 0.35), moderate (r= 0.36 to 0.68), and strong (r≥0.69), as previously described [202]. Given previous moderate correlations (r= -0.45) between quadriceps intramuscular adipose tissue (computed tomography) and insulin sensitivity indices (hyperinsulinemic euglycemic clamp) [175], we interpreted the strength of correlation coefficients based on the expected moderate associations (r≥0.36), rather than statistical significance. Statistical significance of t-tests comparing healthy and glucose impaired groups was set as $p<0.05$. All statistical analyses were performed using SPSS (version 26, IBM, USA).

8.3 Results

The glucose impaired group (n=10) presented with a higher BMI (p=0.024) and waist circumference (p=0.04), but not age, height, weight, or blood pressure (Table 8.1) compared to the healthy group (n=22).

Table 8.1. Demographic and anthropometric characteristics

	All (n=32)	Healthy (n=22)	Glucose impaired (n=10)	p-value
Age, y	75.3 (7.9)	73.5 (7.0)	79.3 (8.7)	0.057
Height, m	1.73 (0.07)	1.73 (0.08)	1.72 (0.06)	0.586
Weight, kg	79.9 (13.2)	77.2 (9.8)	85.8 (18.0)	0.093
BMI, kg/m^2	26.6 (4.0)	25.5 (2.3)	28.9 (5.8)	0.024
Systolic, mmHg	126.6 (9.4)	124.6 (8.8)	131.0 (9.6)	0.074

Diastolic, mmHg	75.8 (7.7)	75.1 (7.8)	77.3 (7.8)	0.464
Waist circumference, cm	99.2 (9.4)	96.9 (6.8)	104.3 (12.6)	0.040

Data are presented as mean (SD). BMI, body mass index

While the glucose impaired group had a higher body fat (p=0.015) compared to the healthy group, there were no differences for appendicular lean tissue mass index, muscle thickness, muscle echo intensity, subcutaneous adipose tissue thickness, or dSAT and sSAT thickness (Table 8.2).

Table 8.2. Body composition characteristics

	All (n=32)	Healthy (n=22)	Glucose impaired (n=10)	p-value
Appendicular lean tissue index, kg/m^2	7.27 (0.87)	7.16 (0.68)	7.51 (1.19)	0.300
Body fat, %	29.6 (4.1)	28.4 (3.5)	32.2 (4.4)	0.015
Anterior upper leg muscle thickness, cm	2.68 (0.66)	2.56 (0.60)	2.93 (0.74)	0.150
Anterior upper arm muscle thickness, cm	3.15 (0.49)	3.11 (0.39)	3.23 (0.69)	0.524
Anterior abdomen muscle thickness, cm	0.87 (0.18)	0.87 (0.20)	0.86 (0.16)	0.925
Anterior upper leg echo intensity, AU	52.4 (13.9)	54.1 (14.1)	48.8 (13.5)	0.327
Anterior upper arm echo intensity, AU	63.4 (11.8)	63.2 (12.5)	64.0 (11.0)	0.857
Anterior abdomen echo intensity, AU	58.4 (18.8)	55.3 (17.6)	65.3 (20.4)	0.166
Anterior upper leg subcutaneous adipose tissue thickness, cm	0.58 (0.25)	0.58 (0.26)	0.58 (0.23)	0.937
Anterior upper arm subcutaneous adipose tissue thickness, cm	0.38 (0.21)	0.35 (0.20)	0.45 (0.21)	0.231
Anterior abdomen subcutaneous adipose tissue thickness, cm	2.13 (0.67)	2.21 (0.53)	1.96 (0.92)	0.351

Superficial subcutaneous adipose tissue, cm	0.64 (0.28)	0.68 (0.29)	0.54 (0.26)	0.189
Deep subcutaneous adipose tissue, cm	1.37 (0.55)	1.41 (0.48)	1.29 (0.72)	0.571
Deep/superficial subcutaneous adipose tissue	2.3 (1.1)	2.3 (1.2)	2.5 (1.1)	0.824

Data are presented as mean (SD). AU, arbitrary unit.

Fasting glucose ($p<0.001$), insulin ($p=0.001$), and c-peptide ($p=0.002$) were elevated in the glucose impaired compared to healthy group (Table 8.3). Similarly, 2-hour glucose ($p<0.001$), Matsuda index ($p=0.001$), glucose AUC ($p<0.001$), insulin AUC ($p=0.002$), c-peptide AUC ($p=0.011$), and glucose-insulin AUC ($p<0.001$) were elevated in the glucose impaired compared to healthy group (Table 8.3). All timepoints for the oral glucose tolerance test were elevated for glucose (Figure 8.2A) and insulin (Figure 8.2B) in the glucose impaired compared with healthy group ($p<0.05$). All timepoints for c-peptide (Figure 8.2C), except for 30 and 60 mins ($p>0.05$), were elevated ($p<0.05$) in the glucose impaired compared to healthy group. No differences were present in the fasting lipid profiles between healthy and glucose impaired groups (Table 8.3).

Table 8.3. Blood metabolic characteristics

	All (n=32)	Healthy (n=22)	Glucose impaired (n=10)	p-value
Fasting glucose, mmol/L	5.3 (0.8)	4.9 (0.3)	6.2 (1.0)	<0.001
Fasting insulin, pmol/L	85.2 (72.0)	58.9 (23.5)	143.0 (105.8)	0.001
Fasting c-peptide, pmol/L	866.3 (409.4)	726.2 (235.1)	1174.5 (543.1)	0.002
Matsuda index	3.72 (4.00)	5.3 (2.6)	2.15 (1.4)	0.001

2-hour glucose, mmol/L	5.8 (2.1)	4.9 (1.3)	7.8 (2.3)	<0.001
Glucose AUC, mmol-min/L	853.7 (220.1)	744.3 (134.4)	1094.4 (176.2)	<0.001
Insulin AUC, pmol-min/L	58267.0 (40018)	44268.4 (18821.3)	89064.1 (56183.9)	0.002
C-peptide AUC, pmol-min/L	312971.2 (86655.9)	287609.2 (71241.7)	368767.6 (94821.7)	0.011
Glucose-insulin AUC, $mmol^2$-min^2/L^2	53.8 (48.9)	34.1 (18.5)	97.1 (66.6)	<0.001
Triglycerides, mmol/L	0.94 (0.44)	0.86 (0.29)	1.10 (0.24)	0.162
HDL-C, mmol/L	1.2 (0.3)	1.2 (0.3)	1.1 (0.2)	0.173
LDL-C, mmol/L	2.5 (0.8)	2.7 (0.7)	2.3 (0.7)	0.226
Total cholesterol, mmol/L	4.1 (0.9)	4.2 (0.8)	3.8 (0.8)	0.196

Data are presented as mean (SD). AUC, area under the curve; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

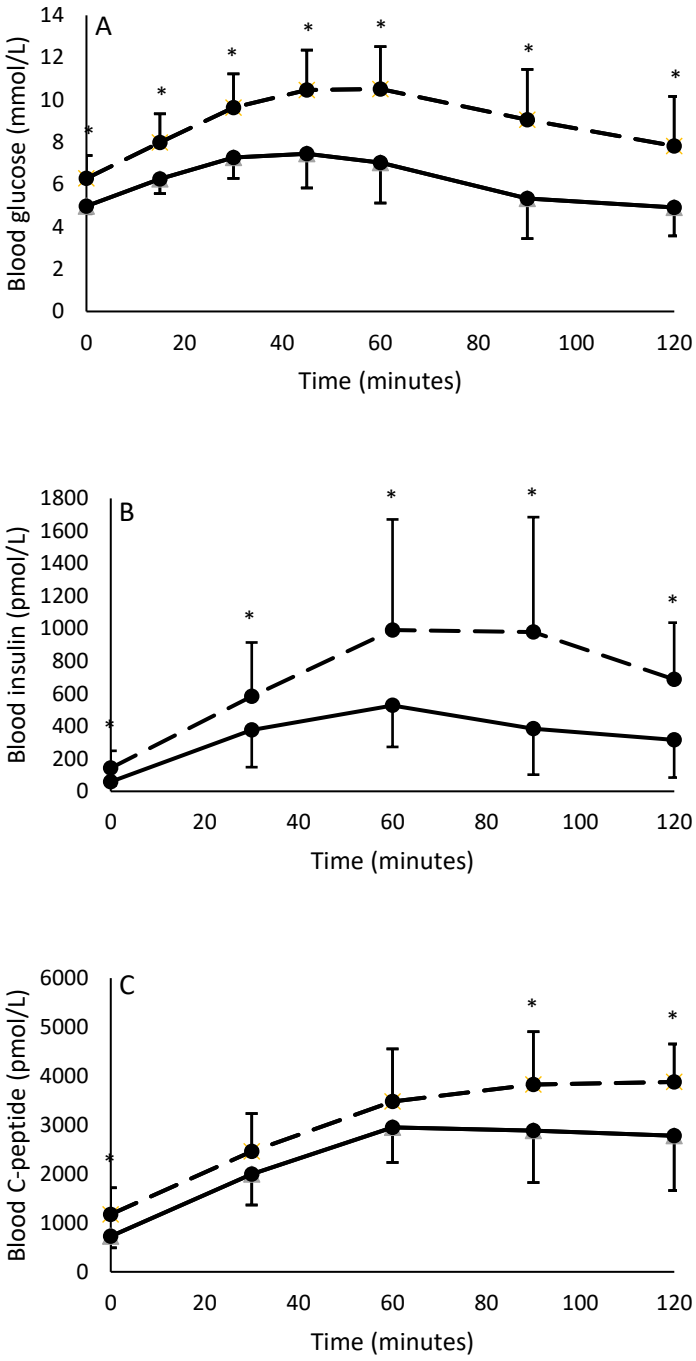

Figure 8.2. Oral glucose tolerance test curves for glucose (A), insulin (B), and c-peptide (C). Solid line depicts the healthy group, and the dashed line depicts the glucose impaired group. Error bars represent SD. * indicates significance difference between healthy and glucose impaired groups.

Within the healthy group, only the anterior abdomen muscle thickness displayed a moderate (r= -0.36) association with glucose-insulin AUC (Table 8.4). In contrast, the glucose impaired group displayed several moderate associations between muscle thickness and fasted glucose (r=0.46 to 0.71), glucose AUC (0.25 to 0.61), Matsuda index (-0.32 to -0.68), and glucose-insulin AUC (0.37 to 0.73), however, only weak associations were observed for 2-hour glucose (Table 8.4).

Table 8.4. Correlation coefficients between muscle thickness and blood glucose metrics

		Fasted glucose	2-hour glucose	Glucose AUC	Matsuda index	Glucose-insulin AUC
Healthy (n=22)	Anterior abdomen	0.13	-0.27	-0.22	0.14	**-0.36**
	Anterior upper arm	0.11	-0.27	0.08	-0.10	-0.05
	Anterior upper leg	0.27	-0.08	0.10	-0.17	-0.06
Glucose impaired (n=10)	Anterior abdomen	**0.46**	-0.07	0.25	-0.32	**0.37**
	Anterior upper arm	**0.62**	0.03	**0.61**	**-0.57**	**0.73**
	Anterior upper leg	**0.71**	-0.11	**0.50**	**-0.68**	**0.69**

Moderate correlation coefficients (r≥0.36) are bolded. AUC, area under the curve.

For skeletal muscle echo intensity, the healthy group displayed moderate positive correlations for 2-hour glucose and anterior abdomen (r= 0.36) and anterior upper leg (r= 0.59) echo intensity, but not the anterior upper arm (r= 0.07) (Table 8.5). Anterior upper leg echo intensity was also positively associated with glucose-insulin AUC (r= 0.43). In comparison, the glucose impaired group demonstrated several negative associations between muscle echo intensity and fasted glucose (r= -0.36 to -0.79), glucose AUC (r= 0.03 to -0.61), and positive

associations with Matsuda index (r= -0.04 to 0.53). However, for glucose-insulin AUC, anterior abdomen displayed a positive association (r= 0.47) (Table 8.5).

Table 8.5. Correlation coefficients between muscle echo intensity and blood glucose metrics

		Fasted glucose	2-hour glucose	Glucose AUC	Matsuda index	Glucose-insulin AUC
Healthy (n=22)	Anterior abdomen	0.11	**0.36**	0.18	0.07	0.14
	Anterior upper arm	0.10	0.07	-0.05	0.22	-0.11
	Anterior upper leg	0.01	**0.59**	0.21	-0.10	**0.43**
Glucose impaired (n=10)	Anterior abdomen	**-0.36**	-0.01	0.03	-0.04	**0.47**
	Anterior upper arm	**-0.45**	0.06	**-0.52**	**0.46**	**-0.67**
	Anterior upper leg	**-0.79**	-0.09	**-0.61**	**0.53**	**-0.4**

Moderate correlation coefficients (r≥0.36) are bolded. AUC, area under the curve.

Waist circumference displayed a negative association with Matsuda index (r= -0.36) in the healthy group (Table 8.6). In the glucose impaired group, fasted glucose displayed moderate to strong associations with appendicular lean tissue (r= 0.40), body fat (r= 0.71), dSAT-sSAT ratio (r= 0.48), and waist circumference (r= 0.41). Similarly, Matsuda index exhibited moderate to strong associations with appendicular lean tissue (r= -0.54), body fat (r= -0.91), dSAT-sSAT ratio (r= -0.64), and waist circumference (r= -0.74). Glucose-insulin AUC also presented with moderate to strong associations with appendicular lean tissue (r= 0.83), body fat (r= 0.62), and waist circumference (r= 0.71).

Table 8.6. Correlation coefficients between body composition metrics and blood glucose homeostasis

		Fasted glucose	2-hour glucose	Glucose AUC	Matsuda index	Glucose-insulin AUC
Healthy (n=22)	Appendicular lean tissue index	0.12	-0.32	0.03	-0.11	-0.10
	Body fat	0.03	0.11	-0.14	0.13	-0.16
	Deep/superficial subcutaneous adipose tissue	-0.13	-0.17	-0.07	-0.01	0.01
	Waist circumference	0.24	0.051	0.19	**-0.36**	0.06
Glucose impaired (n=10)	Appendicular lean tissue index	**0.40**	-0.29	0.24	**-0.54**	**0.83**
	Body fat	**0.71**	0.03	0.33	**-0.91**	**0.62**
	Deep/superficial subcutaneous adipose tissue	**0.48**	-0.25	-0.33	**-0.64**	-0.07
	Waist circumference	**0.41**	-0.27	0.09	**-0.74**	**0.71**

Moderate correlation coefficients (r≥0.36) are bolded. AUC, area under the curve.

8.4.1 Discussion

Here, we observed that in healthy older males who have normal glucose homeostasis, elevated skeletal muscle echo intensity of the anterior upper leg and anterior abdomen was associated with poorer glucose tolerance during a 2-hour oral glucose tolerance test. In contrast, in older males with prediabetes or diabetes, increased skeletal muscle echo intensity was associated with better glucose homeostasis, whereas increased muscle thickness was associated with indices of poorer glucose control. Similarly, the dSAT to sSAT ratio was positively associated with elevated fasting glucose and negatively associated with Matsuda index in the glucose impaired group, but not the healthy group. Despite these divergent associations between the normal and glucose impaired groups, no differences in muscle thickness, echo intensity, dSAT to sSAT ratio, or adipose tissue thickness were observed between healthy and glucose impaired participants.

8.4.2 Skeletal muscle echo intensity displays divergent associations with glucose in older males with normal or impaired glucose control

The ageing-related shift in skeletal muscle composition towards increased intramuscular adipose is associated with poorer glucose homeostasis [175]. While the underlying etiology of this association is not entirely understood, several secreted substances, including free-fatty acids and adipokines, are likely important factors contributing to the regulation of skeletal muscle insulin sensitivity due to its physical proximity [203]. Although several groups have demonstrated associations between skeletal muscle echo intensity and intramuscular adipose tissue [138], [185], [201], the relation between echo intensity and metabolic health, particularly within older adults, is unclear. Harris-Love et al. (2018) [185] observed that both intramuscular

adipose tissue (computed tomography) and rectus femoris echo intensity displayed similar strength of associations (r= 0.38 and 0.43, respectively) with 2-hour glucose following a 75 g oral glucose tolerance test in healthy individuals without metabolic abnormalities. While we observed a slightly stronger association between rectus femoris echo intensity and 2-hour glucose (r=0.59) in our healthy group, there was a similar magnitude correlation for rectus abdominis echo intensity and 2-hour glucose (r=0.36).

AUC metrics of the oral glucose tolerance test are arguably more indicative of glucose homeostasis, as it provides a comprehensive view of glucose, insulin, and c-peptide processing throughout the entirety of the test, rather than a single timepoint. While we observed only weak associations between skeletal muscle echo intensity and glucose AUC, which has been observed by others using computed tomography [204], there was a moderate association between the anterior upper leg echo intensity and the glucose-insulin AUC, indicating that the commonly used quadriceps muscles may be the best choice for evaluating muscle composition in relation to glucose homeostasis. These moderate correlation coefficients between glucose homeostasis metrics and muscle echo intensity are of similar strength of previously observed associations between thigh intramuscular adipose tissue and indices of skeletal muscle insulin sensitivity [80], [175]; which further add to the validity of echo intensity and as a valid metric of muscle composition in individuals with normal glucose homeostasis.

In contrast, we observed that increased muscle echo intensity (i.e. poorer muscle composition) was moderately associated with better glucose homeostasis in the glucose impaired group, including fasting glucose, glucose AUC, glucose-insulin AUC, and Matsuda index. While these divergent associations between the healthy and glucose impaired groups are

unexpected, some methodological limitations of muscle echo intensity may confound their interpretation. The thickness of the subcutaneous adipose tissue overlaying the muscle may decrease muscle echo intensity due to beam attenuation in deeper tissues, which would be interpreted as improved skeletal muscle composition [138], [139], [200]. However, there were no differences in the subcutaneous adipose tissue thickness between the healthy and glucose impaired groups, which indicates that the confounding influence of adipose tissue thickness is unlikely to be the primary influence for these divergent associations. Speculating, there may be differences in the degree of beam attenuation through the subcutaneous adipose tissue due to unknown differences between the groups (e.g. adipose inflammation), which may have influenced the glucose impaired group to a greater extent. Given the consistent associations between intramuscular adipose tissue (computed tomography) and glucose homeostasis across healthy, glucose intolerant, and diabetic individuals [79], these divergent associations question the validity of echo intensity as a metric of muscle composition in glucose impaired individuals.

8.4.3 Skeletal muscle thickness is marginally associated with glucose homeostasis in the healthy group, but displays negative associations in the glucose impaired group

The influence of skeletal muscle mass on glucose homeostasis is controversial. Several publications have demonstrated that increased muscle mass or lean tissue is associated with improved glucose homeostasis [71]–[73], however, many others have demonstrated none or even negative associations [74]–[77]. Furthermore, many of the publications that observed positive correlations between muscle mass and glucose homeostasis normalize muscle mass relative to body weight, confounding the interpretation [75]. Here, we observed that muscle thickness was not indicative of glucose homeostasis in the healthy group, with the exception of

a moderate correlation between anterior abdomen and glucose-insulin AUC. In line with these findings, Ido et al. (2015) [110] observed that risk of metabolic syndrome was negatively associated with the anterior abdomen muscle thickness, but not appendicular lean tissue or 8 other muscle thicknesses, in middle-aged and older obese men and women. While the role of overall skeletal muscle mass in relation to glucose homeostasis is unclear, analyses focusing on site-specific muscle mass in relation to metabolic health are needed to clarify these findings. In the glucose impaired group, muscle thickness was positively associated with poorer glucose homeostasis, which has been previously observed in diabetic individuals [205]. Furthermore, several cross-sectional studies have demonstrated that diabetic individuals have elevated muscle and lean tissue mass in comparison to glucose tolerant individuals [81], [199], [206]–[208]; however, this is not universally observed [209]. While we did not observe significant differences in muscle thickness between the healthy and glucose impaired groups, lean tissue and fat mass are positively correlated with each other [210]. Thus, the associations between increased muscle thickness and poorer glucose homeostasis may be confounded by increased whole body adiposity, which was significantly higher in the impaired glucose group.

8.4.4 Deep to superficial subcutaneous adipose tissue ratio is moderately associated with impaired glucose homeostasis in the glucose impaired, but not healthy group.

While there were no differences in dSAT and sSAT thickness between the normal and glucose impaired groups, the ratio of dSAT to sSAT displayed moderate associations with elevated fasting glucose and reduced Matsuda index in the glucose impaired group, but not the healthy group. Others have reported positive associations between dSAT and sSAT area (computed tomography) and fasting glucose and homeostatic model of insulin resistance in

individuals with diabetes [211] and metabolic syndrome [212], which is in agreement with our findings in the glucose impaired group. However, Kelley et al. (2000) [90] also observed a negative association between dSAT area and glucose disposal (r= -0.64) in healthy lean and obese young males and females, whereas we observed no associations with glucose homeostasis in the healthy group. These differences may be due to age groups evaluated (younger vs older adults), but also related to differences in methodology used (cross-sectional area vs. thickness), as dSAT and sSAT thickness is only moderately correlated with cross-sectional area [46]. Indeed, Marinou et al. (2014) [32] observed weak associations between dSAT or sSAT thickness and fasting glucose in middle-aged adults. Further clarifications are needed to better understand the relation of dSAT and sSAT thickness in relation to glucose homeostasis.

8.4.5 Limitations

There are several limitations to this study. First, we recruited a relatively small sample size, which was further divided due to the divergent associations between the healthy and glucose impaired groups. Furthermore, most of the individuals within the glucose impaired group were in the prediabetic stage, which limits the applicability of these findings to those individuals with poorer glucose homeostasis (i.e. diabetics). Our primary analyses focused on skeletal muscle mass and composition; however, metrics of glucose homeostasis derived from an oral glucose tolerance test are not singularly regulated by skeletal muscle insulin sensitivity. Several factors, including liver and adipose tissue insulin sensitivity and pancreatic release of insulin, are important regulators of blood glucose, which would confound the comparisons with skeletal muscle thickness and echo intensity.

8.4.6 Conclusions

Lower skeletal muscle echo intensity, indicative of less intramuscular adipose tissue infiltration, is associated with better glucose homeostasis in individuals with healthy glucose tolerance. Whereas in individuals with impaired glucose homeostasis, elevated skeletal muscle echo intensity is associated with better glucose homeostasis and muscle thickness correlated with poorer glucose management. These divergent associations with echo intensity and metabolism between those individuals with normal or impaired glucose homeostasis require further clarification.

CHAPTER 9

INTEGRATED DISCUSSION

9.1 Current state of sarcopenia assessments

This thesis aimed to compare how site-specific and traditional measures of muscle tissue differ between younger and older adults, and how these indices relate to muscle function and metabolism in older adults. Characterization of ageing-related declines in skeletal muscle mass, composition and function is a critical aspect for identification of sarcopenia in older adults. The European Working Group on Sarcopenia in Older People have recently updated their definition and protocol for identifying sarcopenia in clinical practice, which is endorsed by a range of international societies [213]. These current guidelines indicate probable sarcopenia in the presence of low muscle strength, which is then confirmed by low muscle mass [10]. While several different modalities can be used for identifying low skeletal muscle mass, the most commonly suggested metric is appendicular lean tissue mass. However, given the emerging understanding of non-uniform changes in skeletal muscle tissue across the body, using whole-body indices of skeletal muscle mass may not be the ideal approach for assessing muscle loss with ageing. Yet, there is a paucity of literature comparing how site-specific and traditional whole-body indices relate to the functionality of muscle in older adults.

9.2 Site-specific skeletal muscle thickness and echo intensity of the anterior abdomen and upper leg display robust differences between older and younger adults

It is well-established that appendicular lean tissue mass declines with advancing age [65]. Here, we observed that compared with younger adults, both older males and females

displayed decreased appendicular lean tissue mass when matched for subcutaneous adipose tissue thickness. However, when matching for *relative* appendicular lean tissue mass using age- and sex-specific normative data, only minor, non-significant differences existed for appendicular or regional lean tissue mass between younger and older males. On the contrary, muscle thickness of the anterior upper leg and anterior abdomen was observed to be consistently and robustly lower in older compared to younger adults, regardless of if participants were matched for relative muscularity or absolute adipose tissue thickness. Importantly, differences were not evident between older and younger males for other muscle groups. These robust site-specific differences in skeletal muscle thickness suggest that the anterior upper leg and anterior abdomen may be useful indicators for identifying sarcopenia in older adults compared with traditional whole-body indices (i.e. appendicular lean tissue mass). However, when the older males were grouped based on previously established cutpoints for low or normal appendicular lean tissue mass, significantly smaller muscle thickness were evident across several muscle groups. Therefore, identification of low muscle mass using appendicular lean tissue or site-specific muscle thickness may be an adequate approach.

In regards to skeletal muscle composition, several publications have demonstrated that older adults display elevated muscle echo intensity in comparison to middle-age and younger adults [16], [136], [194], [214]. While some have attempted to account for the confounding influence of subcutaneous adipose tissue thickness on skeletal muscle echo intensity through the use of a correction factor (derived comparing MRI intramuscular adipose tissue and echo intensity) [138], [141], it is unclear if this correction factor can be universally applied across all ultrasound devices, protocols and setups. To mitigate the influence of SAT thickness on echo

intensity, we matched older and younger adults for absolute SAT thickness and observed that the anterior upper leg and anterior abdomen, but not the anterior upper arm, displayed elevated muscle echo intensity in the older adults compared to younger adults. Interestingly, these findings align with the degradation of the muscle thickness across these similar sites. Even in older adults who were not matched for subcutaneous adipose tissue thickness with younger adults, elevated echo intensity was observed across several muscle groups, including the anterior upper arm. The elevated muscle echo intensity in the older adult group, when not matched for subcutaneous adipose tissue thickness, could potentially be due to the increased overall adiposity, which is positively associated with intramuscular adipose tissue [69]. Speculating, there are likely competing influences for the effects of subcutaneous adipose tissue thickness on muscle echo intensity. The positive associations between adiposity and intramuscular adipose tissue suggest that obese individuals would display elevated echo intensity, due to poorer muscle composition; however, the elevated subcutaneous adipose tissue overlying the muscle of interest would attenuate the pixel intensity values in the deeper tissues, artificially decreasing echo intensity. These competing factors make it challenging to definitively interpret differences in muscle echo intensity.

9.3 Ultrasound muscle thickness and appendicular lean tissue display similar magnitude association with strength, but echo intensity requires further clarification as a valid metric of muscle composition

Contrary to our hypotheses, site-specific muscle thickness and DXA appendicular lean tissue provided similar magnitude associations with indices of muscle strength and power, indicating that both measures are potentially useful in the identification of sarcopenia.

However, given the stronger, albeit non-significant, associations of regional lean tissue mass compared to muscle thickness or appendicular lean tissue mass in relation to muscle strength, regional lean tissue mass from DXA should be further evaluated for the identification of sarcopenia. Furthermore, changes in the upper leg lean tissue exhibited a greater degree of atrophy with advancing age compared to appendicular lean tissue mass. However, it should be noted that changes in thigh muscle cross-sectional area or volume may not be adequately accounted for using DXA thigh lean tissue mass [215], highlighting the disconnect between muscle and lean tissue.

Compared with ultrasound muscle thickness, the stronger associations we observed for regional lean tissue mass with muscle strength is likely attributed to the fact that muscle thickness reduces a 3-dimensional muscle structure (i.e. volume or mass) to a 1-dimensional linear distance (thickness). Whereas regional DXA measurements capture the entirety of the lean tissue mass within that area, which may be more representative of the contractile tissue mass of the muscle. However, by utilizing panoramic ultrasound, muscle cross-sectional area can be readily obtained using clinically available ultrasound units, which displays strong associations with MRI and CT measured muscle area [216]. Yet, these measures of muscle area will still be confounded by the inability to distinguish IMAT from contractile muscle tissue, which would over-estimate the degree of contractile tissue in older adults or clinical populations.

The additional benefit that ultrasound offers over DXA, is the ability to quantify surrogates of skeletal muscle composition. We observed that muscle echo intensity was associated with glucose homeostasis in healthy individuals, whereas appendicular lean tissue

was not. While metabolic health is not a component of sarcopenia assessment, several groups have evaluated features of sarcopenia in relation to glucose homeostasis [28], [66], [77]. Importantly, within the healthy group, the association between muscle echo intensity and indices of glucose tolerance were of similar magnitude to those observed using more direct measures of intramuscular adipose tissue (e.g. CT) and glucose homeostasis [80], [175], adding to the validity of skeletal muscle composition as a surrogate of muscle composition. However, the divergent associations between glucose homeostasis and muscle echo intensity for healthy and glucose impaired individuals questions the validity of muscle echo intensity as a surrogate for muscle composition. This is particularly an issue given that intramuscular adipose tissue measures from CT or MRI display similar associations across lean, obese, and diabetic individuals [80], [175]. Future work implementing additional features of muscle composition using ultrasound, such as texture analysis or backscatter modelling, may be useful to clarify the use of ultrasound for muscle composition analysis.

9.4 Ultrasound and dual-energy x-ray absorptiometry provide unique benefits and limitations for identification of sarcopenia in clinical settings

Sarcopenia is increasingly being encountered within clinical practice and with its recent designation into the International Classification of Disease, an operational definition that can be applied in clinical centres is needed [213]. While both ultrasound and DXA offer valid options for assessing skeletal muscle mass in research settings, the feasibility of these tools in clinical settings need to be taken into consideration. Considerations should include cost, ease of use, time required for assessment, training requirements, reliability, validity, accessibility, portability, and availability of normative data. Ultrasound has clear advantageous over DXA for

cost, accessibility, and bedside portability, however, there is a lack of standardization for how to obtain measurements and the training required to obtain reliable and valid results. These challenges limit the ability to develop normative data and cutpoints for identifying low skeletal muscle mass using muscle thickness or cross-sectional area. On the contrary, due to the well-established protocols for DXA, rapid assessment times (~6 minutes/scan), standardized training (e.g. medical radiation technologists), and excellent reliability have resulted in a wealth of normative data [65]. Yet, the lack of portability limits DXA's accessibility for clinical settings, unless these scanners are available on site. Furthermore, there are differences in manufacturer protocols and software algorithms, which make comparisons across different units challenging [217]. Ultimately, while both modalities are feasible and valid options for use in research settings, further work is needed to clarify their usability in clinical settings for the identification of sarcopenia.

9.5 Overarching limitations

Site-specific analysis of skeletal muscle mass is an overarching theme of this thesis. The primary metric we utilized to evaluate mass of specific muscle groups was thickness; however, analysis of muscle cross-sectional area or volume is a more accurate representation of mass than thickness. While evaluation of cross-sectional area or volume may be more representative of mass, the analysis of muscle thickness represents a much more clinically feasible approach, as even using panoramic ultrasound for assessing cross-sectional area requires additional training and time for assessment. Similarly, a primary metric of interest throughout the thesis is skeletal muscle composition, which we evaluated using muscle echo intensity. However, given several limitations associated with the interpretation of muscle echo intensity (e.g.

subcutaneous adipose tissue thickness), more direct evaluation of intramuscular adipose tissue using established imaging modalities (CT, MRI) may be needed to better understand ageing related changes in site-specific muscle composition.

For Study 4 (Chapter 9), our primary interest was in relating skeletal muscle echo intensity to indices of muscle glucose homeostasis. However, indices derived from the oral glucose tolerance test are not solely a factor of skeletal muscle, as liver, adipose tissue, and pancreatic function are significant regulators of glucose tolerance. A more direct measure of muscle glucose homeostasis, such as a hyperinsulinemic euglycemic clamp, would be a more appropriate comparison in relation to muscle echo intensity. However, given the more invasive nature of these clamps, this was not a feasible option.

Lastly, we recruited a relatively small sample size of older males. This limited sample size not only restricts the comparisons to a single sex (for Studies 1, 3 and 4), but also limits the statistical power to detect differences between older and younger adults or in relation to muscle function or metabolism. Furthermore, the older adult cohorts represented a wide age range (65-92 years of age), during which substantial changes in body composition occur. Therefore, combining both 'young' (65-79 years) and 'old' (>80 years) older adults into a single group may confound the interpretation of our findings, particularly given that adults over 80 years of age may experience accelerated skeletal muscle atrophy and deposition of intramuscular adipose tissue. Lastly, while several older males from the prospective cohort displayed low appendicular lean tissue mass, very few would be identified as sarcopenic based on recent guidelines, as the majority of recorded grip strengths were above cutpoints for low muscle strength. Therefore, while we were interested in understanding how site-specific

measures of muscle mass and composition compare to traditional appendicular lean tissue mass in relation to identification of sarcopenia, the lack of sarcopenic individuals limits our ability to extrapolate to these individuals.

9.6 Future directions

The most obvious next steps are to confirm these findings using more representative measures of site-specific skeletal muscle mass (e.g. cross-sectional area or volume) and composition (e.g. intramuscular adipose tissue) in both older males and females. However, given the positive associations between muscle thickness and cross-sectional area and between muscle echo intensity and IMAT, I would hypothesize similar findings would be observed, except for skeletal muscle echo intensity in individuals with poor glucose homeostasis. Ultimately, if the purpose of assessing site-specific indices of muscle mass and composition is for the identification of individuals with sarcopenia, I believe the critical next steps are to: 1) evaluate these features of site-specific muscle mass and composition in sarcopenic and non-sarcopenic older males and females, 2) determine if these site-specific body composition features are useful for identifying older adults with mobility or functional capacity limitations in comparison to traditional appendicular lean tissue mass, and 3) examine the feasibility and practicality of implementing different modalities for evaluating site-specific body composition in clinical settings.

9.7 Conclusions

In comparison to younger adults, older adults present with smaller muscle thicknesses at the anterior upper leg and anterior abdomen, indicating these sites are largely impacted by advancing age and may be useful for identification of sarcopenia. However, in older adults

displaying low appendicular lean tissue, all muscle thickness sites are decreased relative to older adults with normal amounts of appendicular lean tissue. In contrast, muscle echo intensity is elevated across several muscle groups of the body, suggesting that muscle composition deteriorates across the body, rather than being focused at specific muscles. Importantly, both ultrasound muscle thickness and DXA lean tissue provide similar magnitude associations with indices of muscle strength and power, which provide validity for their use in assessment of sarcopenia. Furthermore, elevated skeletal muscle echo intensity is indicative of poor glucose homeostasis in healthy individuals but displays divergent associations in individuals with impaired glucose metabolism. These divergent associations require clarification to further validate skeletal muscle echo intensity as a valid metric of muscle composition. Overall, this thesis highlights the potential usefulness of site-specific muscle mass and composition for the identification of sarcopenia.

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APPENDICIES

APPENDIX A1 DETAILED METHODS

Plasma was analyzed for glucose at the 0, 15, 30, 45, 60, 90, and 120 minute timepoints and for insulin and c-peptide at the 0, 30, 60, 90, and 120 minute timepoints. Glucose was analyzed in triplicate using a peroxidase-glucose oxidase enzymatic reaction. To each test tube, 2.5 mL of a reagent solution containing peroxidase-glucose oxidase and o-dianisidine dihydrochloride (Thermo Fisher Scientific, East Providence, RI) was added and incubated with 10 µL of plasma or glucose standard at 37° C for 30 minutes. The absorbance of the product, oxidized o-dianisidine, was read at 450 nm (Cytation 5, BioTek, Winooski, VT) and glucose concentration was calculated based on a standard curve. Time points with coefficients of variation >5 % were repeated.

Plasma insulin and c-peptide were analyzed in duplicate using commercially available radio-immunoassay kits (MilliporeSigma, Billerica, MA) for human insulin and c-peptide, respectively. To each test tube, 100 µL of assay buffer, sample or standard, hydrated ^{125}I-labelled insulin or c-peptide, and human insulin or c-peptide antibody, which was incubated for 24 hours at either room temperature (insulin) or 4° C (c-peptide). After incubation, 1 mL of precipitating reagent was added and centrifuged (3000 g) for 20 minutes at 4° C. The supernatant was decanted, and remaining pellets measured for gamma radiation at 1-minute intervals. Insulin and c-peptide concentrations were calculated from a standard curve. Time points with coefficients of variation >15 % were repeated.

Triacylglycerides were analyzed in duplicate using commercially available triacylglyceride-GPO reagent set (Pointe Scientific, Canton, MI). In each test tube, 1 mL of

reagent was incubated with 10 µL of sample or standard for 5 minutes at 37° C. The absorbance of the resultant product was read at 500 m (Cytation 5, BioTek, Winooski, VT). Total cholesterol and high-density lipoprotein cholesterol were analyzed in duplicate using commercially available cholesterol and precipitating reagent set (Pointe Scientific, Canton, MI). Total cholesterol reagent (1 mL) was incubated with 10 µL of sample or standard for 5 minutes at 37° C. The absorbance of the resultant product was read at 500 m (Cytation 5, BioTek, Winooski, VT). For the high-density lipoprotein cholesterol analysis, 500 µL of serum was incubated with 500 µL of precipitating reagent and centrifuged for 5 minutes (2000 g), which precipitates the all non-high-density lipoprotein fractions. Following precipitation, 50 µL of supernatant was then analyzed in the same fashion as total cholesterol.

APPENDIX A2 DETAIL ULTRASOUND LANDMARKS

Table A1. Overview of ultrasound-based landmarks for analysis of site-specific body composition

Landmark	Location	Tissues of interest	Type of analysis
Anterior upper arm	• Anterior and posterior surface, 60% distal from the acromial process of the scapula to the lateral epicondyle of the humerus	• Biceps brachii • Brachialis	• Muscle thickness • Muscle echo intensity • Adipose thickness
Posterior upper arm		• Triceps brachii	• Muscle thickness • Muscle echo intensity
Anterior forearm	• Anterior surface, 30% distal from the head of the radius to the ulnar styloid process	• Flexor compartment	• Muscle thickness
Anterior trunk	• 3 cm lateral to the umbilicus • 5 cm lateral to umbilicus (dSAT, sSAT)	• Rectus abdominis • sSAT • dSAT	• Muscle thickness • Muscle echo intensity • Adipose thickness • dSAT and sSAT thickness
Anterior thigh	• Lower third between the anterior superior iliac spine and superior border of the patella	• Rectus femoris • Vastus intermedialis	• Muscle thickness • Muscle echo intensity • Adipose thickness
Posterior thigh	• Posterior surface, 50% between the greater trochanter and lateral epicondyle of the femur	• Biceps femoris	• Muscle thickness
Anterior lower leg	• Anterior and posterior surface, 30% distal between the lateral tibial condyle and the lateral malleolus	• Tibialis anterior	• Muscle thickness • Muscle echo intensity
Posterior lower leg		• Gastrocnemius • Soleus	• Muscle thickness • Muscle echo intensity

APPENDIX A3 APPROVAL FOR STUDY 2

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