Developing measures of muscle health in women diagnosed with breast cancer and in a healthy, young adult reference cohort

by

Egor Avrutin

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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:	Kristin L. Campbell, PT, PhD Professor, Department of Physical Therapy, University of British Columbia
Supervisor(s):	Marina Mourtzakis, PhD Associate Professor, Department of Kinesiology, University of Waterloo
Internal Member:	Monica R. Maly, PT, PhD Associate Professor, Department of Kinesiology, University of Waterloo
	Joe Quadrilatero, PhD Professor, Department of Kinesiology, University of Waterloo
Internal-External Member:	Alfred C.H. Yu, PhD Professor, Department of Electrical and Computer Engineering, University of Waterloo

Author's 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

Egor Avrutin was the sole author of Chapters 1-5, and 9 which were written under the supervision of Dr. Marina Mourtzakis. This thesis consists in part of three manuscripts written for publication. Exceptions to sole authorship of material are as follows:

Research presented in Chapter 6

This research was a secondary analysis of an existing database of DXA scans which were collected as part of research studies under the supervision of Dr. Marina Mourtzakis and Dr Clark R. Dickerson. Egor Avrutin, under the supervision of Dr. Marina Mourtzakis, was responsible for the conception of the study, data analysis, interpretation of results and drafting of the manuscript. Egor Avrutin, Amanda G. Pfeiffer, Schuyler Schmidt, Lisa Bos, Michael T. Paris, Jacquelyn M. Maciukiewicz, and Alicia L. Nadon contributed to participant recruitment and data collection. All authors contributed to critical review and revision of the manuscript.

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Research presented in Chapters 7 and 8

This research was conceptualized and conducted at the University of Waterloo by Egor Avrutin under the supervision of Dr. Marina Mourtzakis. Dr. Monica R. Maly provided critical input on the conception of the studies as well as laboratory space and equipment. Michael T. Paris and Dr. Kirsten E. Bell contributed to participant recruitment and data collection. Michael T. Paris assisted with image analysis for Chapter 7. Egor Avrutin conducted data analysis and drafted both manuscripts. All authors contributed to critical review and revision of the manuscript based on Chapter 8.

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Abstract

Skeletal muscle health is an important predictor of survival, treatment toxicity, and physical function in cancer patients and survivors. While accelerated rates of muscle wasting are often observed in patients with advanced cancer, the links between lower than optimal muscle mass and adverse outcomes have been reported across diverse cancer types and stages. With improvements in detection strategies and treatment, the 5-year survival rate in breast cancer patients is relatively favorable. With detrimental body composition changes that occur during treatment and extend into survivorship, mitigating derangements in muscle health may help improve quality of life, mobility and metabolic outcomes. For this purpose, it is important to not only understand the alterations in muscle characteristics that occur throughout the breast cancer trajectory, but also potential links between muscle features such as size, composition and architecture with respect to muscle strength and function. Computed Tomography (CT) and Dual-energy X-ray Absorptiometry (DXA) are two tools commonly used to study muscle mass or muscle size. DXA assesses lean mass at the whole body or regional level and offers an opportunity to evaluate functionally relevant muscle groups. The first research study of this thesis examined DXA-based body composition profiles of breast cancer patients with lower that normal muscle mass, along with common strategies proposed to help adjust for differences in body size, in relation to muscle strength. The focus of studies 2 and 3 is on use of ultrasound-based imaging methods for evaluating skeletal muscle size, composition and architecture features in breast cancer patients and in a healthy, young adult reference cohort. Ultrasound is a portable, relatively inexpensive and accessible modality that may evaluate muscle size, composition and architecture, this modality has not been used for body composition analysis in cancer patients to date. Overall, finding of the studies in this thesis highlight several areas that warrant further method development both for DXA-based lean tissue mass and ultrasound-based muscle features, in order to help the assessment of muscle size, composition, architecture and strength in breast cancer patients in- and post-treatment.

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List of Abbreviations

ADP	Air Displacement Plethysmography
ALM	Appendicular Lean Mass
ALT	Appendicular Lean Tissue
ALTI	Appendicular Lean Tissue Index
AU	Arbitrary Units
BC	Breast Cancer
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CI	Confidence Interval
CSA	Cross-Sectional Area
CT	Computer Tomography
DXA	Dual-energy X-ray Absorptiometry
FM	Fat Mass
HU	Hounsfield Unit
ICC	Intraclass Correlation Coefficient
ICU	Intensive Care Unit
IMAT	Intermuscular Adipose Tissue
IPAQ	International Physical Activity Questionnaire
L3	Third Lumbar Vertebra
LM	Lean Mass
MRI	Magnetic Resonance Imaging
MVC	Maximum Voluntary Contraction

SD	Standard Deviation
SPPB	Short Physical Performance Battery
WC	Waist Circumference
%CV	Percent Coefficient of Variation

Chapter 1

Overview

Body composition assessment, and specifically measurement of muscle characteristics, is increasingly used to help better understand tissue level perturbations that occur during illness. Muscle health, which encapsulates muscle size, composition, architecture and function, is an important mediator of both primary (i.e. survival, treatment toxicity and post-operative complications) as well as secondary outcomes (i.e. development of comorbidities, physical function, and quality of life) in disease. In cancer, reduced muscle size is specifically associated with impaired physical function, increased risk of treatment toxicity, delayed treatment regimens and overall survival. Loss of muscle mass may also be linked with increased risk of diabetes and cardiovascular disease. Nutrition and exercise interventions can be utilized to help reduce the common detrimental effects of cancer and cancer treatment on muscle health during the disease trajectory and into survivorship. Yet, development and validation of methods to enable effective and practical assessment of muscle characteristics is needed. This step is critical for establishing an in-depth understanding of muscle health at baseline and in cancer patients, and to help track the effectiveness of future interventions. This thesis is comprised of an investigation into the methodology of muscle size, composition and architecture measurements and the relationship of these muscle features with respect to muscle strength.

Muscle size, composition and architecture are important determinants of muscle function. Body composition assessment plays an increasingly important role in helping identify

derangements in muscle health in clinical populations, and in settings where effective testing of muscle function may not be feasible. Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Dual-energy X-ray Absorptiometry (DXA) are well accepted tools that can be used to measure lean tissue mass, muscle size and attenuation. Furthermore, ultrasound is an emerging method that provides a practical and accessible alternative to reference modalities and further offers the ability to capture muscle architecture. Chapters 2 and 3 outline fundamental concepts in skeletal muscle tissue characteristics and body composition assessment methodology, respectively.

With the increasing use of body composition assessment methods, studies were able to precisely determine *in-vivo* tissue specific alterations that occur in patients with cancer in- and post-treatment. Chapter 4 contains a review of literature relevant to skeletal muscle during the cancer trajectory. Adverse clinical outcomes have been reported across a diverse range of cancer types and severities. Due to improvements in cancer screening and treatments, there is a need to study not only the immediate biology of the tumour and primary treatment, but also better understand the detrimental effects of cancer treatment which may extend into survivorship. Specifically, lower than normal muscle mass is a predictor of survival, treatment toxicity, and physical function in breast cancer patients and survivors. Effectively identifying patients at risk of poor physical function and evaluating the underlying relations between muscle size, composition and architecture with respect to strength can help guide exercise and nutrition interventions. Chapter 5 further discusses the important gaps, rationale and the objectives of the thesis, and outlines the objectives and hypotheses of the research studies.

Chapters 6-8 contain manuscripts detailing the original research that represent this thesis. These chapters investigate DXA-based classifications of low lean tissue mass in relation to muscle strength, and strategies to help adjust for differences in adiposity, in a cohort of breast cancer patients in- and post-treatment. This work also evaluates ultrasound-based muscle size, composition and architecture measurements in breast cancer patients and in a reference young healthy adult cohort. In turn, Chapter 9 includes an overview of key findings, an integrative discussion, limitations and future directions as well as conclusions.

Chapter 2

Muscle tissue characteristics in health and disease

Skeletal muscle comprises about 40% of total body weight [53, 61]. Contraction of skeletal muscle is important for movement, breathing and balance [53, 91, 112]. Muscle tissue function extends beyond contraction because it is involved in metabolic regulation, cytokine signaling, energy expenditure and thermogenesis [61, 170]. In aging individuals, loss of muscle mass and concurrent declines in muscle contractile function may lead to mobility limitations, risk of falls and inability to complete activities of daily living [35, 44, 67, 90]. There are multiple examples where derangements in muscle function, composition or structure are involved in disease states including organ failure, respiratory disease, sepsis, burns and cancer [52, 60, 112, 170].

Examination of muscle mass alone may by insufficient in elucidating the full extent of the perturbations that occur in this tissue over the course of aging or disease [60, 102]. Muscle quality is a general term used in the body composition literature to describe muscle characteristics other than quantity [41, 60]. Skeletal muscle health can be determined by its composition (i.e. infiltration of muscle with non-contractile adipose or fibrotic tissue), and structure (i.e. architectural orientation muscle fascicles), as well as the overall integrity of the tissue (i.e. functionality of muscle based on its composition and structures). Together, muscle tissue characteristics such as mass, composition and structure, along with muscle

function can be referred to as muscle health. Muscle health is increasingly recognized for its role in mobility- related outcomes as well as multiple clinical endpoints. In the absence of adequate maintenance of muscle health, when muscle features and function are sub-optimal, the ability of the body to handle and respond to disease or stressors is reduced [52, 102, 170].

Skeletal muscle is a highly organized tissue system [53, 61]. Yet, the links between muscle characteristics as they relate to muscle function are not completely understood. The make-up of skeletal muscle is complex and involves multiple layers of organization, which include molecular, cellular and tissue levels [53, 61, 112]. While muscle features are commonly studied using conventional laboratory approaches such as animal models and cell culture, several studies using human skeletal muscle tissue have also been performed. Skeletal muscle biopsies are popular for studying the various properties of skeletal muscle [71]. Traditionally, biochemical and histological examination of tissue samples ex-vivo was necessary to evaluate these composition features [71, 92, 112]. However, this approach is invasive and localized to a small area. *In-vivo* measurements are well-suited for studying muscle features in a non-invasive fashion in healthy and clinical populations [71]. Thus, body composition techniques are advancing in their protocols to provide more detailed information about skeletal muscle during illness. While body composition assessment modalities are reviewed in Chapter 3, the current chapter provides the underlying concepts essential to interpreting body composition outcomes in healthy and clinical populations.

2.1 Overview of skeletal muscle tissue characteristics

Skeletal muscle tissue is predominantly composed of water (75%) and protein (20%) [61]. Skeletal muscle protein is involved in a structural, contractile or regulatory role, and amounts to approximately 80% of non-water muscle content. Muscle protein is the main amino acid reserve in the body, and alterations in protein turnover are extensively involved in muscle remodeling [61]. Skeletal muscle cells (also called myocytes) contain deposits of stored glycogen and lipid (termed intramyocellular fat) [3, 53, 92, 170]. Although the rela-

tive contribution of glycogen and lipids to overall tissue weight is small, given that muscle makes-up a substantial proportion of total body weight, both glycogen and lipids are important mediators of not only muscle-specific but also whole body energy and metabolic homeostasis [170].

At the cellular level, skeletal muscle is comprised of muscle fibres, which are a syncytium of muscle cells [37, 53, 92]. The assembly of muscle fibres has been studied extensively; during embryonic development this tissue is formed through the fusion of progenitor cells. Muscle fibres contain multiple nuclei, the contractile apparatus, organelles, signaling components [36, 53]. The distinctive striated appearance of skeletal muscle is due to a precise arrangement of the various components of the sarcomere, which is the main functional unit that forms myofibrils and generates contraction [53, 61]. Myocytes also contain specialized organelles that are crucial to the proper function of these cells [53, 61]. The sarcolemma (plasma membrane of muscle cells) along the T-tubule network are involved in excitation of the cell which is the stimulus for muscle contraction. The sarcoplasmic reticulum (SR) and the mitochondrial network are both structures within the muscle cells that are key to calcium storage or oxidative metabolism, respectively. The mitochondria in particular, have been extensively researched for their role in various metabolic derangements which occur during obesity, prolonged disuse or with aging [53, 61].

With a negative protein balance, reduced muscle protein content results in a decrease in muscle fibre size, and muscle mass as well as the loss of organelles and cytoplasm [53, 61, 92]. The complexity of muscle remodeling makes evaluating atrophy challenging, and the discrepancies between measurements of protein content, fibre cross sectional area, as well as wet weight versus dry weight have been highlighted in a recent review [71]. Conversely, muscle growth is thought to occur due to muscle fibre hypertrophy (increase in size), while fibre hyperplasia (increase in the number of fibres) is limited [71]. Muscle adaptations are not limited to growth or atrophy, but also include a shift in phenotype. Muscle fibre types include slow oxidative (type I) as well as fast intermediate (type IIa) and fast glycolytic (type IIx) in humans, which have been classified based on an array of features such as the myosin isoform, type of motor unit and metabolic profile among others [53, 61]. Shifts in fibre type have been featured extensively in research evaluating muscle adaptation to training, disuse or the alteration during aging or disease states.

Beyond the contents and structure of the myocytes, organization of the muscle tissue is another critical component of skeletal muscle health [92, 112]. Muscle fibres are encased in collagenous connective tissue termed the extracellular matrix, which plays a role both in the structural organization of the tissue and muscle function [63]. The extracellular matrix has been characterized in terms of 3 inter-connected layers of organization. The endomycium is a layer of connective tissue surrounding each muscle fibre. At a more macroscopic level of arrangement, perimycium encases bundles of muscle fibres, these bundles are commonly referred to as muscle fascicles. Furthermore, the epimysium layer of the extracellular matrix encases muscle groups [63]. The extracellular matrix merges with the tendons anchoring muscle groups. Thus, it is a key component of the elastic properties of skeletal muscle tissue as well as propagation of the force generated during contraction to the tendon [63].

The extracellular compartment also contains non-contractile structures that are nonetheless essential for the function of skeletal muscle, these include neuronal connections and blood vessels [63]. In addition, the extracellular space contains mononucleated cells that are extensively involved in tissue remodeling [53, 63]. Satellite cells are the most known example and these progenitor cell populations are responsible for muscle repair in response to injury or tissue damage, by contributing nuclei to terminally differentiated muscle fibres. When activated satellite cells enter the cell cycle and proliferate, this is followed by differentiation and fusion with existing muscle fibres or into myotubes [53]. Fibroblasts are another type of mononucleated cell found in skeletal muscle, whereby these cells are thought to play a role in the maintenance of collagen and the extracellular matrix [63]. Interestingly, several recent studies identified cellular mechanisms which enable satellite cells and perhaps fibroblasts to trans-differentiate towards the adipogenic fate [161]. This mechanism is an intriguing possible explanation for the cellular origin of the intermuscular adipose tissue, which alongside intramyocellular lipids accumulates with fatty infiltration of skeletal muscle tissue [3, 161].

The alignment of muscle fascicles relative to the direction of force generation (muscle architecture) is important and is not in all cases parallel. Three common muscle geometry types have been described in humans, parallel (all the fascicles are in line with the direction of force production), unipennate (all the fascicles in a muscle are oriented in one direction) and multipennate (there direction of fascicle insertion is varied across the muscle) [93, 111].

Muscle groups that feature fibres aligned at an angle relative to the tendon are common, the angle at which the fibres insert into the fascia surrounding the muscle is termed pennation angle [91–93, 112]. The angled alignment of muscle fascicles is an adaptation to help pack as many contractile elements as possible in a given area, or in other words sarcomeres in parallel, along the length of the tendon [84, 112]. An additional aspect of muscle geometry is fascicle length, which corresponds to the distance between the insertion of a fascicle at the deep fascia layer and the corresponding superficial insertion point of the same fascicle [93, 112]. While the resting operating length of each sarcomere (i.e. amount of overlap between the thick and the thin filaments) *in-vivo* is varied across different muscles, higher fascicle length values correspond to an increase in the number of sarcomeres in series [91, 93]. The number of sarcomeres in series or in parallel have been identified as an important determinant of contraction velocity and contraction force, respectively [91, 93]. It is thought that muscle architecture factors further elaborate the links between tissue characteristics to muscle function, as compared to simple cross-sectional area measurements [112].

2.2 Assessment of skeletal muscle health

Experimental methods such as animal models and cell culture, together with studies utilizing muscle biopsies, have revealed various properties of skeletal muscle as well as adaptations that may occur with training, disuse, aging or disease states [71]. However, much of our knowledge is based on invasive methods that include acquisition of tissue samples or is challenged by generalizing smaller experimental models to larger populations. Emerging *in-vivo* measurements of muscle characteristics using several of the body composition modalities discussed in the next chapter are needed to further expand the understanding of muscle health, particularly in populations where invasive methods are less feasible. As evidenced in studies that examine muscle characteristics in older adults, alterations in muscle properties such as size, function, composition (i.e. presence of fibrosis or fatty infiltration) or muscle architecture may be independent predictors of poor muscle health [60, 102, 113].

Changes in muscle contractile function may not always align with changes in muscle

mass [60, 102]. Loss of muscle strength has been found to be a more prominent predictor of mortality, as compared with muscle mass, in older adults [115]. A longitudinal study of 1880 older adults who were followed over a span of 3 years, and had leg lean mass (measured using DXA) and strength assessed, demonstrated that the decline in muscle strength was approximately three times greater than the decline in muscle mass [66]. These findings have been further supported by other studies [47, 77, 102]. It is increasingly recognized that examination of skeletal muscle quantity alone without consideration of other features may provide an incomplete perspective [60, 76]. Several molecular mechanisms are linked with the detrimental changes in muscle function, composition and structure [102, 113]. For instance, Type II muscle fibres atrophy to a greater extent than Type I fibres [102, 113]. An increase in muscle fat infiltration or changes in protein turnover are also involved in the process of aging [3, 102, 113]. Changes in muscle architecture have been reported during aging, and therefore differences in muscle structure may in-part account for the decline in muscle contractile capacity [93, 112]. Neuronal changes, such as motor unit remodeling and alterations in excitation contraction coupling properties, also contribute to a decrease in muscle strength and power [35, 126, 160]. While there is a plethora of evidence identifying the perturbations in muscle health that take place during aging, these are not limited to this cohort alone. Derangements in muscle characteristics and function have also been reported in several disease states.

An integrative approach is needed to evaluate the full scope of perturbations in muscle characteristics and muscle function, and how they mediate poor health outcomes. The contractile capacity of skeletal muscle tissue may be assessed as isometric strength, muscle power or using physical function tests [60, 91]. Nonetheless, these approaches may be limited due to multiple factors that include the availability of facilities and equipment, as well as the personnel training required to administer the various tests. Many of the contractile function tests also require the physical capacity and motivation to achieve maximal exertion levels needed to accurately determine muscle strength or power. *In-vivo* measurements of muscle size, composition and structure may potentially bridge this gap and may advance our understanding of the alterations that occur in skeletal muscle not only with aging but also various disease states [91, 92, 112]. Currently, an integrative knowledge base that maps the various muscle characteristics with respect to their influence

on muscle function is lacking and requires further method development to enable future research.

Chapter 3

Body composition assessment

Body composition analysis is a generic term that is used to describe assessment of the constituents of the human body [74]. Body composition may be examined at one of 5 levels of organization: the atomic, molecular, cellular, tissue and whole body levels [163]. In various circumstances and disease states, measurements using anthropometric methods such as body mass index (BMI), or waist circumference (WC), lack the specificity and precision to effectively evaluate muscle mass [155]. Assessment tools such as computed tomography (CT), magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DXA), or ultrasound may facilitate muscle quantification in both healthy and clinical cohorts [76, 128]. CT, MRI and ultrasound measure muscle quantities at the tissue level, while DXA is used to determine lean tissue mass at the molecular level [72, 75]. Skeletal muscle tissue is a term used to refer to the tissue/organ level of organization, while fat-free mass or lean mass is examined at the molecular level. These terms all refer to muscle quantities, yet both lean tissue and fat-free mass are comprised of more than skeletal muscle tissue [128].

The lack of a standardized single measurements for muscle health, is in part due to the practical considerations of assessing muscle characteristics *in vivo*. In many cases, body composition measurements rely on the use of modalities that are also used for medical care purposes. Considerations such as availability of facilities and personnel, as well as costs of operating the equipment are all factors which might limit the accessibility of those

modalities.

3.1 Multi-compartment models

The multi-compartment model approach to body composition measurements is a family of methods aimed at fractioning total body weight into 2 or more partitions [73]. At the molecular level, the human body is made up largely of 5 main groups of molecules, these are lipids, proteins, carbohydrates, minerals and water [73, 99]. Using a combination of measurement methods in an attempt to discern the underlying molecular properties, such as density, may support quantification of the relative proportion of various components.

3.1.1 Two compartment models

The two compartment model, which segments the body into fat versus fat-free mass, has been used for hydrostatic weighing, air displacement plethysmography and skinfold assessments. This approach centres around the measurement of water displacement, based on Archimedes' principle, to determine body volume which is then used to calculate density [72, 73]. Whole body density is then used together with assumed density constants for fat and fat-free tissue to determine the proportion or fat to non-fat mass [72, 73]. Beyond the assumed density values this approach requires a correction for air contained in the lungs and the intestines. Commonly this is achieved by instructing the subject to exhale fully then estimating the vital capacity of the lungs based on a predictive equation given the height and sex of the individual. A constant value of 0.1L is a commonly used estimate of air in the gastro-intestinal system [73, 99].

Beyond the specialized facilities that this approach requires, hydrostatic weighting relies on the subject being fully submerged and then exhaling maximally. This limits such assessment to individuals who are able to accomplish that, and it is often impractical in children, elderly and in some clinical populations [74]. More recently, air displacement plethysmography (ADP) has been developed as an alternative method of determining body

volume [73, 99]. Although it relies on similar underlying principles, in particular the relationship between pressure and volume under constant temperature (i.e. Boyle's law). This measurement eliminates the need for the subject to submerged, and instead a specialized sealed chamber is used to determine the volume of the participant placed inside [73, 99]. In addition, body volume measurements can be also performed using 3D scanning or DXA, which provides further flexibility with respect to the method used to derive volume and density measures that are needed not only for the two compartment model but also for more sophisticated multi compartment approaches [72, 73, 99].

3.1.2 Three compartment models

The density estimates used as part of the two compartment methods has been a subject of debate, and a few different assumed values have been reported by researchers [73]. While the density of fat that is reported in the literature is relatively consistent, estimates of fat-free tissue density are heterogeneous. The two compartment model relies of stable proportions of the various constituents that make up the fat-free compartment, which limits the generalizability of the density assumption outside the healthy reference range [73]. This limitation serves as a major motivating factor for the development of methods to enable further compartmentalization. Isotope dilutions (i.e. deuterium or tritium) emerged as an approach to measure water content *in-vivo*, and these were later used to establish a three level model which builds upon the two compartment model, where body weight is partitioned into fat, water and residual fractions [73].

3.1.3 Four compartment models

The development of photon absorptiometry and consequently dual energy x-ray absorptiometry have enabled researchers to further extend the multi-compartment models through providing an *in-vivo* measurement of bone mineral content, whereas the two and three compartment models lean tissue and bone minerals are grouped together as non-fat and residual fractions, respectively [72, 73]. Thus, the four compartment model is based on measurements of body weight and volume together with water and bone mineral content values

which can be used to calculate the relative proportions of fat, bone minerals, water and residual masses, where the residual mass by and large reflects soft lean tissue [73, 99]. Since multi-compartment models rely on the assumption that the density of each compartment is consistent across all individuals assessed, multiple physiological or pathological states can influence the validity of these assumptions and in-turn the accuracy of models [72]. A four compartment model is commonly considered adequate for effectively determining body composition not only in a general adult population, but also in children as well as in clinical cohorts [73, 99]. This method can be used to study body composition across various clinical populations because it accommodates pathological states the result in altered hydration status, loss of bone mineral content or lean tissue wasting. Beyond the four compartment approach, newer models incorporate additional measurements to provide the resolution required to determine five or even six different compartments [73].

3.1.4 Limitations of multi-compartment models and practical alternatives

A key limitation of some multi-compartment models is that they rely on several distinct assessments that need to be performed, for instance a combination of body volume measurement, DXA scan and isotope dilution [73, 99]. In most circumstances performing the required assessments is labor intensive and time consuming, it requires specialized facilities and multiple visits. Alongside the development of multi-compartment models a number of field based alternatives that include skinfold calipers and bioelectrical impedance analysis (BIA) have been established to help address this gap [99, 128]. Skinfold calipers can be used to estimate the thickness of subcutaneous adipose tissue layer and in conjunction with easily available co-variates such as age, sex, height and weight are used in a predictive equation calibrated against multi-compartment based measurements of fat mass [74]. Predictive equations are also involved in most of the body composition tests performed using BIA. This modality measures the impedance to an electrical current which travels between 2 electrodes, often placed on the wrists and ankles [74, 89]. Since lean mass is a better conduit of an electrical current as compared to bone or adipose tissue, a BIA estimate of resistance and reactance (i.e. the 2 components of impedance) are used together with height, sex,

age and weight in a predictive equation [74, 89]. Even though various reference modalities have been used to calibrate the BIA based predictive equations, multi-compartment models are commonly used for this purpose in the body composition literature [89]. Portable measurements are relatively effective in assessing various aspects of body composition, yet they are limited to the population wherein they were validated. Several reports highlight that in clinical cohorts, measurements such as bioelectrical impedance analysis commonly yield errors that are larger than what is expected in the general healthy population [51, 128].

3.2 Imaging modalities in the assessment of body composition

3.2.1 Dual-energy X-ray Absorptiometry (DXA)

While DXA is primarily used in the assessment of bone health, it is also a popular method for the assessment of fat and lean tissue mass [74, 99, 128]. DXA relies on the assessment of x-ray attenuation to determine molecular characteristics of the tissues and tissue mass is computed using proprietary algorithms [74]. Bone minerals, fat and lean tissues differently attenuate the passing x-rays. The recorded attenuation may be related to expected attenuation values of the respective tissues, and using empirically established density values the tissue masses are computed [74, 75]. The 2 different energies are used to compute the masses of the 3 compartments (bone mineral, fat and lean tissue). Researchers and technicians using this modality generally do not have access to either the raw attenuation values or the algorithms used to derive the body compositional metrics [74]. Due to the relatively small radiation exposure per scan, DXA is commonly used to analyze body composition in prospective cross-sectional and longitudinal studies. Another advantage of DXA-based measurements is the ability to conveniently assess whole body and regional tissue mass.

DXA does not assess skeletal muscle directly, rather it determines the lean tissue (which consists of muscle, connective tissue, and visceral organs) [76, 128]. An important caveat of these approaches is that the fat-free mass or lean mass compartments also encompass

connective tissue as well as visceral organs [128, 163]. In the extremities, skeletal muscle is the major constituent of lean tissue. Therefore, due to the confounding effects of visceral organs in the torso region, appendicular lean tissue is often used to assess muscularity rather than whole body lean tissue [76, 128]. The ability to segment the body into limbs, torso and head has set DXA apart from a number of alternative compartment-based approaches. DXA-based appendicular lean tissue mass is calculated by summing the lean mass of the legs and arms [76, 127]. However, similar to whole body weight, on average individuals with a larger stature will also carry more lean mass. In order to compare muscularity across different individuals, appendicular lean tissue mass (ALM) is often adjusted, most commonly it is expressed as ALM per height squared [15, 130]. This adjustment strategy borrows the approach used to calculated BMI values (weight per height squared), and the resulting value is termed appendicular lean tissue index (ALTI). While ALTI is the most common DXA-based measurement of muscularity, there are multiple additional adjustment methods that have been proposed to help capture muscularity relative to body size. DXA based lean tissue mass can be scaled to total body weight (i.e. percent lean tissue mass), to BMI or expressed a ratio of fat to lean masses [29, 50, 79, 101, 150].

3.2.2 Magnetic Resonance Imaging (MRI)

MRI offers the capability to specifically and precisely assess skeletal muscle and adipose tissue features in 2D and in some cases 3D. MRI relies on the use of a strong magnetic field to influence and consequently measure the intrinsic properties of hydrogen nuclei [74]. Specifically, the MRI scanner generate strong magnetic fields, which align the spin directions of the protons within hydrogen atoms [74]. A pulse ratio frequency is then applied to the tissues in order to alter the direction of spin. Once the pulsed radio frequency is switched off the protons return to the orientation induced by the magnetic field, this process is termed relaxation [74]. During relaxation, protons emit energy that is subsequently detected by the MRI scanner. Importantly, the time from the application of the pulsed radio frequency to relaxation is different depending on tissue composition [74]. For instance, protons within fat relax at a faster rate than protons in an aqueous medium [74]. The MRI scanner detects and determines the spatial orientation of the resonance of hydrogen

nuclei, the MRI terminal then uses this information to recreate 2D images of the tissue assessed. Measurement of multiple consecutive transverse sections may be used to recreate 3D images of the body [74].

Measurement of muscle tissue cross sectional areas on MRI images is performed in a fashion similar to CT images. Specialized software is used to help segment the areas based on grey level intensity ranges [72, 99]. Furthermore, multiple signal processing approaches may be used to help examine intermuscular adipose tissue, which similar to muscle area may be segmented using gray-scale thresholding during the image analysis stage [76]. MRI offers some advantages relative to CT image, including the lack of radiation exposure [49, 128]. Absence of radiation exposure from MRI has contributed its use in examining whole body tissue volumes, whereas CT scans are often limited to a specific region of the body (i.e. abdominal, thoracic, head and neck) to minimize radiation exposure. With the lack of radiation from MRI, it may be used in body composition of healthy reference cohorts and/or in longitudinal, prospective studies. However, MRI is also associated with a number of limitation including: the cost of operating the equipment, the time required to capture a regional and/or whole body image, and exclusion of those with metal implants [49, 128].

3.2.3 Computed Tomography (CT)

CT imaging is a medical modality introduced in the early 1970's [75, 100]. The basis of CT imaging is the attenuation of x-rays as they penetrate different tissues. The attenuation of the x-rays is dependent on the density and the composition of each tissue. Differences in these physical attenuation properties help distinguish between different tissue types [74, 128]. The scanner consists of an x-ray source and a detector, which both rotate relative to the individual being scanned to provide images of the participant from multiple directions. The detector of a CT scanner measures the attenuation of x-rays and digital reconstitution assigns each pixel in the image the corresponding attenuation value [74, 128]. Once the entire image is reconstituted, the attenuation values may be used to distinguish between the tissues [74, 128]. Additionally, multiple images may be reconstructed consecutively across a specific region, such as the abdomen, or in rare cases, the whole body to provide a 3D image [75, 100].

The cross-sectional area (CSA) of tissues may be determined with specialized software [100]. Two approaches may be used to determine tissue CSA, manual planimetry and semi-automated segmentation. Manual planimetry is the process of manually tracing the borders of the tissue, performed by an analyst while visually evaluating the images [100]. This approach has been used to determine the dimensions of various internal organs such as kidney, liver and spleen. Manual planimetry of CT images compared to autopsy measures demonstrated that this method is highly accurate [72, 75]. However, when tissues with irregular shapes, such as skeletal muscle, need to be analyzed manual tracing of tissue borders may be difficult. Semi-automated image segmentation is an alternative approach that may be performed using thresholds of specific attenuation value ranges [72, 100]. This approach is based on distinguishing between the gray-scale values of the pixels in the image. X-ray attenuation is measured using a Hounsfield Unit (HU) scale, where water corresponds to 0 and air is -1000 HU. Each pixel of an image is assigned a HU value, which is relative to air and water. The HU range of -29 to 150 is often used to select skeletal muscle tissue [72, 100]. Thus, the image is commonly analyzed using a combined process where the analyst visually selects the muscle tissue while specialized software helps filter all pixels that are outside the predetermined HU range. This image analysis method is highly reproducible. Coefficients of variation of 2% for fat and skeletal muscle tissues and less are commonly reported in the literature [99, 155].

Beyond measurements of muscle area, CT images in the abdominal or mid-thigh regions may be used to examine muscle fat infiltration [12]. A decrease in mean HU values of skeletal muscle tissue has been shown to correspond to an increase in fatty-acid infiltration [3, 12]. Additionally, adipose tissue that is found between muscle groups may be segmented separately from muscle tissue during image analysis. For instance, on CT images muscle appears within the -29 to 150 HU range, while intermuscular adipose tissue (IMAT) on CT images appears in the -190 to -30 HU range [3, 12]. There are multiple limitations associated with CT image analysis. These include high radiation exposure, equipment cost and availability, as well as the necessary resources to analyze each image [74, 155]. As such, alternative methods of image analysis are needed to provide detailed images in a cost-effective and safer manner.

3.2.4 Ultrasound

The ultrasound is a medical device that has multiple diagnostic and clinical uses [76, 155]. The ultrasound probe creates an inaudible sound wave. Ultrasound waves are high frequency sound waves that are produced by the piezoelectric crystals within the transducer probe [120, 123]. The beam travels through the various tissues and it is reflected due to the acoustic impedance of tissue interfaces [120, 123]. The refracted waves, also known as echoes, are captured by the receiver built into the ultrasound device [74, 76]. B-mode (brightness) ultrasonography is a type of ultrasound that recreates 2D images of the underlying tissues, and analysis of the images permits identification of numerous tissue characteristics including, dimensions, composition and structure [41, 106, 156].

Ultrasound imaging may be used to examine the dimensions of skeletal muscle groups. Most often muscle thickness is assessed, while more advanced equipment is capable of capturing muscle cross-sectional area [107, 143, 155]. The dimensions of prominent muscle groups may be assessed in order to examine the physical capacity of participants. For instance, low muscle thickness values are related to poor physical function scores in older adults [116, 156]. Ultrasound may also be used to examine the composition of skeletal muscle [41, 76, 106]. Fat and fibrotic infiltration found within the muscle reflect the ultrasound waves differently compared with muscle itself. The area associated with muscle tissue will appear dark, while fat and perhaps fibrotic tissue will be represented by white or bright gray areas. Specialized computer software can be employed to measure echogenicity as the grey-scale value associated with the area of the muscle group analyzed, which would provide information about muscle composition [41, 76, 106]. Additionally, skeletal muscle tissue structure may be examined using ultrasonography. While muscle dimensions and composition are typically imaged in the transverse plane, ultrasound images acquired in the longitudinal plane may be used to determine fascicle length and pennation angle [91, 111].

There are several limitations associated with the use of musculoskeletal ultrasound. The thickness measurements are operator-dependent [76, 116, 156]. Aspects such as landmarking, tissue compression and probe tilt, as well as inconsistencies in manual image segmentation can all contribute to high variability between measurements [76, 116, 156].

For this reason, reliability studies are critical for the development of ultrasound-based methods. There are multiple reviews that suggest that measurements of muscle thickness, echogenicity and architecture with the use of ultrasound have good reliability [41, 88, 116, 156]. However, due to the site-specific nature of ultrasound-based measurements there may be heterogeneity in the inter- and intra-rater reliability between different landmarks [88, 116]. Different ultrasound equipment manufacturers, as well as imaging settings such as gain and dynamic range, further limit the generalizability between studies that examine ultrasound-based measures of muscle characteristics [124, 152]. Also, the use of predictive equations may lead to error when applied to a cohort different from the one wherein they were developed [51]. The major advantages of ultrasonography are that this modality is portable, easy to use, relatively inexpensive and does not expose the subjects to radiation [106]. The ability to measure muscle architecture using ultrasound is another advantage that sets this modality apart from other imaging methods such as CT or MRI.

3.2.5 Validation of muscle size or mass measurements

Medical imaging modalities used in the assessment of muscle mass are indirect measurements, and therefore require validation studies. Validation studies rely on comparison of skeletal muscle measurements using these modalities versus direct measurement of cadaveric or animal tissue to help confirm their effectiveness [72]. CT, MRI and DXA have been extensively validated during the 1980's and 90's [75]. DXA has been extensively validated versus the chemical composition of animal carcasses in pigs, rhesus monkeys and rats [72, 75]. DXA validation in animal carcasses, and in relation to multi-compartment models as well as CT and MRI firmly establish this modality as an accurate method to measure bone mineral density, fat and lean tissue mass [72, 75].

Unlike DXA, CT and MRI seldom capture the entire body due to the cost, and in the case of CT, due to the radiation exposure; instead, a typical image contains a 2-dimensional axial cross-section of the region of interest. A series of consecutive 2D images can be used to digitally reconstruct a 3-dimensional scan. Only a small portion of studies using CT or MRI imaging examine whole body volume, most commonly cross-sectional area in 2D is measured. In 1998, Mitsiopoulos *et al.* confirmed that skeletal muscle CSA calculated using

CT images at the level of the mid-thigh corresponds to both MRI measured CSA as well as muscle CSA analyzed in cadavers [103]. Prior to this study, researchers have evaluated the shape of tissues that are more simple to trace, such as liver or kidney [75]. Due to the complex shape of skeletal muscle groups manual tracing of the borders of this tissue is difficult, and computerized approaches are used in order to assist with segmentation.

Since whole body CT scans are rare, a single transverse scan at the level of the third lumbar vertebra (L3) is often used as an index instead of 3D volume. In a landmark study Shen *et al.* examined whole body MRI scans in a large cohort of healthy adults and demonstrate that the CSA of skeletal muscle and adipose tissue in the abdominal area is strongly associated with whole body volume [148]. The highest coefficient of correlation was observed 5 cm above the L4/L5 level for skeletal muscle CSA versus volume, while adipose tissue 5 cm below the L4/L5 level yields the strongest association with whole body adipose tissue volume [148]. This work provides the evidence supporting the use of a single abdominal scan to examine whole body muscle mass. The CSA values measured using a single scan are typically expressed as an index value by adjusting CSA to height squared, similar to BMI [49, 128], to partially account for the stature of the individual.

Ultrasound imaging requires a different approach to quantifying muscle area. The width and the depth of the ultrasound probe is a key determining factors for the sort of analysis that can be performed on images acquired using the ultrasound device. In most cases large muscle groups may not fit horizontally on a single image, limiting the analysis to 1 dimension (i.e. muscle thickness) [106, 156]. Another factor involves the depth and the overlaying structure for any muscle group that is assessed with ultrasound imaging. For instance, the psoas muscle group is deep within the abdominal cavity, there are also several structures between the surface of the skin (where the probe is placed) and the psoas muscle. Since the energy of sound waves decreases as they travel deeper beneath the skin, it is often difficult the definitively identify the outlines of deep muscle groups. A commonplace strategy to evaluating muscle mass with ultrasound uses a predictive equation in conjunction with muscle thickness measures to capture whole body or regional muscle mass [116, 156]. Validation and use of such predictive equations rely on criterion metric of muscle mass, such as MRI or DXA, to which ultrasound muscle thickness values are then calibrated against. Multiple predictive equations are currently described in the literature.

These predictive equations may be differentiated according to the cohorts wherein the equations were developed, as well as the number of sites assessed. Ultrasound protocols in the literature range from one thickness measurement to upwards of nine predefined landmarks, and commonly include co-variates such as age, sex and height [107, 116, 156].

3.3 Assessment of skeletal muscle health in clinical populations

The role of skeletal muscle in numerous disease states has been highlighted by a large body of research demonstrating a robust association between muscle depletion and the risk adverse clinical outcomes [14, 52, 121]. In many cases, these results extend on research evaluating muscle characteristics over the course of aging. The use of commonplace body composition assessment tools to evaluate muscle characteristics in clinical populations needs to accommodate several potential confounding factors, related to both patient and equipment availability [49, 140, 155]. For example, factors such as limited mobility, altered hydration status, scarring due to surgery or other treatment types may introduce additional challenges and variability into the measurement of muscle characteristics in patients of specific health conditions. Furthermore, a limited participant pool, heterogeneity in the patient cohort and availability of facilities, equipment and personnel may also create logistical challenges.

Currently, there is not a single tool that is most optimal, and without limitations, for the assessment of muscle characteristics in clinical cohorts [128, 155]. For this reason, it is important to consider the nuances of each individual body composition assessment modality when comparing across research studies. Development of practical methods as well as research evaluating the agreement and accuracy between the different tools used is commonly performed in studies evaluating body composition in clinical cohorts [51]. This extensive validation work has established CT, MRI and DXA as reference body composition modalities. In turn, these methods are commonly used as part of criterion validation studies for emerging body composition assessment methods, such as ultrasound [74, 99, 128].

Beyond helping prognosticate risk of adverse clinical outcomes, measures of muscle

characteristics may be used to help better understand the functional changes that occur in parallel with muscle depletion. Functional outcomes, such as poor contractile function or metabolic derangements may further elucidate how disease states impact muscle health [32, 34, 102]. There is mounting evidence that muscle size is not the only important factor in muscle health, but also muscle composition and architecture are useful indicators of muscle function [41, 112, 156]. Thus, it is essential to incorporate measurements of these characteristics along with measurements of muscle function to gain a comprehensive understanding of the links between muscle health and clinical outcomes.

Chapter 4

Skeletal muscle during the cancer trajectory

Cancer survivors, a population that includes individuals who have been diagnosed with cancer and are currently undergoing treatment or are in remission, experience alterations in body composition throughout the treatment trajectory [129, 140]. Tumour-related effects and treatment side effects may contribute to perturbations in the function of a few different tissues [8]. The loss of muscle mass in particular appear to be a key feature of body composition changes observed in cancer survivors [9, 82, 140]. In individuals with cancer and cancer survivors CT images are often obtained as part of routine hospital care, for purposes that include diagnosis, treatment planning and monitoring [45]. Thus, analysis of CT images serves as an opportunity to examine tissue level alterations in skeletal muscle that occur during the cancer trajectory [14, 129]. This non-invasive method facilitates the examination of human *in-vivo* tissue characteristics, where otherwise the approaches require biopsies and other invasive procedures, and often necessitate the reliance on animal models or tissue culture studies. Recently, the increasing use of CT image-based analysis of skeletal muscle mass in cancer patients has led to a rapid growth in the interest in muscle wasting in this cohort [30, 85, 140, 145]. According to recent reviews, there are over 100 publications that have examined muscle characteristics in cancer patients and survivors [30, 34].

Measurement of skeletal muscle mass at multiple timepoints during the cancer trajectory and during treatment, has helped establish that the rate of muscle atrophy is elevated in this population [85, 129]. Age-related muscle atrophy rate (beyond the age of 40) is believed to be approximately 0.5-1% per year, whereas muscle losses of 3-5% per 100 days have been observed in some cancer cohorts [14, 129]. Prado *et al.* evaluated change in skeletal muscle area in several cancer cohorts including lung, colorectal, and pancreatic [132]. Their findings demonstrated that the rate of muscle loss is highest in a subset of the population who survived the shortest period of time following a cancer diagnosis [132]. Loss of muscle mass manifests in a large portion of patients with advanced cancer, and it is primarily influenced by negative energy and protein balance resulting from a combination of reduced caloric intake, reduced weight-bearing activity and increased catabolic processes [14, 105].

Loss of muscle mass has several adverse consequences including increased risk of mortality as well as treatment related risks, such as chemotherapy toxicity and surgical complication [30, 138, 145]. Beyond these clinical outcomes, muscle wasting is linked with poor physical function and an increased risk of developing comorbidities such as cardiovascular disease and type 2 diabetes [30, 34]. While the loss of muscle mass during the disease trajectory is commonly observed in patients with advanced cancer, it may occur across a diverse range of disease stages and cancer types [45, 85]. Clinical researchers today recognize that muscle wasting is not manifested as a single phenotype, rather the rates are varied across different patients [55, 145]. The factors that may be involved in influencing the severity of muscle wasting include cancer type and severity, sex, age, genetic factors, comorbidities and treatment related catabolic effects [55, 145]. The inter-individual differences in the relative contribution of these factors, and how they may affect the rate of muscle atrophy, are yet to be fully understood. However, despite the increasing research into muscle wasting in this cohort, a number of areas related to alterations in muscle characteristics during cancer trajectory remain elusive.

4.1 Muscle atrophy during the cancer trajectory

Perturbations in the balance between muscle protein synthesis and degradation are key mechanisms mediating cancer-related muscle wasting [8, 83, 141]. In cancer patients and survivors, increased muscle protein turnover and amino acid release may occur with decreased availability of nutrients (food intake, loss of appetite, etc.), and reduced physical activity levels that lead to disuse-related atrophy. Changes in the endocrine milieu also contribute to a catabolic environment via inflammation, insulin resistance and glucocorticoids. Treatment may also lead to direct side effects in skeletal muscle, both systemic (chemotherapy) and localized (radiation and/or surgery) [8, 83, 141]. Thus, the large heterogeneity in the prevalence of low muscle mass, or excessive rates of muscle atrophy, may be explained by inter-individual differences in the exposure to various mediating factors [54].

Muscle wasting in cancer patients involves both a decrease in synthesis and increase in muscle protein degradation [17, 82]. Muscle protein is involved in numerous roles which include structural, contractile and signaling functions [9, 17, 170]. Perturbations in muscle protein turnover may not only decrease the weight of the muscle per-se, but also carry deficits related to such functional aspects [17, 82]. Although degradation of contractile components can affect muscle contraction force, degradation of muscle proteins that are involved in structural or signaling roles may also have widespread effects on muscle cells and their function [83]. Skeletal muscle also supplies amino acids as building blocks or sources of energy during prolonged starvation or catabolic stress [8, 170]. Muscle depletion may contribute to a disruption of homeostasis and inadequate reserves, and thus lead to limited capacity to respond to injury and physiological stress [8, 170].

4.1.1 Mechanisms mediating the loss of muscle mass in cancer patients and survivors

During cancer treatment, muscle atrophy may occur with a decrease in the availability of nutrients caused by reduced food intake, loss of appetite, and nausea [9, 18]. Reduced availability of nutrients, or more specifically amino acids like leucine, may shift muscle

metabolism towards a negative protein balance. This reduced availability of amino acids leads to protein degradation in muscle, which would release amino acids into the circulation to help support protein synthesis in other tissues [8, 54, 122]. An energy deficit will also decrease the availability of growth factors such as IGF1 [8, 54, 122]. In addition to the direct influence of protein synthesis or degradation pathways, individuals with this disease also appear to be less responsive to branched-chain amino acid (BCAA) induced stimulation of muscle protein synthesis. Therefore, the anabolic response to an intake of protein is diminished further exacerbating the negative consequences of the decrease in food intake (i.e. malnutrition) [8, 54, 122].

Patients undergoing treatment may have decreased levels of physical activity. Disuse is a potent independent factor associated with muscle atrophy [9, 17]. Muscle disuse, intrinsically through decreasing the levels of contractile activity, will promote an insulin resistance state within skeletal muscle and therefore blunt the activation of signaling cascades involved in muscle protein synthesis [17, 32]. Disuse also leads to an increase in the activity of ubiquitin proteasome pathway (UPP) via regulating the transcription of MuRF1 and MAFbx [9, 40, 141]. Cancer therapy is shown to also cause a decrease in physical activity [17, 34, 134]. This may be due to the burden of treatment such as recovery from surgery or radiation therapy, as well as side effects such as fatigue. This decrease in physical activity in of itself may lead to muscle atrophy via a decrease in protein synthesis [17, 34, 134]. In addition, the effects of cancer and treatment related decreases in functional capacity may confound over the disease trajectory. In extreme cases the depleted reserves cause an inability to complete activities of daily living thus leading to further disability and disuse [17, 34, 134].

Cancer as well as cancer treatment may lead to a catabolic environment, where stimuli such as inflammatory cytokines and insulin resistance have a negative influence on net protein balance [83, 122]. Alterations in the levels of endocrine and paracrine factors is a large area within cancer related muscle atrophy field. Early studies demonstrated that tumour-bearing animals had circulating factors (i.e. proteolytic-inducing factors) that caused wasting. Media incubated with macrophages from tumour bearing animals appear to mediate the endocrine effects [122]. TNFa and IL6 were consequently identified as the mediators driving this mechanisms and suggesting that muscle wasting may be

partly attributed to the interactions between tumour and immune system cells [83, 122]. Further research identified multiple other inflammatory cytokines that may be elevated over the course of cancer and treatment related muscle atrophy [82, 83, 122]. Inflammatory cytokines may act via several distinct pathways to activate proteolytic mechanisms in skeletal muscle.

Furthermore, several cancer treatments have been shown to cause muscle dysfunction at the cell level, leading to a loss of muscle overall. Abiratorone, is an agent used in prostate cancer therapies, acts by suppressing testosterone activity [31]. Chemotherapy drugs also appear to have a number of toxic effects in skeletal muscle [45, 151]. Sorafenib is a multi-kinase inhibitor, which may suppress the activity of pathways involved in muscle anabolism [7, 78]. Mk-0646 is an anti-IGF1R drug, that similar to other examples may also prevent adequate activity of anabolic signaling cascades [56]. Multiple drugs appear to disrupt proper mitochondrial function in skeletal muscle and may cause metabolic derangements in this tissue[9, 151]. Also, glucocorticoids are potent factors contributing to muscle atrophy, and these are often used during cancer treatment to manage side-effects [19]. The heterogeneity observed in rates of muscle wasting in cancer patients undergoing treatment may be partially attributed to diverse effects of these various treatment types.

Research into the cellular mechanisms involved in the loss of muscle mass that occurs over the cancer treatment and survivorship trajectory is done *in-vitro* and in animal models. Thus, there are challenges associated with generalizing these findings to human subjects [82, 109]. A few recent clinical trials evaluating interventions aimed at reducing muscle wasting using targeted pharmacological approaches have yielded inconclusive results [13, 43, 135]. In turn, the inconclusive findings of human trials highlight the need to continue developing tools such as ultrasound imaging that allow non-invasive, *in-vivo*, assessment of muscle characteristics that occur over the cancer trajectory. Identification of strategies that may help attenuate the excessive loss of muscle mass and the related metabolic and nutritional consequences, will perhaps require a further understanding of the underlying tissue-specific as well as whole-body mechanisms.

4.2 Cross-sectional classification of lower than normal muscle mass

Measurement of muscle atrophy is challenging due to the need of multiple, longitudinal quantification of skeletal muscle; thus, developing a cross-sectional approach that uses muscle mass measurements to demonstrate the detrimental effects of muscle depletion is important [30, 45]. CT image analysis is commonly used to identify cancer patients who have less than optimal muscle quantities, and who are at risk of adverse clinical outcomes. Lower than normal muscularity in cancer patients has been demonstrated to be a predictor of poor survival outcomes [30, 45]. Low muscle mass, independent of rate of muscle atrophy, is a predictor of mortality risk across several different cancer types and disease severities [30, 138, 145].

A systematic review and meta-analysis of 38 studies and a total of 7779 patients, performed in 2016, demonstrated that participants with cancer who have low skeletal muscle values also have poorer overall survival [145]. Although the prevalence of low muscle mass in various cancer cohorts is reported to range from 11% to 90% of the population examined, cancer patients with low muscle index values have a hazard ratio of 1.44 (95% CI = 1.32 - 1.56) when compared with cancer patients classified as having normal skeletal muscle index values [145]. The links between lower than normal muscularity and overall survival has been observed across several different cancer types, both in advanced and early stage disease [30, 138, 145]. These findings suggest that muscle wasting occurring in the early stages of diseases is clinically significant, similar to wasting observed in late stage disease. Both low muscle mass and excess adiposity are independent predictor of mortality in cancer patients [4, 30, 129]. With the increasing use of CT image analysis, delineating skeletal muscle mass from adipose tissue is important and sarcopenic obesity has been associated with poor clinical outcomes in patients with cancer [28].

Beyond mortality, low skeletal muscle index values are reported to be linked with adverse clinical outcomes such as treatment toxicity, length of hospital stay and post-surgical complications [34, 140]. Patients who fall below clinical muscle index thresholds have a higher risk of dose limiting toxicities, these include dose delays, dose reduction and in

some cases treatment termination [30, 45]. To date, this relationship has been reported in breast, renal, liver, lung, colorectal, thyroid, and melanoma cancer types [30, 45]. Another group of risk factors that are elevated in cancer patients with low muscle mass is the risk of adverse post-operative outcomes. These appear to include post-operative infections, hospital length of stay, complications, risk of hospital readmission following discharge, as well as higher costs of medical care [30, 45]. In cancer patients, low muscle mass is not only related to chemotherapy toxicity and surgical outcomes, it has also been linked to poorer quality of live related outcomes [34].

Cross-sectional stratification of muscle mass using a single measurement may be performed using pre-established threshold values to dichotomize the cohort into those with lower than optimal muscle quantities and patients with adequate muscularity [133, 138]. One strategy used to define lower than optimal muscle mass cut-off values employs an iterative stratification process to identify the threshold value, typically used for CT-based skeletal muscle index values. The optimal stratification approach attempts to maximize the log-rank statistic of time-to-survival [133, 138]. There are numerous published skeletal muscle index value thresholds. Prado *et al.* used optimal stratification to illustrate an increased risk of mortality in obese cancer patients with low skeletal index values [133]. Martin *et al.* subsequently used optimal stratification to develop skeletal muscle index, muscle attenuation as well as rate of weight loss cut-points [97]. These latter cut-points were developed separately in 4 groups characterized as underweight, normal weight, overweight and obese based on BMI values [97]. There are inconsistencies between published cut-off values, as they may be developed in different cohorts of cancer patients and survivors [138, 145]. Additionally, these threshold values are specifically optimized to prognosticate risk of mortality, and may perhaps not be optimized in relation to other adverse clinical outcomes [138, 145].

Lower than optimal muscle mass may be also identified using a comparison to a healthy reference cohort. Classification of lower than normal muscle mass based on values observed in a reference healthy cohort is a strategy originally used by Baumgartner *et al.* [15]. Appendicular lean mass index less than 7.26 kg/m^2 in men, and 5.45 kg/m^2 in women, fall 2 standard deviations below the mean of a young healthy cohort. These values are an often utilized muscle depletion thresholds [15]. A more contemporary set of reference

values, and perhaps more representative of the general North American population, is based on DXA values measured as part of NHANES III [86]. The NHANES III reference data was collected in 20552 individuals of diverse ethnic backgrounds, and an age range of 8 to 85 and over. Recently, CT skeletal muscle index values of a healthy reference cohort have also been published [158]. Despite the prevalent use of muscle mass cut-points in the oncology literature, there is currently a lack of consensus regarding what threshold may be optimal in classifying patients at risk of adverse clinical outcomes [121, 138]. The inconsistency of published cut-off values may lead to difficulties directly comparing the findings across different articles [138, 145]. These disparities result in vast heterogeneity in the reported prevalence of lower than normal muscle mass in cancer patients and survivors [138, 145]. Importantly, these limitations perhaps prevent the translation of research evidence into clinical practice.

4.3 Alterations in muscle composition in cancer patients and survivors

Muscle protein constitutes approximately 75-80% on the non-water compartment of skeletal muscle cells and changes in muscle protein turnover are a critical mediator of muscle loss [61, 170]. Yet, further muscle composition alterations at molecular, cellular and tissue levels are increasingly researched for their role in muscle depletion that occurs throughout the cancer trajectory. Given the invasiveness of muscle biopsies, CT image analysis has primarily been used to study not only muscle size but also muscle composition [12, 45].

The utilization of muscle biopsies has led to the identification of important molecular and cellular level muscle composition derangements that occur during the cancer trajectory. Zampieri *et al.* compared muscle biopsy samples that were collected during colorectal cancer surgery and prior to the initiation of chemotherapy in 10 patients with samples obtained in patients who had abdominal surgery for reasons other than cancer [172]. Although there were no differences in myofibre cross-sectional areas between the two groups, there was a difference in the frequency of centrally located nuclei [172]. In another study, muscle biopsy samples from pancreatic cancer patients with high rates of weight

loss [142] had a 45% lower content of myosin heavy chain than cancer patients who were weight-stable [142]. Furthermore, cancer cachexia is also reported to be associated with an increase in intramyocellular lipid droplets in muscle samples [153]. While these studies demonstrate that a range of muscle composition derangements may be evident in this cohort, the methodological challenges associated with performing this level of mechanistic research, results in small sample sizes and thus limited generalizability. Generalizability is particularly challenging because of the wide heterogeneity in tissue properties between different cancer types, disease stages and treatment options.

In-vivo analysis of muscle composition at the tissue level can be performed based on CT images also used for the measurement of muscle quantity. As mentioned previously, HU reflects x-ray attenuation and these values are commonly used to gauge ectopic fatty infiltration into skeletal muscle. While analysis of muscle attenuation values is not as commonplace as measurements of muscle size in cancer patients and survivors, a recent systematic review has identified 40 studies which evaluated CT-based muscle composition in this population [6]. Similar to low muscle mass, patients classified as having low muscle attenuation had a higher risk of overall mortality. This corresponded to a hazard ratio of 1.75 (95% CI: 1.60 - 1.92) [6]. This association is most prominent in patients with gastro-esophageal, colorectal and pancreatic cancer [6]. However, a systematic review suggests that muscle attenuation is linked with survival in other cancer types as well [6]. Besides mortality risk, muscle attenuation is extensively studied in relation to chemotherapy toxicity [6, 45, 129]. Evidence available to date highlights that in multiple cases muscle attenuation is an independent predictor of patient outcomes. Thus, further research is needed to help better understand tissue level muscle composition characteristics in cancer patients and survivors, and the importance of assessing composition alongside muscle size to identify patients in a higher risk group.

4.4 Muscle health in the breast cancer trajectory

Breast cancer is among the most common types of cancer in women. According to the most recent estimates by Statistics Canada, 26900 women were expected to be diagnosed

in 2019. Cumulatively, this corresponds to a lifetime incidence of 1 in 8 women [26]. The prevalence of breast cancer across the world is approximately 2.1 million newly diagnosed cases each year [20]. Deleterious changes in body composition, such as gain in fat mass or obesity, have been identified as a risk factor for developing breast cancer and recurrence after treatment [147, 159]. Furthermore, weight gain is commonly observed in women undergoing treatment [147, 159], and excess adiposity may increase the risk of developing comorbidities [23, 149]. With the increasing use of body composition measurements, in particular the ability to distinctly assess the characteristics of muscle and adipose tissue, it has been demonstrated that both adiposity and muscle health are independent mediators of patient outcomes in this cohort [23].

The highest rates of muscle wasting are commonly observed in cancer patients with advanced disease, this is common in pancreatic, lung and gastrointestinal cancer types [132]. However, it was also demonstrated that the link between muscle depletion and adverse clinical outcomes is observed across different cancer types, stages and treatment modes [138, 145]. With improvements in detection strategies and treatment the 5-year survival rate in breast cancer patients is relatively favorable and corresponds to 88% according to estimates by Canadian Cancer Statistics Advisory Committee [26]. In this cohort, skeletal muscle health has been explored within several distinct domains, including links between muscle characteristics and clinical outcomes such as overall mortality, chemotherapy toxicity or treatment complications [4], as well as quality of life, mobility and metabolic outcomes [34].

Skeletal muscle has been strongly associated with mortality in breast cancer patients. In 3241 patients with non-metastatic breast cancer, Caan *et al.* associated muscle mass, using CT images acquired during diagnosis, with overall survival [23]. Patients classified as having lower than normal muscle mass were at an increased risk for overall mortality, with a hazard ratio corresponding to 1.41 (95% CI: 1.08 - 1.69) after adjustment for several confounding factors [23]. These results corroborate the findings of other authors who also used CT-based muscle mass measurements and report a similar association, albeit in a smaller sample [48]. Villaseñor *et al.* used DXA-based appendicular lean tissue index measurements in order to classify women diagnosed with breast cancer into a low and normal ALTI groups within 12 months after diagnosis [162]. In this cohort the hazard

ratio for patients with lower than normal muscle mass corresponded to 2.86 (95% CI: 1.67 - 4.89) [162], thus further highlighting that muscle health is a mediator of overall mortality in breast cancer patients.

Interestingly, findings of studies evaluating CT-based muscle measurements in women with metastatic breast cancer suggest that there may be discrepancies in the association between muscle characteristics and survival across cohorts with different disease stages and treatment histories. In patients with metastatic breast cancer, muscle attenuation, which is indicative of lipid infiltration, was associated with overall survival, while skeletal muscle index was not correlated with survival [139]. In this subgroup, the combination of lower than normal muscularity and low muscle attenuation was also linked with treatment toxicity [131, 146]. Chemotherapy regimens are often prescribed based on body size, and these findings highlight that muscle depletion may be masked due to excess adiposity [6, 45, 129]. In these circumstances, body composition measurements that are able to determine muscle characteristics may help improve treatment and reduce the risk of toxicity.

The importance of assessing muscle function alongside muscle strength is often emphasized by studies evaluating muscle health over the course of general aging. Skeletal muscle has multiple pertinent functional roles, while some studies have identified that breast cancer patients and survivors may exhibit muscle weakness, insulin resistance and dyslipidemia [16, 87]. Few studies have evaluated associations between muscle characteristics and functional derangements in this cohort. Recently, Aleixo *et al.* evaluated CT-based muscle index and muscle attenuation in 99 women with early breast cancer with respect to scores on functional tests such as Timed Up and Go as well as the Short Physical Performance Battery [5]. Their findings indicate that patients with prolonged Timed Up and Go scores also had lower mean HU values, an indicator of increased fatty infiltration [5]. Another report, by the same group, evaluated longitudinal changes in DXA-based appendicular lean tissue mass, and changes in grip strength and gait speed in a large cohort that included patients with several cancer types and stages, which included breast cancer patients [168]. The results highlight the importance of assessing physical function in this cohort by demonstrating that slow gait speed was associated with a 1.44 hazard ratio (95% CI: 1.05 - 1.98) of increased mortality risk, and a 1.70 (95% CI: 1.08 - 2.68) higher risk of disability [168]. Thus, measurements of muscle characteristics are emerging as a useful determinant

of multiple risk factors in breast cancer patients, which may be valuable in identifying those who may benefit from lifestyle interventions, such as exercise and nutrition.

Chapter 5

Rationale

Muscle depletion is common in cancer patients [30, 34], including breast cancer patients and survivors [4], and it is exacerbated by treatment side-effects and persists into survivorship [129, 140]. Accelerated rates of muscle wasting are often observed in patients with advanced cancer stages, with the highest rates reported in the months preceding death [85, 132]. The robust relationship between lower than normal muscularity and poor outcomes, including mortality, treatment toxicity and hospital length of stay [138, 145], has emerged due to increased use of precise body composition tools. Computed Tomography (CT) and Dual-energy X-ray Absorptiometry (DXA) are two tools commonly used to identify patients with muscle depletion [45, 49]. CT images can be used to measure muscle cross-sectional area at the level of the third lumbar vertebra (L3), while DXA is often used to assess the lean tissue of the arms and legs (appendicular lean tissue) [49, 128]. Both CT-based muscle index and DXA-based appendicular lean tissue index effectively represent whole body muscle mass [108, 155]. Today, classification of patients at risk of adverse outcomes that is based on poor muscle health is widely reported in the literature, across diverse cancer types, disease stages and treatments. However, little evidence is available to help establish the physiological mechanisms or to demonstrate causal links between low muscle mass with respect to mortality, morbidity and physical function.

Assessment of muscle characteristics using tools such as CT and DXA is also associated with several limitations. These limitations include cost, availability of the modality, expo-

sure to radiation and personnel training [49, 128]. Ultrasonography is an emerging modality that is portable, accessible, easy to use, and does not emit radiation, and it may help assess various distinct muscle characteristics [106, 155]. Images of prominent muscle groups can be used to determine muscle thickness or cross-sectional area [106, 143, 156]. Ultrasound imaging can also be used to describe features other than muscle size. Echogenicity, for example, represents muscle composition, including perturbations such as increased fatty infiltration or fibrosis [41, 123]. Pennation angle and fascicle length provide architectural measures of skeletal muscle, which have been identified as key determinants of contractile strength [111, 156]. With the potential to comprehensively assess size, composition and architecture, ultrasonography may be a practical and useful tool towards understanding muscle health in cancer patients.

Skeletal muscle tissue is fundamental to different functional roles including muscular contraction to generate movement, energy metabolism and hormonal signalling as well as immune and inflammatory responses [52, 80, 170]. Increased muscle mass strongly correlates with muscle strength [71, 102], and research is currently ongoing to further characterize the potential links between muscle features with respect to metabolic and immune roles [17, 105]. In older adults, muscle strength has been identified as a predictor of mortality, highlighting the importance of physical function in overall health [115]. Conversely, the significance of adequate muscle function in regard to clinical outcomes and mortality in cancer patients is poorly characterized. Classification of lower that normal muscle mass aided by CT and DXA relies on examination of whole body or regional muscle size. However, it is not clear if this approach is also optimal for examining functional derangements. For example, lower body muscles are involved in gait and mobility while abdominal muscles are important in stability and posture. Thus, in order to characterize the links between muscle depletion and poor physical function a more targeted approach may be warranted.

The distinction between whole body or regional versus site-specific measurements is an important methodological consideration for ultrasound-based muscle feature assessment that has not been extensively studied in cancer patients. Since ultrasound images are captured at a specific landmark, estimation of whole body or regional muscle size often entails protocols that combine measurements at one or more landmarks as part of a predictive equation, which commonly also includes other covariates such as height, sex and age [2,

107, 119]. Currently, there are no ultrasound protocols specifically developed in breast cancer patients, or any other cancer cohorts. There are several potential protocols, which have been developed in the general population, that rely on a distinct set of landmarks used for images as well as different co-variables [2, 119, 156]. Further research is needed to validate a protocol by determining the landmarks which optimize between ease of image acquisition and agreement with reference measurement. It is not known if muscle wasting in patients undergoing cancer treatment or survivors is selective towards certain muscle groups or conversely whether the effects are systemic. Despite the current gaps in the evidence related to the use of this modality, the versatility of ultrasound-based measurements of muscle characteristics makes it an attractive target that can be potentially used to study muscle features in breast cancer patients and survivors.

The aims of this thesis are:

1. To better understand the relationships between muscle mass or size with respect to muscle function at both the whole-body and site-specific levels.
2. To further test the use of ultrasound-based imaging methods for studying skeletal muscle composition and architecture features both in breast cancer patients in- and post-treatment as well as in a young, healthy reference cohort.

The specific study objectives are outlined below:

Study 1 (Chapter 6)

In a cohort of women who are currently undergoing or recently completed breast cancer treatment, our objectives were to:

1. Characterize and compare body composition features in participants with lower than normal muscle mass versus participants classified in the normal range, as well as a healthy reference cohort.

2. Compare muscle strength in participants who have lower than normal muscle mass with participants who have muscle mass in the normal range.

3. Examine the associations of unadjusted, height normalized, weight normalized and body mass index (BMI) normalized measures of lean tissue mass with muscle strength.

Hypotheses:

1. Classification of lower than normal DXA-based appendicular lean tissue mass index will be independent of body size and adiposity values.

2. Participants with lower than normal muscle mass will have lower muscle strength scores.

3. Lean mass when evaluated as a continuous variable will be correlated with strength.

4. Weight and BMI normalized lean tissue measures will have higher coefficients of correlation versus strength as compared to unadjusted and height normalized values.

Study 2 (Chapter 7)

In a cohort of breast cancer patients who are currently in treatment or who have recently completed treatment (<2 years), our objectives were to:

1. Evaluate the feasibility of acquiring ultrasound measurements of muscle size, composition and architecture.

2. Examine the inter- and intra-rater reliability of image analysis protocols used for the measurement of muscle features.

3. Assess the agreement between appendicular lean mass (ALM) values predicted using ultrasound-based muscle thickness measures versus observed DXA-based ALM.

Hypotheses:

1. Images of landmarks of the 5-site protocol, as well as the anterior and lateral aspects at the mid-thigh level, can be obtained in all participants.
2. The coefficient of variability of inter- and intra-rater reliability for ultrasound-based muscle thickness, echogenicity, pennation angle and fascicle length will be less than 5%.
3. Predicted ALM when compared to observed DXA-based values will not be biased, and have limits of agreement of within the -3.2 to 3.2 kg interval.

Study 3 (Chapter 8)

In a healthy, non-obese cohort of young adults, the objectives were to:

1. Examine the inter-relationships between ultrasound-based muscle size, composition and architecture features.
2. Determine the association of ultrasound-based muscle echogenicity, pennation angle and fascicle length measurements with respect to:
    a. DXA-based fat and lean tissue mass.
    b. Muscle strength and power.

Hypotheses:

1. Ultrasound-based muscle thickness will be inversely correlated with echogenicity values, and directly correlated with pennation angle and fascicle length values.
2. Echogenicity values will be inversely correlated with pennation angle and fascicle length values.
3. Echogenicity will be directly correlated with DXA-based leg fat mass.
4. Pennation angle and fascicle length will be directly correlated with DXA-based lean mass.

5. Muscle thickness, pennation angle and fascicle length will be directly correlated, while echogenicity will be inversely correlated, with isometric strength, isotonic power or velocity.

Chapter 6

Classification of low lean tissue mass relative to adiposity in relation to muscle strength in a cohort of breast cancer patients in- and post-treatment

Abstract

Background: Breast cancer patients commonly lose lean mass (primarily as skeletal muscle) during and post-treatment. Lower than normal muscle mass is linked to an increased risk of mortality, treatment toxicity and lower quality of life. This study sought to comprehensively evaluate the body composition profiles of breast cancer patients with lower than normal muscle mass, along with common strategies proposed to help adjust for differences in body size, in relation to muscle strength.

Methods: We evaluated dual-energy X-ray absorptiometry (DXA) scans in 95 breast cancer patients. A subset of the cohort (n=48) also completed knee extension or elbow flexion isometric muscle strength assessment. DXA scans in a reference cohort of women, 18-30 years old (n=52), without pre-existing chronic health conditions were also collected.

Results: Breast cancer patients with low lean mass (measured as appendicular lean tissue index (ALTI) < 5.45 kg/m^2) had similar fat mass (FM) and body mass index (BMI) values as the reference cohort. Patients with ALTI ≥ 5.45 kg/m^2 had higher BMI and FM values compared to those who had low ALTI and the reference group. DXA-based lean tissue mass also correlated with fat mass (r=0.74, p<0.001). The residuals of the regression between appendicular lean and fat mass correlated with muscle strength (r=0.5, p<0.001).

Conclusion: Classifying individuals with lower than normal muscle mass or poor muscle function may be a result of a smaller body size, and likely less fat mass. To optimally classify breast cancer patients at higher risk of poor muscle function, an adjustment for adiposity may be needed.

Introduction

Improved screening strategies and advancements in treatments have resulted in a high (88%) 5-year survival rate for women with breast cancer [26]. However, patients undergoing breast cancer treatment commonly gain fat and lose muscle mass during treatment, and these body composition changes persist for several years in survivorship [48, 147, 169]. These deleterious changes in body composition may support the development of comorbidities, reduce quality of life into survivorship [127, 159] and increase the risk of breast cancer recurrence. Importantly, lower than normal muscularity or excess adiposity have been linked with a range of adverse clinical outcomes such as chemotherapy toxicity, post-surgical complications, prolonged length of hospital stay and risk of mortality in this cohort [4, 23, 30, 145].

Several different modalities have been used to assess body composition in breast cancer patients including computerized tomography (CT), dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) and anthropometry [21, 129]. CT and DXA distinguish between fat and lean tissue or muscle with excellent precision and reliability [155]. Although CT can examine specific muscle groups with high resolution, DXA has the capacity to readily measure whole-body and regional lean tissue mass [155] with much less radiation than CT. Appendicular lean tissue index (ALTI) can also be calculated using

DXA, which includes the sum of lean tissue mass of the arms and legs relative to height squared [129, 155]. ALTI is commonly used to identify those at a higher risk of poor muscle function, mobility limitations, morbidity and mortality in older adult population [128, 167]. The development of cut-points for identifying those with low lean mass have typically been based on comparisons with a healthy reference cohort, or threshold values that optimally stratify patients based on risk of mortality [15, 138, 145, 167]. However, the effectiveness of classifying risk based on lean tissue index values alone may not be optimal. Multiple adjustment strategies have been proposed to help evaluate muscle depletion relative to the size of the individual [29, 81, 101, 114, 150, 166].

Body composition measures may provide more valid classifications if muscle and fat mass are co-considered in determining the risk of mortality and morbidity in clinical populations [23, 28]. Given that breast cancer survivors generally lose muscle and gain fat mass, it may be important to assess a potential bias whereby those with low muscle mass are generally those with a smaller body size (i.e. lower BMI) and vice versa for those who are generally obese. This relative assessment of muscle and fat tissue may be particularly important when evaluating strength. Here, we evaluate body composition profiles using DXA in a cohort of women undergoing or who recently completed treatment for breast cancer. We compared the DXA-based body composition profiles of patients classified as having lower than normal muscularity with patients in the normal range, and with respect to a reference cohort of young healthy women. In a subset of participants, we evaluated the correlation of unadjusted lean mass, lean mass to BMI ratio, and fat mass to lean mass ratio with respect to muscle strength.

Methods

Study Design

This study is a retrospective cross-sectional observational study that examined DXA scans performed in the Department of Kinesiology at the University of Waterloo. We evaluated 95 breast cancer patients or survivors, who took part in research studies and completed a DXA scan. All women were currently undergoing or had recently completed treatment

for breast cancer (any cancer subtype or stage) of any form (chemotherapy, radiation, surgery, and/or hormonal treatment). A subset of this cohort (n=48) also completed a muscle strength assessment. In addition, we examined 52 scans from a reference cohort of women, 18-30 years old, without pre-existing chronic health conditions. The healthy reference cohort was recruited from the University of Waterloo campus community using posters and word of mouth. The study was approved by the institutional ethics board and written informed consent was obtained from all study participants.

Body Composition Measurements and Calculations

Height and weight were measured using a stadiometer and a scale, respectively. Participants were asked to change into a hospital gown in order to limit potential scan artifacts. Body composition was assessed using a full-body DXA (Hologic Discovery QDR 4500) scan. The scan images were segmented into head, arms, torso and legs to obtain region specific tissue mass and area values. A DXA scan determines the weight of three tissue compartments, lean, fat and bone; a sum of all 3 values was calculated to obtain the cumulative tissue weight in each region. Participants who did not fit on a single image (n=13), completed two scans to ensure all body segments were measured; one participant required three scans. In patients with multiple scans, only values from segments that are captured fully (i.e. those that fit in the scan area) were used. If any segment was fully captured in more than one scan, the corresponding average value was calculated.

Strategies used to express lean mass relative to body size include evaluating the ratio of muscle or lean tissue mass to height squared [15, 108], kilogram bodyweight [79], BMI [29, 101], or as a ratio of whole-body fat mass to lean tissue mass [50, 150]. Appendicular lean tissue index (ALTI) values were calculated by summing lean mass weights of the arms and legs to determine appendicular lean tissue (ALT) mass, and then dividing by height squared. Whole-body or regional percent fat, or percent lean mass, were calculated by dividing the respective tissue mass values by the cumulative tissue weight. The ratio of ALT to BMI and whole-body fat mass to lean mass (FM/LM) were also calculated as suggested previously [29, 101, 150]. The residuals of a regression analysis between lean tissue mass to fat mass were used to help identify those individuals whose observed lean

tissue values are less than predicted based on corresponding fat mass [114, 166]. Since lean mass in the torso region also contains visceral organs, the residual values of a simple regression that included ALT as the dependent and limb fat mass as the independent variables were used to assess the effectiveness of this proposed approach.

Strength Assessment

Isometric maximal voluntary contraction torque (MVC) was measured bilaterally for knee extension and elbow flexion using linear variable differential transformer (LVDT). For the lower body trials, participants were seated with their knees at 90 degrees, while for the upper arm trials the limb was placed on a flat table with the elbow at 90 degrees of flexion. The participants were instructed to gradually ramp-up the exerted force until reaching maximum, to ensure controlled movement. Peak values for each trial were recorded, and the participants were given sufficient recovery after each trial. Moment arm was measured from the centre of the joint to the point of attachment in order to calculate torque. To ensure reliability and accuracy, the average force generated in three trials was calculated. In cases where the %CV of the three trials was higher than 10%, the two values with the smallest %CV were used.

Statistics

Data analysis was performed using R (R Foundation for Statistical Computing, Vienna, Austria). Cohort characteristics are reported as mean $\pm$ sd for continuous or n (%) for categorical variables, unless stated otherwise. Lower muscle mass was classified as < 5.45 kg/m^2 based on previous literature [15]. Group differences were assessed using an independent samples t-test (for two groups) or using one-way ANOVA followed by a Tukey post-hoc comparison (for three or more groups). For categorical variables a chi-squared test was used to evaluated group differences. Pearson correlation coefficients were calculated to determine the strength of an association between two variables, semi-partial correlation was used to test the association between two variables while accounting for the influence of an additional variable on one of the two. For all tests a two-tailed p-value of 0.05 was used as the threshold of statistical significance.

Results

The mean age of the breast cancer patient cohort was 55.3 ± 10.5 years. Mean height and weight were 1.64 ± 0.06 m and 74.42 ± 16.77 kg respectively. The majority of the cohort, 63.1%, were overweight or obese; 16.8% of the women had appendicular lean tissue index (ALTI) values below the 5.45 kg/m^2 threshold, which is recognized as a cut-point for identifying sarcopenic individuals [15]. The reference group (n=52) had a similar mean height, but a lower proportion (13.5%) of participants who were overweight or obese as compared with the breast cancer cohort. The proportion of participants who had ALTI values that were $<$ 5.45 kg/m^2 was similar between groups (Table 6.1).

Characterization of body composition profiles in patients with low appendicular lean tissue index values

Breast cancer patients that were classified as the low ALTI group ($<$ 5.45 kg/m^2) not only had significantly less lean tissue, but they also had less fat tissue mass and lower BMI values compared with patients in the normal ALTI group. However, there were no differences between the groups when lean tissue mass or fat mass were evaluated as a proportion of total whole-body or regional tissue weight (Table 6.2).

When comparing breast cancer patients in the low ALTI group with the reference cohort, the low ALTI group had similar mean BMI and fat mass, but lower lean tissue mass. Conversely, the normal ALTI group had greater BMI and adiposity values but were not different with respect to lean tissue quantities compared with the reference group (Figure 6.1A-D). Both the low and normal ALTI groups of breast cancer patients had higher percent body fat and lower percent lean tissue values than the young reference group (Figure 6.1E-F). Since DXA separates tissue weight into 3 compartments, with the bone compartment contributing a relatively small fraction of the total (3.3 ± 0.6 %) weight, percent lean mass and percent fat mass are strongly and inversely related $r > 0.99$ ($p < 0.001$) (Supp Figure 6.5). Upon closer examination both percent fat and percent lean mass are also closely related to FM/LM ratio (Supp Figure 6.5).

Associations between absolute and adjusted lean mass measurements with respect to muscle strength

The low ALTI group had weaker upper and lower body strength. Interestingly, when strength was expressed relative to limb-specific lean tissue mass, whole body lean tissue, total weight or BMI, no significant differences existed between the 2 groups (Table 6.3). A moderate correlation between MVC and ALTI r = 0.45 (r^2 = 0.2, p = 0.001, 95% CI: 0.19 - 0.66) was observed, which aligns with the results of the group-based comparison used in Table 6.3. While the correlation coefficient between MVC and BMI was r = 0.28 (r^2 = 0.08, p = 0.058, 95% CI: -0.01 - 0.52), neither the FM/LM nor the ALT/BMI ratios correlated with muscle strength, r = 0.03 (r^2 = 0, p = 0.82, 95% CI: -0.26 - 0.32), r = 0.24 (r^2 = 0.06, p = 0.10, 95% CI: -0.05 - 0.49), respectively (Figure 6.2).

Strong associations existed between lean mass and fat mass (r = 0.74; Figure 6.3A), as well as ALTI to BMI (r = 0.79; Figure 6.3B). The correlations between FM/LM or ALT/BMI ratios with respect to fat mass were consistently stronger than with LM, as indicated by non-overlapping 95% confidence intervals (Table 6.4). The observed semi-partial correlation between total MVC and the residuals of a regression between ALT and appendicular fat mass corresponded to r = 0.50, p $<$ 0.001 (Figure 6.4). We further classified breast cancer patients into one of 2 groups based on residual values: 1) those less than -1 standard deviation (sd) below the expected value, and 2) those greater than or equal to -1 sd (Table 6.5). Overall, individuals whose observed values -1sd below the predicted values or less had lower MVC, absolute lean mass and relative lean mass.

Discussion

In this study, we demonstrated that in a cohort of women currently undergoing or who have recently completed treatment for breast cancer, lower ALTI measures were associated with lower body size (i.e. BMI and/or fat mass). DXA-derived measurements of lean tissue strongly correlated with fat mass. These findings were further supported with comparisons of low and normal ALTI groups with a healthy young reference cohort. Those

with lower than normal lean tissue had similar measures of adiposity as the reference cohort. Conversely, the breast cancer cohort with adequate lean tissue mass had greater fat mass than the reference group. Importantly, the proportion of lean or fat tissue as percent of total weight for the low and normal ALTI groups were not statistically different. In a subset analysis, we examined the MVC of the knee extensors and elbow flexors, which are representative muscle groups for lower and upper body strength, respectively. While there was a moderate correlation between ALTI and strength, when we correlated fat to lean mass ratio, or appendicular lean tissue mass per BMI, the correlation no longer existed. Unadjusted lean tissue index values may be biased in identifying only women based on overall body size, rather than women who truly have low muscle per-se. Thus, we suggest that an alternative approach that accounts for total body size is needed to help identify poor muscle strength.

Several reports emphasize the importance of evaluating specific tissues, rather than body weight and BMI, when examining the relations between body composition and outcomes in breast cancer patients in- and post-treatment [30]. Since both fat gain and loss of muscle mass have been shown to occur in this cohort, it is critical to understand not only the independent contribution, but also the interplay between fat and lean tissue. We compared lean mass, fat mass or percent body fat between the lower than normal ALTI group and women with adequate ALTI values, as well as in contrast to young healthy women. We were able to identify that although the low ALTI group had similar BMI values to the reference cohort, they also had less lean tissue mass. When compared to the normal ALTI breast cancer patient group, those with low ALTI had similar percent body fat and percent lean mass values. Importantly, breast cancer patients and survivors who were determined to have lean mass comparable to a young reference cohort on average had BMI values that would classify them as overweight. These findings emphasize that muscularity and adiposity need to be co-considered when examining the risk of adverse outcomes in this cohort.

Both the fat mass to lean mass ratio and the ALT to BMI ratio were strongly related to fat mass, and to a greater extent than to lean mass. Neither adjusted metric correlated with upper body, lower body or combined muscle strength in our cohort. These discrepancies between findings previously reported in the general, older adult cohorts [29, 50, 79], and

our study may be due to disease-specific body composition or muscle function changes. Otherwise, perhaps these can also be attributed to differences in outcomes used. We directly tested muscle strength, where in other circumstances functional testing such as gait speed or grip strength were the outcomes of interest [5, 101].

To identify individuals whose lean tissue mass was lower than expected given their body size, we examined an alternative approach that is based on the use of residual values of a linear regression between fat mass and lean mass [46, 114, 166]. In our cohort, a directly proportional correlation related the regression residuals to MVC values. This suggests that breast cancer patients whose actual observed lean mass values were lower than the values predicted based on their levels of adiposity, also had lower muscle MVC values. When we separated participants into two subgroups $<$ -1sd and $\geq$ -1sd (similar to Z-score analysis), breast cancer participants with high FM/LM ratio or low ALT/BMI ratio were most likely to also have observed lean mass values that were lower than expected. This strategy may help effectively identify insufficient lean tissue mass and/or strength independently of adiposity. When we compared those who would typically be identified as having low muscle mass using the DXA-based ALTI cut-point of < 5.45 kg/m^2, many were misclassified. Only half of those with values $<$ -1sd were also identified as having lower than normal lean tissue index values based on the conventional ALTI cut-point. Given the importance of correctly identifying individuals who are at risk of sarcopenia or malnutrition, this finding needs to be specifically explored in future work to develop new cut-points that account for adiposity.

There are several limitations to our study. This is a retrospective analysis that requires confirmation with a larger and more diverse sample. Use of DXA-based body composition profiles allowed us to evaluate muscle groups directly involved in knee extension and elbow flexion. Despite the breadth of information from DXA, DXA-based lean mass measurements do not capture muscle attenuation, which can predict adverse clinical outcomes in breast cancer patients [4]. Further examination of muscle strength, power as well as physical function testing would also help provide corroborating evidence to support the development of cut-points that effectively identify patients with poor muscle health.

Conclusions

Low muscle mass in breast cancer patients has been demonstrated to effectively identify women who are at risk of poor clinical outcomes, including mortality and treatment toxicity [4, 23]. Moreover, muscle depletion in cancer patients is associated with an increased incidence of comorbidities, poor physical function and quality of life [34, 140]. Identification of lower than normal muscle mass has often been performed using CT-derived muscle index values. CT muscle index values and DXA-based ALTI values are related in cancer patients [108], yet there is no evidence confirming this specifically for breast cancer cohorts. Many of the classification thresholds have been determined by optimal stratification using mortality as an outcome. Risk of mortality may not be the sole outcome of interest to breast cancer patients in- and post-treatment who have a relatively high 5-year survival rate (˜88%) [26]. In contrast, DXA-based ALTI cut-points are determined in relation to a healthy reference cohort, where individuals whose values are -2sd or lower in relation to a sex-specific population mean are classified as having lower than normal muscularity [15, 167]. In the present study, we suggest that approaches evaluating unadjusted lean tissue index values may be biased in identifying women based on overall body size, rather than low muscle per se. Assessment of the amount of lean tissue in context of body size or adiposity is needed to better identify those who fall outside healthy ranges, and may be useful for prescribing targeted interventions such as exercise or nutrition [21, 127].

Table 6.1. Participant characteristics

	Breast Cancer (n = 95)	Healthy Reference (n = 52)	P-value
Age (years)	55.3 ± 10.5	23.1 ± 3.0	**<0.001**
Height (m)	1.64 ± 0.06	1.65 ± 0.07	0.141
Weight (kg)	74.4 ± 16.80	61.4 ± 8.08	**<0.001**
BMI:			**<0.001**
Normal	35 (36.8)	45 (86.5)	
Overweight	29 (30.5)	7 (13.5)	
Obese	31 (32.6)	0 (0)	
ALTI:			0.9427
Low	16 (16.8)	9 (17.3)	
Normal	79 (83.2)	43 (82.7)	

Continuous values are listed as mean ± sd, categorical values are listed as count (%). An independent samples t-test was used to determine significance for continuous variables, chi-squared test was used for categorical variables. ALTI - appendicular lean tissue index, BMI - body mass index.

Table 6.2. Body composition characteristics

	Low ALTI	Normal ALTI	Healthy Reference	P-value
BMI (kg/m^2)	22.47 ± 2.25$^{\mathbf{b}}$	28.73 ± 5.63$^{\mathbf{a}}$	22.37 ± 2.17$^{\mathbf{b}}$	<**0.001**
Total Weight: Whole-Body (kg)	59.25 ± 7.65$^{\mathbf{b}}$	75.44 ± 16.61$^{\mathbf{a}}$	60.15 ± 7.89$^{\mathbf{b}}$	<**0.001**
Lean Mass: Whole-Body (kg)	33.76 ± 2.7$^{\mathbf{ab}}$	41.29 ± 5.52	39.92 ± 5.34	<**0.001**
% Lean Mass	0.57 ± 0.05$^{\mathbf{a}}$	0.56 ± 0.07$^{\mathbf{a}}$	0.67 ± 0.04$^{\mathbf{b}}$	<**0.001**
Fat Mass: Whole-Body (kg)	23.54 ± 5.63$^{\mathbf{b}}$	31.94 ± 12.01$^{\mathbf{a}}$	18.04 ± 4.08$^{\mathbf{b}}$	<**0.001**
% Fat Mass	0.39 ± 0.05$^{\mathbf{a}}$	0.41 ± 0.07$^{\mathbf{a}}$	0.3 ± 0.05$^{\mathbf{b}}$	<**0.001**
ALTI (kg/m^2)	5.09 ± 0.22$^{\mathbf{ab}}$	6.53 ± 0.73	6.24 ± 0.81	<**0.001**
Lean Mass: Arms (kg)	3.17 ± 0.26$^{\mathbf{ab}}$	3.99 ± 0.55	3.81 ± 0.69	<**0.001**
Fat Mass: Arms (kg)	2.88 ± 0.68$^{\mathbf{ab}}$	3.92 ± 1.65$^{\mathbf{a}}$	1.92 ± 0.56$^{\mathbf{b}}$	<**0.001**
Lean Mass: Legs (kg)	10.6 ± 1.01$^{\mathbf{ab}}$	13.5 ± 2.07	13.33 ± 2.24	<**0.001**
Fat Mass: Legs (kg)	9.98 ± 2.6	12.1 ± 4.85$^{\mathbf{a}}$	7.67 ± 1.88$^{\mathbf{b}}$	<**0.001**

All values are listed as mean ± sd. Group differences were assessed using a one-way ANOVA followed by Tukey's Post-Hoc test. ALTI - appendicular lean tissue index, BMI - body mass index.

a Different from Healthy Reference group

b Different from Normal ALTI group

Table 6.3. Strength characteristics of patients with low ALTI

	Low ALTI	Normal ALTI	P-value
MVC: Arms (Nm)	52.19 ± 12.93	63.24 ± 21.3	**0.05**
MVC: Legs (Nm)	162.89 ± 31.05	207.19 ± 50.58	**0.001**
MVC: Total (Nm)	211.24 ± 39.21	270.43 ± 65.87	**0.001**
MVC to Lean Mass ratio: Arms (Nm/kg)	16.56 ± 3.51	15.94 ± 4.39	0.65
MVC to Lean Mass ratio: Legs (Nm/kg)	15.3 ± 2.31	15.8 ± 3.7	0.60
MVC to Lean Mass ratio: Total (Nm/kg)	6.2 ± 0.92	6.66 ± 1.46	0.23
MVC to ALTI ratio: ($Nm/kg*m^{-2}$)	41.8 ± 7.56	41.45 ± 9.85	0.90
MVC to limb weight ratio: Arms (Nm/kg)	0.87 ± 0.22	0.88 ± 0.29	0.92
MVC to limb weight ratio: Legs (Nm/kg)	2.74 ± 0.55	2.91 ± 0.82	0.42
MVC to weight ratio: Total (Nm/kg)	3.52 ± 0.63	3.79 ± 1.02	0.30
MVC per unit BMI ($Nm/kg*m^{-2}$)	9.37 ± 2.12	9.75 ± 2.88	0.65

Values are listed as mean ± sd. An independent samples t-test was performed to determine statistical significance. ALTI - appendicular lean tissue index, BMI - body mass index, MVC - maximal voluntary contraction.

Table 6.4. Associations between adjusted body size measurements with respect to lean and fat mass

	Lean Mass	Fat Mass
BMI	0.75 (r^2=0.56, p>0.001, 95% CI: 0.64 - 0.82)	0.93 (r^2=0.86, p>0.001, 95% CI: 0.90 - 0.96)
Fat to Lean ratio	0.43 (r^2=0.18, p>0.001, 95% CI: 0.25 - 0.58)	0.91 (r^2=0.83, p>0.001, 95% CI: 0.87 - 0.94)
ALT to BMI ratio	0.01 (r^2=0.00, p=0.910, 95% CI: -0.19 - 0.21)	-0.55 (r^2=0.30, p>0.001, 95% CI: -0.68 - -0.39)

Values are Pearson correlation coefficient (coefficient of determination, p value, 95% confidence interval). ALT - appendicular lean tissue mass, BMI - body mass index

Table 6.5. Comparison between groups stratified based on fat-adjusted lean mass values

	$<$ -1sd	$\geq$ -1sd	P-value
Height (m)	1.6 ± 0.06	1.65 ± 0.06	$<$**0.001**
BMI (kg/m^2)	26.82 ± 5.03	28.23 ± 6.03	0.23
Total Weight: Whole-Body (kg)	67.42 ± 12.39	75.79 ± 17.88	**0.01**
Lean Mass: Whole-Body (kg)	35.75 ± 3.32	42.42 ± 5.60	$<$**0.001**
Fat Mass: Whole-Body (kg)	29.62 ± 9.18	31.14 ± 12.79	0.50
Fat to Lean ratio	0.81 ± 0.19	0.72 ± 0.21	**0.02**
ALTI (kg/m^2)	5.72 ± 0.68	6.61 ± 0.78	$<$**0.001**
ALT to BMI ratio	0.56 ± 0.07	0.65 ± 0.09	$<$**0.001**
Lean Mass: Arms (kg)	3.32 ± 0.31	4.14 ± 0.51	$<$**0.001**
Fat Mass: Arms (kg)	3.74 ± 1.30	3.77 ± 1.72	0.92
Lean Mass: Legs (kg)	11.35 ± 1.27	13.95 ± 2.07	$<$**0.001**
Fat Mass: Legs (kg)	11.67 ± 3.59	11.82 ± 5.11	0.87

Values are listed as mean (sd). An independent samples t-test was performed to determine statistical significance. ALT - appendicular lean tissue mass, ALTI - appendicular lean tissue index, BMI - body mass index.

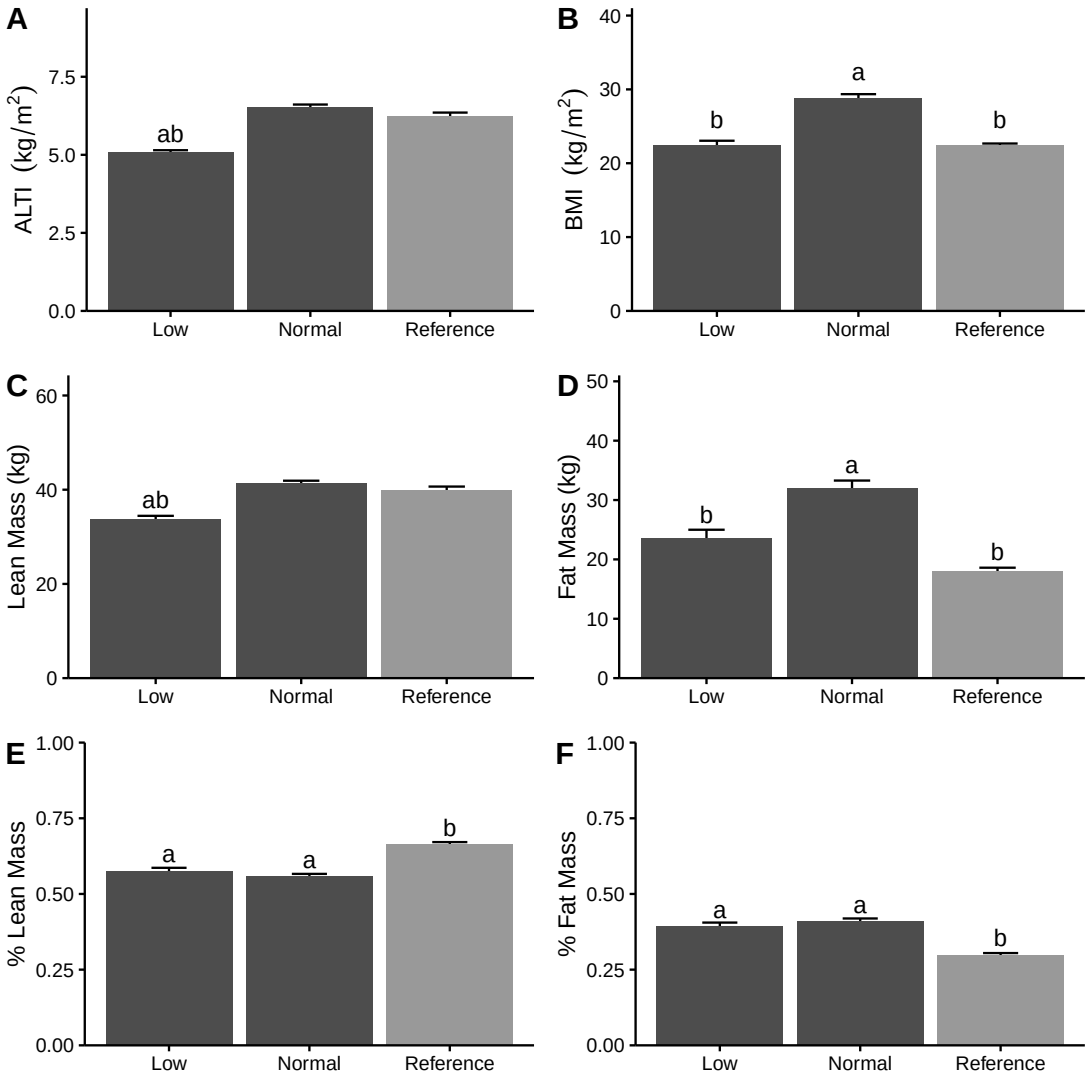

Figure 6.1. Body composition profiles of breast cancer patients in the Low and Normal ALTI groups, and the Healthy Reference cohort. (A) Appendicular lean tissue index (ALTI). (B) Body mass index (BMI). (C-D) Whole-body lean and fat mass, respectively. (E-F) Percent lean mass and percent fat mass.

a - different from Healthy Reference group, **b** - different from Normal ALTI group.

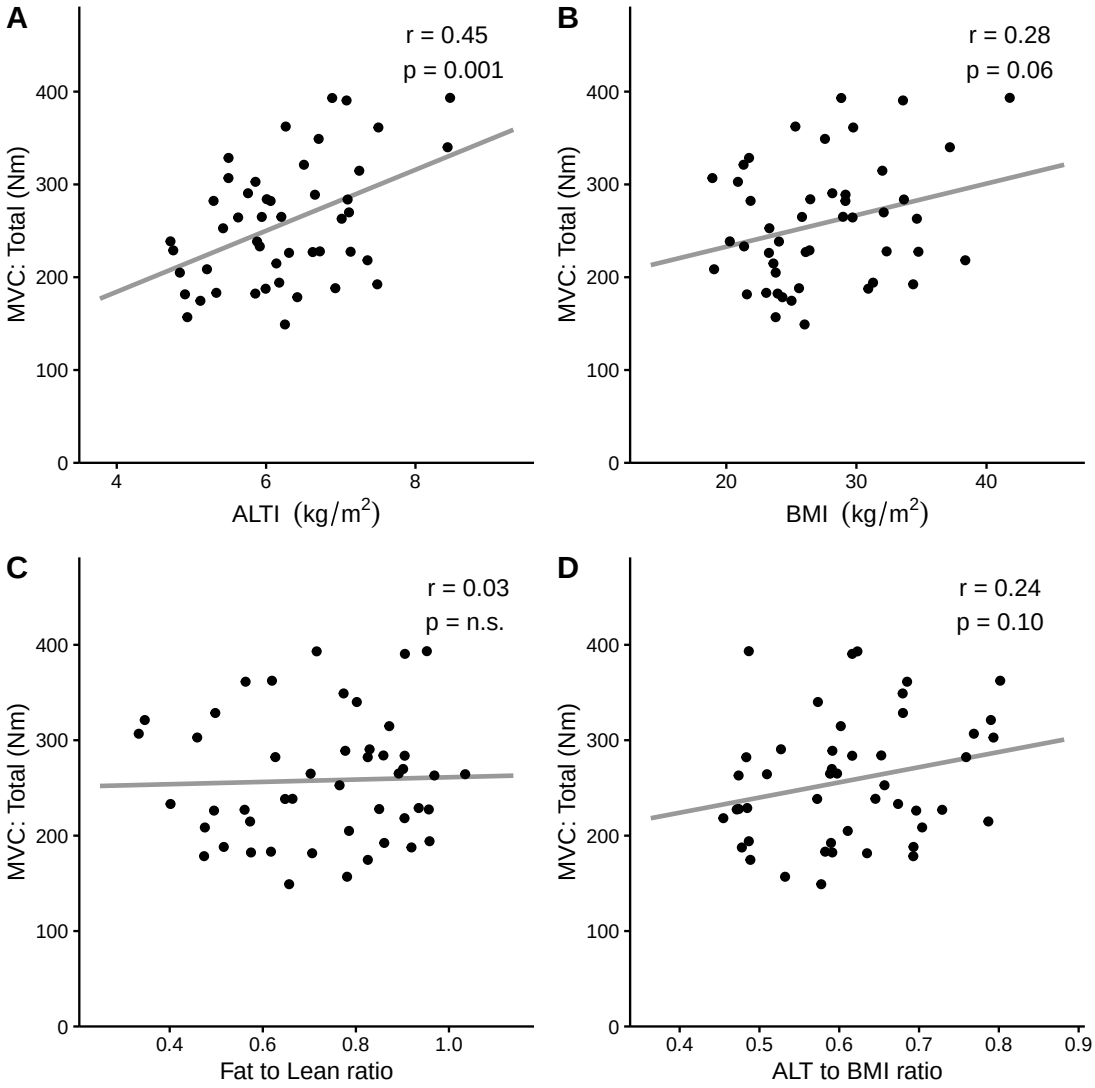

Figure 6.2. Correlations between appendicular lean tissue index (A), body mass index (B), as well as the relative lean mass measures of fat-to-lean ratio (C) and appendicular lean tissue mass (ALT) to BMI ratio (D), with respect to muscle strength represented as sum of maximal voluntary contractions (MVC) for the arms and legs.

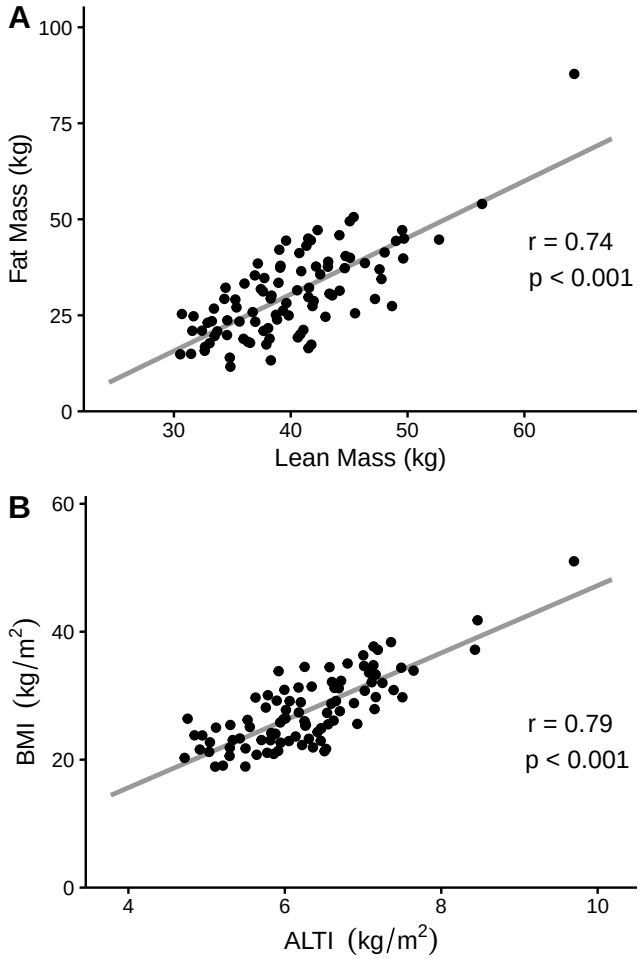

Figure 6.3. Pearson correlations between whole-body lean tissue mass to fat mass (A) and between appendicular lean tissue index and body mass index (B).

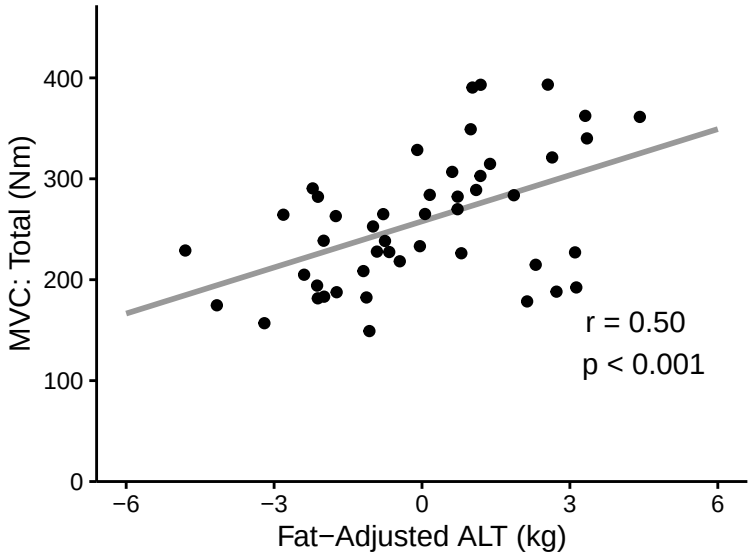

Figure 6.4. Association between fat-adjusted appendicular lean tissue (ALT) residual values and the sum of maximal voluntary contractions (MVC) for the arms and legs.

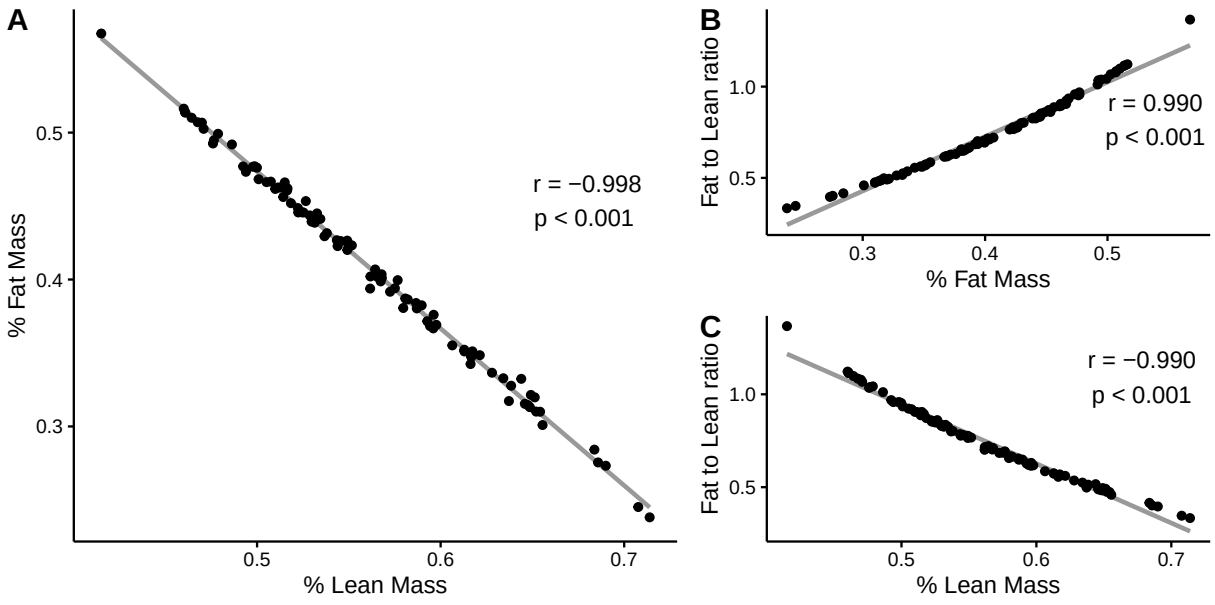

Figure 6.5. Pearson correlations between percent lean mass and percent fat mass (A), and between the fat-to-lean ratio with respect to percent fat mass (B) or percent lean mass (C).

Chapter 7

Evaluating skeletal muscle characteristics using ultrasound-based measurements in breast cancer patients in- and post-treatment: A pilot feasibility study

Abstract

Skeletal muscle health is an important predictor of survival, treatment toxicity, and physical function in breast cancer patients and survivors. While ultrasound is a portable, relatively inexpensive and accessible modality that may evaluate muscle size, composition and architecture, this modality has not been used for body composition analysis in breast cancer patients in- and post-treatment. This cross-sectional pilot feasibility study examined the feasibility of acquiring ultrasound-based measures of muscle characteristics, including thickness, echogenicity, pennation angle and fascicle length. Furthermore, we assess the reliability of image analysis as well as the agreement between appendicular lean

mass (ALM) observed with DXA and ALM predicted using ultrasound muscle thickness, in a cohort of 5 breast cancer patients and a reference group of 17 healthy women. Overall, the coefficient of variability of ultrasound-based muscle thickness measurements was less than 1%, for both intra- and inter-rater reliability. Echogenicity varied between two independent raters, or the same rater who repeated the analysis, by no more than 5.36%. However, the reliability of pennation angle and fascicle length segmentation was greater than 10%. Based on Bland-Altman analysis, the average bias between actual and predicted ALM values was 1.08 kg with a 95% confidence interval of -0.418 kg to 2.57 kg, and limits of agreement of -1.47 kg to 3.63 kg. Overall, our results indicate that ultrasound images can be obtained in this cohort, while measurements of muscle thickness are reliable and can be used to assess the size of functionally relevant muscle groups, measurements of echogenicity and architecture require further method development.

Introduction

Weight gain and loss of muscle mass commonly occur in breast cancer patients throughout and following treatment [147, 159]. However, the gain in fat mass that breast cancer survivors typically exhibit may mask muscle atrophy when evaluating weight or body mass index (BMI) [23, 129]. Thus, it is essential that body composition assessment tools have the capacity to distinguish fat from skeletal muscle mass. Measurements of muscle size (including thickness, cross-sectional area or volume) and composition (such as fatty infiltration) may be important predictors of survival, chemotherapy toxicity, and physical function in this cohort [4, 129, 168], and may facilitate in evaluating the effectiveness of lifestyle interventions such as exercise and nutrition.

While there are several different methods to measure body composition, few have combined portability and precision. Computed tomography image analysis and dual energy X-ray absorptiometry (DXA) are two body composition modalities commonly used to examine muscle indices as well as changes in skeletal muscle mass across the cancer trajectory [21, 85, 145]. However, there are some practical constraints related to the use of these modalities. Assessment of skeletal muscle mass in most cancer survivors may be limited

due to accessibility of equipment, cost of operating the equipment as well as radiation exposure [49, 155]. Field methods of body composition assessment, such as BMI, anthropometrics, and bioelectrical impedance analysis (BIA), may lack the specificity, accuracy and/or precision to quantify skeletal muscle mass, especially in clinical populations [49, 128]. However, ultrasonography has been used to measure skeletal muscle mass in aging, critically ill, and patients with muscle dystrophies [106, 120, 123, 156]. Ultrasounds are portable, do not emit any radiation and can provide detailed tissue characteristics [76, 107, 156]; however, they have not been used for body composition analysis in cancer patients in- and post-treatment.

Ultrasonography may determine the dimensions of prominent muscle groups, as well as structural features of the muscle assessed [2, 41, 106, 123]. Ultrasound-based muscle thickness measurements may be used to not only examine the dimensions of specific muscle groups, but have also been used to predict regional and whole body muscularity [1, 116, 119, 156]. Also, this modality may aid the assessment of muscle composition using echogenicity. Recent evidence indicates that increased anterior thigh muscle echogenicity values are associated with fatty infiltration, poor strength and physical function scores [41, 69, 123]. Beyond muscle size and composition, muscle architecture features such as pennation angle and fascicle length can also be evaluated and provide further information about muscle function [2, 112]. These ultrasound-based muscle features have not been evaluated in any cancer cohort. Although ultrasound-based measurements of muscle characteristics may serve as a portable and easy to use alternative to CT or DXA, currently there is a lack of evidence corroborating the effectiveness of this modality in breast cancer patients in- and post-treatment.

In this study, we examine muscle size, composition, architecture and function in a cohort of women who are currently undergoing or recently completed treatment for breast cancer in order to pilot the respective ultrasound protocols. We will evaluate the feasibility of obtaining each image, in order to determine whether acquiring ultrasound measures is possible in this cohort. In addition, we assess the reliability of image analysis for measurements of muscle thickness, echogenicity and architecture. We also evaluated the effect size of group differences in body composition measurements between the breast cancer cohort and a group of healthy young women to help inform sample size calculations for a future

study.

Methods

Study Design

This study is a prospective cross-sectional pilot feasibility study in a cohort of community-dwelling breast cancer survivors in- and post-treatment. The participants attended a single data collection session at the University of Waterloo. Outcome measurements included demographic information, anthropometry, DXA scan to assess whole body and regional composition, ultrasound measurement of muscle size, composition and architecture and diverse landmarks, and an isometric maximal voluntary knee extension test. We also recruited a group of healthy young women as a reference cohort. The study was approved by the institutional ethics board and written informed consent was obtained from all study participants.

Participants

In this study we recruited 5 breast cancer patients. The inclusion criteria for the cancer cohort were: >40 years of age and currently undergoing treatment or have been treated for breast cancer within the past 2 years (including chemotherapy, surgery, radiation therapy or hormonal therapy). Breast cancer patients and survivors were excluded if they had: a diagnosis of metastatic or bone cancer, neuromuscular disease or $\geq$1 episode of stroke, insufficient physical ability to perform the Short Physical Performance Battery (SPPB), any counterindication to exercise, DXA or ultrasonography. We also recruited a reference group of 17 women, from the local campus community, who were 18-30 years old and who did not have pre-existing chronic health conditions. All participants were recruited using posters and a newspaper ad.

Anthropometry

Height and weight were acquired using a stadiometer and scale, respectively. The participants were asked to wear light-weight athletic clothing and remove their shoes for both the height and weight measurements.

Isometric muscle strength

Maximal isometric knee extension strength was measured for both legs using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA). The participants were seated in a chair with their knee joint axis of rotation positioned in-line with the axis of rotation of the level arm of the dynamometer; chest, waist mid-thigh and lower leg straps were used to stabilize the participant and isolate the muscles of interest. Isometric maximal voluntary contraction values were obtained using a 3 second maximal contraction at 60° of flexion, with 30 seconds of rest between each trial (3 trials/leg). To ensure reliability and accuracy of strength measurements, the average of 3 trials was calculated. In cases where the percent coefficient of variation (%CV) of the 3 trials was higher than 10%, the 2 values with the smallest %CV were used.

Dual energy X-ray absorptiometry (DXA)

DXA is a non-invasive imaging assessment that provides accurate and reliable measurement of lean mass, fat mass and bone mineral content. The DXA scan was performed using a Hologic Discovery QDR 4500 scanner (Hologic, Toronto, ON) by a certified Medical Radiation Technologist. The participants were placed on the bed of the scanner in supine position with the legs extended and arms resting alongside the body, without coming in contact with the sides of the body. The participants were instructed to remain still for the duration of the scan. A second scan was performed on one participant to ensure that all body segments were captured by the scanner. A single trained analyst segmented the arms, legs, pelvis, and thoracoabdominal regions using Hologic Apex software (version 13.2). Whole-body or regional percent lean mass values were calculated by dividing the respective tissue mass values by the net body weight. Appendicular lean tissue mass (in

kg) was calculated by adding the lean tissue masses for the legs and arms, and appendicular lean tissue index (ALTI) was calculated dividing appendicular lean tissue mass (in kg) by height squared as (m^2).

Ultrasound Image Acquisition

All images were acquired using B-mode ultrasound imaging device (M-Turbo, SonoSite, Markham, ON, Canada) equipped with a multi-frequency linear array transducer (L38xi, 5-10 MHz). Muscle thickness measures at 5 easily accessible sites were obtained according to the protocol described in Paris *et al.* [119]. The landmarks of the 5-site protocol included:

- Site 1 - 60% of the length from the acromial process of the scapula to the lateral epicondyle of the humerus at the anterior aspect of the right upper arm.
- Sites 2 and 3 - 50% of the length between the anterior superior iliac spine (ASIS) and the midpoint of the superior edge of the patella on the right and left thigh.
- Sites 4 and 5 - 2/3 of the length between the ASIS and the midpoint of the superior edge of the patella, bilaterally.

These measures together with age, sex and height were used to predict ALM [119]. Since there are no equations estimating ALM specifically in breast cancer patients, we evaluated the accuracy of the previously published 5 site predictive equation in this study [119]. To determine muscle thickness and echogenicity, we landmarked and obtained transverse images at the mid-thigh level (50% of the distance between the greater trochanter and the lateral condyle of the knee) for both the anterior (rectus femoris and vastus intermedius) and lateral (vastus lateralis and vastus intermedius) aspects of the thigh. Ample transmission gel and minimal skin compression was used during image acquisition. Probe angle was adjusted to maximize the visibility of the bone. Images of muscle architecture were acquired at the lateral mid-thigh landmark, with the probe oriented parallel to the direction of the fibres (longitudinally) and perpendicular to the skin without compression of underlying tissues. All ultrasound settings were held constant across all study participants, at their default values preset by the manufacturer.

Ultrasound Image Processing

Ultrasound images were saved in the Digital Imaging and Communications in Medicine (DICOM) format and transferred to a computer for further analysis. Muscle thickness was obtained by measuring the perpendicular distance between the upper margin of the femur and the lower boundary of the most superficial muscle group in the image. Subcutaneous fat thickness was determined by measuring the vertical distance between the superior border of the muscle fascia and the inferior border of the skin at the medial aspect of the image, the centre and the lateral aspect, the 3 measurements were averaged. To determine echogenicity a rectangular region of interest was placed in the superficial muscle (rectus femoris or vastus lateralis) to maximize the area of the region without including any of the surrounding fascia. The individual gray scale intensities (expressed as values between 0 - black and 255 - white) for all pixels in the region of interest were averaged to calculate mean echogenicity. To determine muscle architecture, the analyst placed a straight line such that it best fits the orientation of the deep and superficial fascia of vastus lateralis, as well the most visible representative fascicle. The angle of each line relative to horizontal was determined, the angle between the deep fascia and the representative fascicle was calculated to compute pennation angle. In order to calculate fascicle length, the distance between the superficial and deep fascia at the centre of the image was determined, fascicle length was computed as fascia distance divided by sine of pennation angle. All image segmentation and analysis was performed using a custom Python script. Images that were not collected, or where the structures could not be identified during analysis, were recorded and tallied in order to assess the feasibility of obtaining ultrasound-based muscle features.

Statistics

Data analysis was performed using R (R Foundation for Statistical Computing, Vienna, Austria). Demographic characteristics are reported as mean along with standard deviation (sd) for continuous or n (%) for categorical variables, unless stated otherwise. Due to the descriptive, pilot approach used in this study we have not preformed hypothesis testing on group differences in body composition variables. Effect size calculations were performed using cohen's d in order to assess the magnitude of the difference in means between the

breast cancer cohort and the healthy reference group. Reliability of the ultrasound image segmentation was evaluated both within a single analyst who evaluated each image independently on 2 occasions more than a week apart, to assess inter-rater reliability the results of 2 difference analysts were compared. Reliability was evaluated using percent coefficient of variability statistics and intra-class correlation coefficients. Features for which the image was successfully acquired and segmented was qualified as feasible. Bland-Altman analysis was preformed to examine the bias and the limits of agreement between appendicular lean tissue predicted using ultrasound-based muscle thickness values, age, height and sex in relation to the DXA-based ALM values observed directly. In addition, we used a Pearson correlation to evaluate the association between ultrasound-based fat thickness values and mean echogenicity.

Results

In this feasibility pilot study, we evaluated a convenience sample of 5 breast cancer participants who are currently undergoing or have recently completed treatment for breast cancer. The cancer cohort had a mean ± sd age of 60 ± 15 years, height of 1.64 ± 0.09 m and weight of 80.68 ± 21.93 kg. Mean body mass index (BMI) was 29.47 ± 4.65 kg/m^2 (Table 7.1). The reference healthy young cohort included 17 women with a mean age of 23 ± 4 years, height of 1.64 ± 0.07 m, weight of 59.45 ± 9.00 kg and BMI of 22.12 ± 2.71 kg/m^2 (Table 7.1).

Images for all landmarks of the 5-site protocol, as well as the mid-thigh transverse and longitudinal images, were successfully obtained and analyzed for all participants. We observed excellent reliability of muscle thickness measurements at both the anterior and lateral aspects of the thigh. At the anterior mid-thigh landmark the %CV values were 0.31% for intra- and 0.90% for inter-rater reliability. At the lateral landmark these corresponded to 0.29% and 0.65%, for intra- and inter-rater reliability, respectively (Table 7.2). Mean echogenicity measurements at the same landmarks yielded an intra-rater CV of 5.36% at the anterior mid-thigh and 1.80% at the lateral thigh site. The inter-rater reliability corresponded to 2.09% at the anterior site and 2.69% at the lateral landmark. However, the

reliability of muscle architecture segmentation was poor for pennation angle and fascicle length values, corresponding to %CV values in breast cancer patients that were greater than 12% and ICC values of 0.504 or lower (Table 7.2). In addition, we repeated the segmentation of these muscle characteristics on images acquired in the healthy reference cohort, the values in this group are in line with the results observed in the breast cancer cohort (see Table 7.2).

The ALM values predicted using the 5-site equation in breast cancer patients were not different from actual DXA measures of ALM with an average bias of 1.08 kg and a 95% confidence interval of -0.418 kg to 2.57 kg. Based on Bland-Altman analysis (Figure 7.1), the limits of agreement between DXA and ultrasound-based predicted values are -1.47 kg to 3.63 kg. The healthy reference cohort demonstrated a bias of -0.336, with a precision interval corresponding to -1.20 - 0.532 kg, and limits of agreement of -3.66 - 2.99 kg.

When comparing the DXA-based body composition characteristics between the breast cancer and reference cohorts, we observed a large ($\geq$0.8) effect size [38, 39] in total weight and fat mass (Table 7.3), where the breast cancer group had higher whole body and regional tissue mass. Group differences in lean tissue mass and ALTI were in the medium ($\geq$0.5 to $<$0.8) effect size [38, 39] range, however, the breast cancer group had the higher mean value compared to the reference cohort. The ratio between fat mass to lean mass was calculated to estimate differences in the relative quantity of adipose to lean tissue. In the breast cancer patient cohort, the values for whole body and regional fat-to-lean ratios were greater, above the 0.8 threshold indicating a large effect size.

We observed a large effect size for differences in group means for peak isometric muscle strength, with a difference of -89.29 $\pm$ 72.76 Nm and effect size of -1.23 (Table 7.4). Ultrasound images were analyzed to evaluate 3 types of muscle characteristics. We evaluated muscle thickness as a measure of muscle size, mean echogenicity to assess muscle composition, and images of the lateral thigh with the probe oriented longitudinally were analyzed to examine muscle architecture (pennation angle and fascicle length). Mean muscle thickness measurements were lower in the breast cancer patient cohort compared with the healthy young reference group, both at the anterior and lateral aspect of the thigh, corresponding to a large effect size of -1.13 and -0.82, respectively. There were no mean group differences between the 2 cohorts in echogenicity values. We observed a small effect

size ($\geq$0.2 to $<$0.5) in pennation angle, while there were no differences in fascicle length.

To further examine the absence of differences in muscle echogenicity values between the groups, we analyzed subcutaneous fat thickness values and whether fat thickness is possibly confounding the echogenicity values we observed in our study. Indeed, the mean fat thickness values in the breast cancer group was greater at both the anterior and lateral aspects of the thigh, corresponding to a difference of 0.75 $\pm$ 0.37 cm (effect size of 2.02) and 0.83 $\pm$ 0.51 cm (effect size of 1.64), respectively (Supplementary Table 7.5, Figure 7.2A-B). In addition, we tested the association between fat thickness and echogenicity values. At the anterior thigh landmark (rectus femoris) we observed a coefficient of correlation of r = -0.3 (p = 0.05, Figure 7.2C), while at the lateral thigh landmark (vastus lateralis) the correlation coefficient was r = -0.4 (p = 0.01, Figure 7.2D). Thus, further studies are needed to help establish an approach of evaluating muscle composition differences while accounting for any possible confounding effects due to greater fat thickness.

Discussion

In this pilot feasibility study, we evaluated whether ultrasound-based measurements of muscle characteristics in a cohort of community-dwelling breast cancer patients in- and post-treatment is feasible and reliable. To date, the use of musculoskeletal ultrasound in cancer patients and survivors has not been characterized. Our findings highlight that ultrasound image acquisition is feasible for all landmarks examined, and the reliability of image analysis for muscle thickness is similar to that of a reference healthy cohort. Nonetheless, the reliability analysis identified that ultrasound-based measurements of muscle architecture yield inconsistent results both within a single rater when analysis is repeated, or between 2 analysts. Further investigation is needed to help develop protocols that yield consistent results.

While there are a number of distinct muscle characteristics that can be assessed, including measurements of size, composition and structure, currently there is a lack of consensus of which best characterize muscle health. Cancer patients, including breast cancer patients and survivors, who fall below clinically validated muscularity cutoff values are at a higher

risk for adverse clinical outcomes [34, 138, 145, 167]. Our lab group previously developed a protocol which uses ultrasound-based muscle thickness measurements at 5 easily accessible landmarks together with age, sex and height in order to predict appendicular lean mass (as measured by DXA) and to classify those with low lean mass [119]. While this pilot feasibility study is too small to test the effectiveness of the discrete classification predictive equations, we compared continuous ALM values predicted using these published formulas to observed DXA-based appendicular lean mass. The results of Bland-Altman analysis suggest that there is no bias in the prediction and the limits of agreement (i.e. the interval estimated to contain 95% of the error) for this approach is within -1.47 kg to 3.63 kg. Although we used a small sample size in this pilot feasibility study, our findings are similar to the healthy reference group in the present and previous study [119].

Beyond estimating whole body muscularity, ultrasound measurements can be used to examine muscle characteristics in relation to functional outcomes [2, 95, 154], or in comparison to either a reference cohort or age-matched controls. Effect size calculations using the cohen's d formula are commonly used to help estimate the sample size of prospective trials evaluating differences between two groups [38, 39]. With a two-tailed alpha at the 0.05 level and a statistical power of 0.8, to detect an effect of 0.8 (the threshold for large effect size category) one would need 26 participants per group (breast cancer versus young healthy reference cohort). Our findings suggest that differences in muscle thickness between the breast cancer cohort and healthy reference group fall within the large effect size range, while the effects for measures of muscle echogenicity or architecture are small or null. Although these results are preliminary, they indicate that ultrasound-based measurements may be effectively used to study alteration in muscle size which may occur over the cancer treatment trajectory, but potentially not muscle composition or muscle architecture. Further validation and method development is necessary to better understand the interpretation of these findings.

While all muscle architecture images were successfully obtained in our study, we show that the reliability of image segmentation to determine pennation angle and fascicle length was poor, as indicated by %CV values of greater than 10%. The ICC and %CV values were similar for intra- and inter-rater reliability, and in both the breast cancer patient and the healthy reference groups. Other steps involved in the measurements process, such as identi-

fying the exact landmark, positioning the participant, and aspects of the image acquisition such as probe tilt, are all likely to further increase the variability of the measurements. While some reports suggest that ultrasound-based muscle architecture measurements are reliable [88, 116], it is possible that there may be variations between different landmarks and muscle groups. Recently, a number of automated muscle architecture segmentation algorithms have been developed [98, 144], and perhaps these may help eliminate the inconsistencies related to manual analysis of architecture features.

The subcutaneous fat layer may act as a confounding factor with respect to echogenicity measurements of muscle composition [68, 171]. Studies in the general older adult population have reported that increased echogenicity is associated with poor muscle function [25, 62, 165]. In our study, we observed that peak isometric strength in breast cancer patients is lower when compared to the mean of the healthy reference group. Yet, mean echogenicity values both at the anterior and lateral aspects of the thigh were similar across the 2 cohorts. This led us to examine the fat thickness values in the two cohorts, and indeed there is a large difference in mean fat thickness values between the two groups corresponding to 0.75 $\pm$ 0.37 cm and 0.83 $\pm$0.51 cm at the anterior and lateral landmark, respectively. We also observed a statistically significant inverse association between subcutaneous fat thickness and mean echogenicity values, thus suggesting that unadjusted values may be biased in the breast cancer cohort due to greater adiposity. Furthermore, our group recently examined the effect of adjusting the depth setting during image acquisition, which is performed in order to capture the full muscle area and identified that inconsistent image depth and consequently resolution may be an additional contributor to error in multiple ultrasound-based muscle composition measures [118].

Despite that ultrasonography along with CT and DXA are primarily used for clinical assessment, they can concurrently be used for body composition assessment [21, 28, 145]. Ultrasonography is relatively low cost, easy to use and it is a portable modality which may facilitate examination of skeletal muscle features [106, 155]. Ultrasound assessment of skeletal muscle is a potential bedside option for prospective studies of clinical populations, which are often challenging in terms of recruitment, retention and burden on the patients. Further development of precise and accurate methods to enable practical ultrasound-based measures of muscle features is needed as a basis for large scale trials.

Table 7.1. Participant demographic information

	Breast Cancer (n = 5)	Healthy Reference (n = 17)
Age (years)	59.60 ± 14.8	23.18 ± 3.7
Height (m)	1.64 ± 0.09	1.64 ± 0.07
Weight (kg)	80.68 ± 21.93	59.45 ± 9.00
BMI (kg/m^2)	29.47 ± 4.65	22.12 ± 2.71

Values are listed as mean ± sd. BMI - body mass index.

Table 7.2. Reliability of ultrasound-based muscle properties

	Intra-rater		Inter-rater	
	ICC (95% CI)	%CV	ICC (95% CI)	%CV
Breast Cancer				
Thickness: Anterior	1.000 (0.999 - 1.000)	0.31	0.997 (0.989 - 0.999)	0.90
Thickness: Lateral	0.999 (0.997 - 1.000)	0.29	0.998 (0.993 - 1.000)	0.65
Echo: Anterior	0.725 (0.249 - 0.923)	5.36	0.986 (0.949 - 0.997)	2.09
Echo: Lateral	0.990 (0.960 - 1.000)	1.80	0.980 (0.940 - 1.000)	2.69
Pennation Angle	0.380 (-0.055 - 0.696)	14.06	0.309 (-0.134 - 0.652)	17.69
Fascicle Length	0.504 (0.100 - 0.768)	13.13	0.203 (-0.244 - 0.582)	17.42
Healthy Reference				
Thickness: Anterior	0.998 (0.995 - 0.999)	0.54	0.999 (0.997-0.999)	0.45
Thickness: Lateral	0.998 (0.996 - 0.999)	0.54	0.999 (0.997-0.999)	0.41
Echo: Anterior	0.980 (0.960 - 0.990)	2.46	0.971 (0.942-0.985)	3.28
Echo: Lateral	0.980 (0.962 - 0.990)	1.82	0.964 (0.930-0.982)	2.82
Pennation Angle	0.638 (0.473 - 0.759)	12.28	0.453 (0.243-0.622)	11.61
Fascicle Length	0.267 (0.034 - 0.474)	12.21	0.639 (0.474-0.760)	11.52

Ultrasound-based muscle characteristics were measured at the Anterior (thickness and echogenicity) and Lateral (thickness, echogenicity, pennation angle and fascicle length) aspects of the mid-thigh at 50% of the distance between the greater trochanter and the lateral condyle of the knee. %CV - percent coefficient of variation, CI - confidence interval, Echo - echogenicity, ICC - intraclass correlation coefficient.

Table 7.3. DXA-based body composition characteristics

	Breast Cancer	Healthy Reference	Difference	Effect Size
Whole Body				
Total Weight (kg)	78.78 ± 21.29	58.26 ± 8.70	20.52 ± 12.3	1.67
Lean Mass (kg)	42.22 ± 9.22	38.65 ± 5.57	3.57 ± 6.47	0.55
Fat Mass (kg)	34.30 ± 11.92	17.52 ± 4.38	16.78 ± 6.61	2.54
Fat-to-Lean Ratio	0.80 ± 0.11	0.46 ± 0.10	0.34 ± 0.10	3.35
ALTI (kg/m^2)	6.55 ± 0.69	6.07 ± 0.91	0.48 ± 0.87	0.55
Legs				
Total Weight (kg)	26.36 ± 7.03	20.66 ± 3.62	5.70 ± 4.52	1.26
Lean Mass (kg)	13.82 ± 3.02	12.7 ± 2.36	1.13 ± 2.51	0.45
Fat Mass (kg)	11.73 ± 4.03	7.23 ± 1.66	4.50 ± 2.33	1.93
Fat-to-Lean Ratio	0.83 ± 0.14	0.58 ± 0.13	0.26 ± 0.13	2.01
Arms				
Total Weight (kg)	8.57 ± 1.56	5.71 ± 1.07	2.87 ± 1.18	2.42
Lean Mass (kg)	3.99 ± 0.68	3.65 ± 0.76	0.34 ± 0.75	0.45
Fat Mass (kg)	4.27 ± 1.00	1.79 ± 0.56	2.48 ± 0.67	3.68
Fat-to-Lean Ratio	1.08 ± 0.20	0.50 ± 0.17	0.58 ± 0.17	3.32

Values are listed as mean (sd), Cohen's d calculation was used to estimate effect size. ALTI - appendicular lean tissue index.

Table 7.4. Effect size of muscle strength and ultrasound-based characteristics

	Breast Cancer	Healthy Reference	Difference	Effect Size
Knee Extension MVC (Nm)	225.37 ± 48.5	314.66 ± 77.65	-89.29 ± 72.76	-1.23
Thickness: Anterior (cm)	3.16 ± 0.65	3.78 ± 0.52	-0.62 ± 0.55	-1.13
Thickness: Lateral (cm)	3.59 ± 0.65	4.08 ± 0.59	-0.49 ± 0.6	-0.82
Echo: Anterior (AU)	50.34 ± 6.51	50.31 ± 10.66	0.04 ± 9.97	0.00
Echo: Lateral (AU)	46.44 ± 11.79	45.96 ± 6.74	0.48 ± 8.01	0.06
Pennation Angle (degrees)	14.48 ± 1.68	15.82 ± 2.91	-1.34 ± 2.71	-0.49
Fascicle Length (cm)	7.71 ± 1.46	7.91 ± 2.05	-0.2 ± 1.95	-0.10

Ultrasound-based muscle characteristics were measured at the Anterior (thickness and echogenicity) and Lateral (thickness, echogenicity, pennation angle and fascicle length) aspects of the mid-thigh at 50% of the distance between the greater trochanter and the lateral condyle of the knee. Values are listed as mean (sd), Cohen's d calculation was used to estimate effect size. AU - arbitrary units, Echo - echogenicity, MVC - maximal voluntary contraction, Nm - Newton meters.

Table 7.5. Subcutaneous fat thickness

	Breast Cancer	Healthy Reference	Difference	Effect Size
Fat Thickness: Anterior (cm)	1.92 ± 0.47	1.17 ± 0.34	0.75 ± 0.37	2.02
Fat Thickness: Lateral (cm)	1.97 ± 0.81	1.14 ± 0.4	0.83 ± 0.51	1.64

Ultrasound-based fat thickness was measured at the Anterior and Lateral aspects of the mid-thigh at 50% of the distance between the greater trochanter and the lateral condyle of the knee. Values are listed as mean (sd), Cohen's d calculation was used to estimate effect size.

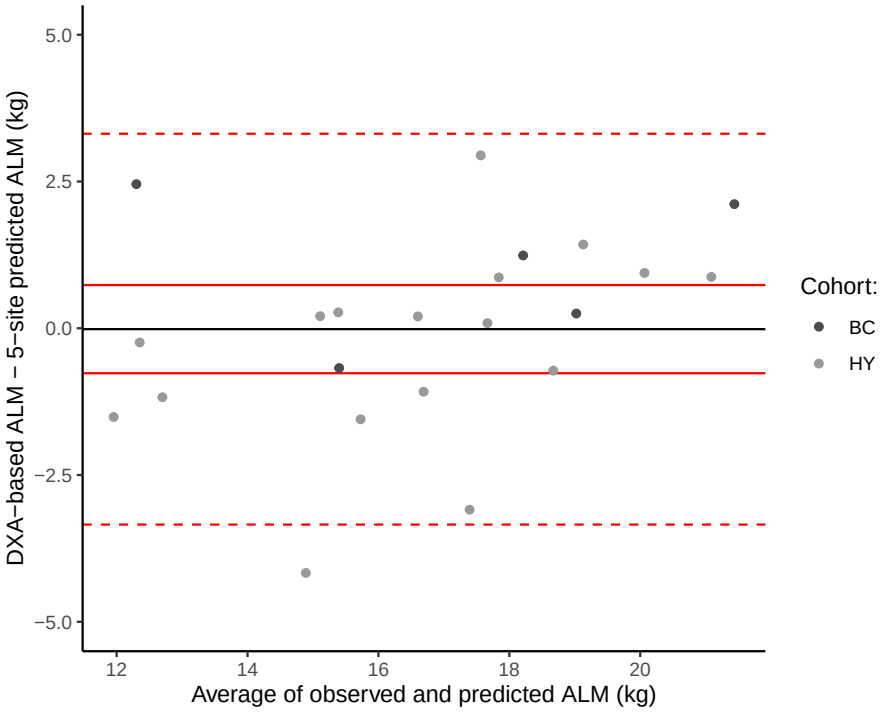

Figure 7.1. Bland Altman plot for DXA-based appendicular lean mass (ALM) values and ALM predicted using ultrasound-based muscle thickness measurements.

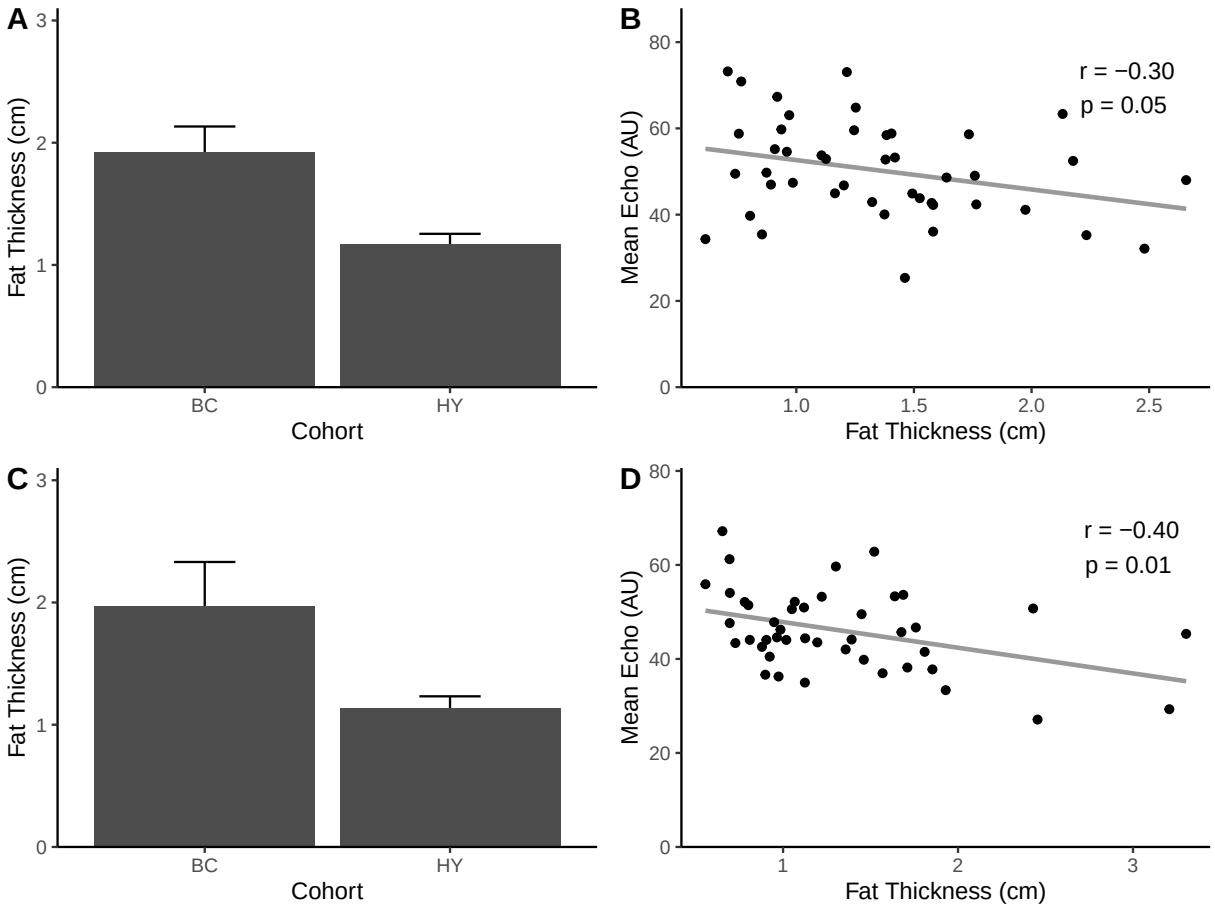

Figure 7.2. Average fat thickness for the breast cancer patients (BC) and the healthy reference (HY) cohorts at the anterior (A) and lateral (C) thigh landmarks. Pearson correlation between fat thickness at the anterior (B) and lateral (D) thigh landmarks with respect to mean echogenicity values.

Chapter 8

Characterization of ultrasound-based muscle size, composition and architecture measurements in young healthy adults

Abstract

Introduction: Ultrasonography is a medical imaging modality that is increasingly being used to evaluate muscle features such as size, composition and architecture. While ultrasound-based measurements of muscle size can be used to classify poor muscle health, there is a paucity of studies that relate muscle function with ultrasound-based muscle composition and architecture. Here, we comprehensively evaluated the associations between ultrasound-based measures of muscle characteristics with lean tissue mass acquired by dual energy X-ray absorptiometry (DXA) as well as knee extensor muscle strength and power.

Methods: We assessed a cohort of 34 young, non-obese, healthy adults (17 male and 17 female) across a wide range of strength training histories. All participants completed: 1) an ultrasound-based assessment of muscle thickness and echogenicity of the anterior and

lateral thigh, as well as pennation angle and fascicle length of the vastus lateralis, 2) a whole-body DXA scan, and 3) knee extensor isometric torque as well as isokinetic power and velocity measurements.

Results: The partial correlation coefficient of appendicular lean mass (ALM) predicted using ultrasound-based muscle thickness measures versus power was r = 0.451 (95% CI: 0.127 to 0.687, adj.p=0.034) and was comparable to the partial correlation coefficient of DXA-based ALM versus power, r = 0.486 (95% CI: 0.172 to 0.711, adj.p=0.021). However, echogenicity and architecture were not significantly correlated with muscle strength or power.

Conclusion: Overall, our results emphasize that further method development is needed to better understand ultrasound-based muscle features beyond size.

Introduction

Individuals with lower than normal muscle mass are likely to experience poor physical function, and may be at risk of mobility limitations or reduced ability to perform activities of daily living [2, 102]. Imaging tools such as computed tomography (CT), dual energy X-ray absorptiometry (DXA), and magnetic resonance imaging (MRI) generate precise measures of skeletal muscle mass and attenuation and are instrumental in the assessment of muscle health. Ultrasonography is increasingly being used to study muscle features such as size, composition and architecture *in vivo* [76, 112] and to quantify changes in various muscle characteristics over time [69, 123]. Several protocols which use ultrasound-based muscle thickness measures in a predictive equation along with common covariates such as age, sex and height, to estimate regional or whole-body muscle size have been published [2, 107, 119]. Although measurements of muscle size have been extensively explored in diverse populations, we have limited knowledge on the relationship between muscle function and ultrasound-based muscle composition and architecture measurements in healthy individuals. This fundamental relationship would be essential for identifying those with poor muscle health.

Fat and fibrotic infiltration, found within the muscle, reflect ultrasound waves and can be identified as regions of bright pixels on images, which are distinct from the homogeneous dark appearance of healthy muscle. It has been reported that ultrasound echogenicity indeed correlates with fat infiltration [137, 171] or fibrous tissue [125]. Echogenicity is also moderately correlated with other muscle composition measures that use magnetic resonance imaging and computed tomography [164, 171]. Manini *et al.* demonstrated that fat infiltration increased in muscle while strength decreased following 4 weeks of lower limb suspension in healthy young adults [96]. Conversely, strength training decreased ultrasound-based muscle echogenicity values, which may be related to decreased fat deposition within trained muscles [24]. Skeletal muscle is highly organized, and fascicle orientation, which can be assessed by ultrasound [88, 111], is an important determinant of muscle force production [93, 112]. Similar to muscle composition, there is evidence highlighting that disuse, such as bed rest or immobilization, decreased both the pennation angle and fascicle length [57, 58, 157]. Conversely, exercise training may increase pennation angle; however, fascicle length adaptations are inconsistent and may depend on the mode of exercise training [57, 58, 157].

To ultimately be able to identify poor muscle health in clinical cohorts, a more solid understanding of the relationship between ultrasound-based measures and muscle function is needed. The association between muscle composition, architecture and contractile function is not consistent or clear. A recent cross-sectional comparison of muscle morphology and architecture between cohorts of long-term strength-trained versus untrained men revealed that the effect size of mean differences in quadriceps volume is substantially larger than that of pennation angle or fascicle length [95]. Strasser *et al.* reported that muscle echogenicity is correlated with muscle strength only in young adults and not older adults, which contradicts previously published evidence [154]. Ultrasound-based measurements of muscle characteristics may be affected by various confounding factors that include age, disease states, and excess adiposity among others [41, 69], but may also be influenced by a range of variables introduced during the image acquisition and segmentation steps [22, 27, 70, 116]. Taken together, it is challenging to generalize results between studies using different protocols, ultrasound devices, and image acquisition settings.

The overarching objective of this study was to evaluate ultrasound-based muscle fea-

tures relative to a reference modality and strength measures in a cohort of healthy non-obese young adults. We examined the associations among ultrasound-based measures of muscle characteristics and evaluated these features in relation to DXA-based lean tissue mass. We also assessed the relationship between muscle characteristics with respect to isometric strength as well as isotonic power and peak velocity. Our hypothesis was that ultrasound-based measurements of muscle size would correlate to muscle strength and power, similar to DXA-based appendicular lean mass (ALM). Also, ultrasound-based muscle thickness will be inversely correlated with echogenicity and directly correlated with pennation angle and fascicle length. We also hypothesize that muscle composition and architecture measurements will be associated with muscle strength and power.

Methods

Study design and participants

This is prospective cross-sectional study examined muscle characteristics in a cohort of young healthy adults across a wide range of strength training histories. Each participant attended 2 sessions that were a minimum of 24 hours and maximum of 7 days apart. The outcomes assessed in this study included a physical activity questionnaire, ultrasound measurements of muscle thickness, composition and architecture, a DXA scan, and assessment of the isometric, isotonic and isokinetic maximal voluntary contraction of the knee extensor muscles. The inclusion criteria were: 18-30 years of age, and BMI of 18.5-29.9 kg/m^2. The exclusion criteria included diagnosis of metabolic, neuromuscular or cardiovascular disease, any contraindication to exercise or to other measurements performed during the study. Participants were recruited from the University of Waterloo campus community using posters and word of mouth. The study was cleared by the University of Waterloo Research Ethics Committee and written informed consent was obtained from all study participants.

Anthropometry, participant information and habitual physical activity

After participants changed into light-weighted athletic clothing and removed their shoes, height and weight were acquired using a stadiometer and balance beam scale, respectively. The participants self-reported their age and frequency of habitual aerobic and resistance training. The International Physical Activity Questionnaire (IPAQ) long form questionnaire was used to determine total METs and minutes of leisure activity per week [42].

Dual energy X-ray absorptiometry

DXA is a non-invasive assessment that determines the weight of 3 tissue compartments, soft fat-free mass (lean mass), fat mass and bone mineral content [128]. DXA scans was performed by a certified Medical X-Ray Technologist using a Hologic Discovery QDR 4500 scanner (Hologic, Toronto, ON). The participants were placed on the bed of the scanner in supine position with the legs extended and arms positioned parallel to, but not touching the trunk. The participants were instructed to remain still for the duration of the scan (˜6 minutes). A single trained analyst segmented the upper limbs, lower limbs, pelvis, and thoracoabdominal regions using Hologic Apex software (version 13.2). The sum of all 3 compartments (lean, fat and bone) was calculated to obtain the cumulative tissue weight in each region. Whole-body or regional percent lean or fat mass values were calculated by dividing the respective tissue mass values by the cumulative tissue weight. Appendicular lean tissue mass (ALM) was calculated by summing the lean tissue mass of the arms and legs; appendicular lean tissue index (ALTI) was calculated by dividing ALM (kg) by height squared (m^2).

Muscle strength and power

Maximal isometric and isokinetic torque, as well as peak isotonic velocity and power, were measured unilaterally for the left and right knee extensor muscles using a dynamometer (Biodex Medical Systems, Shirley, NY, USA). To reduce bias, we randomized the order in which the right and left legs were tested. Following a 5 minute warm-up on a stationary bike at a self-selected moderate intensity, participants were seated in the dynamometer

chair with their knee joint axis of rotation positioned in-line with the axis of rotation of the dynamometer arm. Chest, waist, mid-thigh, and lower leg straps (positioned 5 cm above the inferior aspect of the calcaneus) were used to stabilize the participant and restrict extraneous movement. Isometric maximal voluntary contraction values were obtained using a 5 second maximal contraction at 60° of flexion, with 30 seconds of rest between each trial (3 trials/leg). The mean peak isometric torque of the 3 contractions was calculated. Isotonic contractions were performed at 40% of peak isometric torque value, and participants were instructed to extend their leg as hard and as fast as possible for 3 trials per leg with a 30 second rest between trials. Isokinetic torque was tested at 60°/s and 180°/s. For each velocity, 3 trials were completed with a 30 s rest between the trials. Participants performed all muscle strength and power measurements twice. The first session served to familiarize participants with the protocol. Only measurements obtained on the second session were analyzed. The participants were instructed to avoid exercising for a minimum of 24 hours prior to the study visit, and to refrain from caffeine consumption on the day of the strength assessment. To ensure reliability and accuracy of strength measurements, the average of 3 trials was calculated. In cases where the %CV of the 3 trials was higher than 10%, the 2 values with the smallest %CV were used.

Ultrasound image acquisition

All images were acquired using B-mode ultrasound imaging device (M-Turbo, SonoSite, Markham, ON, Canada) equipped with a multi-frequency linear array transducer (L38xi, 5-10 MHz). The 5-site protocol was used to predict appendicular lean tissue mass, using ultrasound-based muscle thickness measurements at 5 easily accessible sites, along with covariates that include age, sex and height. The landmarks of the 5-site protocol included:

- Site 1 - 60% of the length from the acromial process of the scapula to the lateral epicondyle of the humerus at the anterior aspect of the right upper arm.
- Sites 2 and 3 - 50% of the length between the anterior superior iliac spine (ASIS) and the midpoint of the superior edge of the patella on the right and left thigh.

- Sites 4 and 5 - 2/3 of the length between the ASIS and the midpoint of the superior edge of the patella, bilaterally.

The full details and validation of this protocol is described in Paris *et al.* [119]. Furthermore, we determined muscle thickness and echogenicity on transverse images at the mid-thigh level (50% of the distance between the greater trochanter and the lateral condyle of the knee) for both the anterior (rectus femoris and vastus intermedius) and lateral (vastus lateralis and vastus intermedius) aspects of the thigh. The ultrasound probe was tilted in a caudal-cephalic direction to obtain a perpendicular orientation with the femur (the maximal brightness of the femur cortex) and saved for later analysis. Images of muscle architecture were acquired at the lateral mid-thigh landmark, with the probe oriented parallel to the direction of the fibres (longitudinally) and perpendicular to the skin without compression of underlying tissues. Ample transmission gel and minimal skin compression was used during all image acquisition.

Ultrasound image processing

Ultrasound images were saved in the Digital Imaging and Communications in Medicine (DICOM) format. Muscle thickness was obtained by measuring the perpendicular distance between the upper margin of the femur and the superior boundary of the most superficial muscle group in the image (Supplementary Figure 8.3A). To determine echogenicity, a rectangular region of interest was placed in the superficial muscle (rectus femoris or vastus lateralis) such as to maximize the area of the region without including any of the surrounding fascia (Supplementary Figure 8.3A). The individual gray scale intensities (expressed as values between 0 - black and 255 - white) for all pixels in the region of interest were averaged to calculate mean echo intensity. To determine muscle architecture the analyst placed a straight line such that it best fits the orientation of the deep and superficial fascia of vastus lateralis, as well the most visible representative fascicle. The angle of each line relative to horizontal was determined, the angle between the deep fascia and the representative fascicle was calculated to compute pennation angle. In order to calculate fascicle length, the distance between the superficial and deep fascia at the centre of the image was determined, fascicle length was computed as fascia distance divided by sine of pennation

angle (Supplementary Figure 8.3B). All image segmentation and analysis was performed using a custom Python script.

Statistics

Data analysis was performed using R (R Foundation for Statistical Computing, Vienna, Austria). Cohort characteristics are reported as mean ± standard deviation (SD) for continuous variables, unless stated otherwise. Group differences were assessed using an independent samples t-test. Pearson correlation coefficients were calculated to determine the strength of an association between 2 variables, partial correlation method was used to test the association between 2 variables while accounting for the influence of sex. For all tests a two-tailed p-value of 0.05 was used as the threshold of statistical significance. When testing the significance of the partial correlations between ultrasound-based muscle features with respect to either DXA-derived body composition values or muscle contractile function, we used the Holm's p-value correction to maintain a family-wise alpha at the 0.05 level. Throughout this paper adjusted p-values are denoted as p.adj.

Results

The cohort included 17 men and 17 women, aged 22.6 ± 3.4 years and 23.2 ± 3.7 years, respectively (Table 8.1). Briefly, men were 1.75 ± 0.06 m tall and weighed 73.0 ± 6.8 kg, resulting in mean body mass index (BMI) of 23.8 ± 2.5 kg/m^2. Women were 1.64 ± 0.07 m in height, weighed 59.5 ± 9.0 kg, with a BMI of 22.1 ± 2.7 kg/m^2. Physical activity levels were similar between men and women (p=0.254). We evaluated sex-based differences in DXA outcomes as well as in knee extension strength and power (Supplementary Tables 1 and 2). Men had 54.92 ± 4.64 kg lean tissue mass, 13.72 ± 4.99 kg fat mass and 0.25 ± 0.10 fat-to-lean ratio based on DXA, while women had 38.65 ± 5.57 kg of lean mass, 17.52 ± 4.38 kg of fat mass and a 0.46 ± 0.10 fat-to-lean ratio. Furthermore, men had higher peak isometric strength, isotonic power and isokinetic torque, but there were no sex-based differences in isotonic velocity.

Group differences between men and women were also observed in ultrasound-based muscle characteristics, except for fascicle length (Table 8.2). Women had smaller muscle thickness values at both the anterior and lateral thigh landmarks, corresponding to a difference of -0.9 ± 0.60 cm ($p < 0.001$) and -1.17 ± 0.60 cm ($p < 0.001$), respectively, when compared with men. Mean echogenicity values were 41.91 ± 6.72 AU in men versus 50.31 ± 10.66 AU in women, and 40.78 ± 6.92 AU versus 45.96 ± 6.74 AU, at the anterior ($p=0.011$) and lateral sites ($p=0.034$). Women had smaller pennation angles with mean group difference of -3.38 ± 3.76 degrees ($p < 0.05$). We did not observe a statistical difference in fascicle length values, in our study cohort the average fascicle length for men was 8.73 ± 2.00 cm and 7.91 ± 2.05 cm in women ($p > 0.05$). Due to these group differences all further analyses in this study adjusted for the effect of sex.

We observed significant inverse correlations between mean echogenicity and muscle thickness at the anterior r = -0.556 (95% CI: -0.755 to -0.263, p = 0.001), and lateral r = -0.433 (95% CI: -0.676 to -0.105, p = 0.012) mid-thigh landmarks (Table 8.3). Fascicle length displayed a strong, inverse correlation with pennation angle r = -0.772 (95% CI: -0.882 to -0.584, $p < 0.001$). However, we did not observe any significant correlations between muscle thickness or echogenicity in relation to muscle architecture measures. Our results indicate that echogenicity r = 0.399 (95% CI: 0.064 to 0.653, p = 0.022), but not thickness values r = 0.199 (95% CI: -0.155 to 0.508, p = 0.267), are correlated when examining the relationships between values at the anterior and lateral mid-thigh sites (Table 8.3).

We observed a moderate correlation between muscle thickness at the lateral mid-thigh landmark with respect to DXA leg lean mass r = 0.632 (95% CI: 0.368 to 0.801, adj.p < 0.001), as well as r = 0.558 (95% CI: 0.265 to 0.756, adj.p = 0.017) when comparing thickness with total limb mass (Table 8.4). In our cohort, no correlations existed between anterior thigh muscle thickness to lean mass r = 0.398 (95% CI: 0.064 to 0.652, adj.p =0.462) or with fat-to-lean ratio r = -0.426 (95% CI: -0.671 to -0.096, adj.p = 0.298). DXA-based appendicular lean tissue mass (ALM) was correlated with peak isometric torque r = 0.636 (95% CI: 0.375 to 0.804, adj.p < 0.001) and isotonic power r = 0.486 (95% CI: 0.172 to 0.711, adj.p = 0.021), respectively (Figure 8.1A, B). The partial correlation coefficients for ultrasound-based predicted ALM were r = 0.406 (95% CI: 0.072 to 0.657, adj.p =

0.058) with isometric peak torque, and r = 0.451 (95% CI: 0.127 to 0.687, adj.p = 0.034) with isotonic power (Figure 8.1D, E). The correlation between the DXA-based or the ultrasound-based ALM values with peak isotonic velocity values did not reach significance (Figure 8.1C, F).

When evaluating the associations between ultrasound-based muscle thickness with respect to muscle strength or power, we observed several correlations where the unadjusted p-values were less than the 0.05 threshold. However, when evaluating p-values adjusted for multiple comparisons, muscle thickness at the anterior landmark was not correlated with isotonic power and velocity r = 0.384 (95% CI: 0.047 to 0.643, adj.p = 0.464) and r = 0.364 (95% CI: 0.024 to 0.629, adj.p = 0.595). Similarly, muscle thickness was not associated with isometric torque r = 0.053 (95% CI: -0.296 to 0.389, adj.p=1.000; Figure 8.2A). Muscle thickness at the lateral mid-thigh site was not correlated with isometric torque r = 0.394 (95% CI: 0.059 to 0.65, adj.p = 0.418; Figure 8.2B), or with peak isotonic velocity r = -0.345 (95% CI: -0.616 to -0.002, adj.p = 0.737). We did not observe any significant correlations when evaluating echogenicity, pennation angle or fascicle length with respect to knee extension strength or power (Figure 8.1C-F, Table 8.5).

Discussion

We evaluated ultrasound-based measurements of muscle size, composition, and architecture in a healthy, non-obese young adult cohort. We observed that there is an inverse correlation between muscle thickness and echogenicity at both the anterior and lateral thigh landmarks. While we observed an association between pennation angle and fascicle length, these architectural features were not correlated with measures of muscle size or composition. Similar to DXA-based appendicular lean tissue mass, ALM predicted using the 5-site protocol [119] was associated with knee extension isometric strength, isotonic power, but not peak isotonic velocity. However, each individual site-specific muscle thickness value, measured at the anterior or lateral mid-thigh landmarks, with respect to strength or power did not yield correlation coefficients that were as strong as the results obtained when using the 5-site protocol.

Muscle echogenicity and fascicle geometry reflect tissue composition and architecture, respectively, and may be linked with muscle function [41, 111, 123]. Evaluating the relationship between echogenicity and muscle architecture may present a clinical surrogate for assessment of muscle function in those who are immobile and/or hospitalized. In our study, we observed that echogenicity, pennation angle and fascicle length were not significantly associated with knee extension strength or power in healthy young, non-obese adults. However, previous research indicates that strength training may lead to a reduction in echogenicity, whereas disuse interventions (e.g., detraining or immobilization) may increase echogenicity [24]. Furthermore, echogenicity is elevated in older adults when compared to young adults [11, 119, 154]. Similarly, muscle architecture may also be altered with training or disuse [59, 157]. While we did not observe associations between ultrasound-based measures of muscle composition or architecture and muscle function, it is still possible that changes in echogenicity or architecture values may represent improvement or deterioration of muscle function, as others have shown [25, 33, 164].

While echogenicity measurements were previously shown to be correlated with MRI and CT based intramuscular adipose tissue values [164, 171], in our study there was no correlation between echogenicity with DXA based fat mass. Although we aimed to minimize potential confounders by focusing on healthy non obese young adults, several possible methodological considerations may explain these findings. Different equipment manufacturers as well as imaging settings such as gain and dynamic range limit the generalizability of ultrasound-based muscle echogenicity between studies [124, 152]. In addition, our group previously demonstrated the varying image resolution, or the depth of image acquisition, alters the echogenicity values even when the same region of interest is assessed, with the ultrasound settings and probe positioning held constant [118]. While conventional analysis of ultrasound images targeting measures of muscle composition predominantly employs simple averaging of all the pixel intensities in a region of interest. Additional first order statistics such as histogram standard deviation, skew and kurtosis, higher order texture analysis or feature detection algorithms are all potential methods of analyzing the image, and may help with the assessment of muscle composition [104, 118].

In this study we show that ultrasound-based measures of vastus lateralis muscle architecture were not correlated with muscle size, composition, or knee extension strength and

power. While some studies highlight that muscle geometry may be linked with strength [112, 157], this has been also challenged by others [154]. Improving the reliability of image segmentation with automated algorithms [98, 144] may help enable more accurate assessment of the underlying features. However, it is important to note that the entire length of the fascicle is often not fully visible on an image and therefore is not assessed directly. Indeed, in the present study we projected fascicle length based on pennation angle and the vertical distance between the superficial and deep fascial lines. Extended field of view ultrasonography, which is available in some ultrasound machines may further assess fascicle length directly and accurately [117].

Ultrasonography has the potential for reliable, accurate and specific muscle measurements that can be acquired bedside, noninvasively and prospectively. While ultrasonography offers several practical advantages over reference body composition assessment modalities such as DXA or CT, the use of ultrasonography for the assessment of muscle characteristics requires further method development. The participant cohort in this study was relatively small (n=34) and a rather homogeneous group of healthy young adults, which would not be representative of the broad range of muscle mass and function that exists in older adults or clinical populations. Additionally, perhaps similar to measurements of muscle thickness, use of multiple landmarks alongside simple covariates may be used to further corroborate the links between these muscle features and muscle function.

The results presented in this study highlight that a combination of ultrasound-based muscle thickness measurements together with variables such as age, sex and height effectively predict whole body or regional muscle quantities, and may be used to examine the association between muscle size and muscle contractile function. Yet, site-specific ultrasound features such as thickness, echogenicity, pennation angle, or fascicle length may be limited with respect to their use in capturing the associations with muscle strength or power, in healthy young adults. Future research is needed to establish protocols that use these site-specific features to predict regional or whole-body values.

Table 8.1. Participant characteristics

	All (n=34)	Men (n=17)	Women (n=17)	Difference	P-value
Age (years)	22.9 ± 3.5	22.6 ± 3.4	23.2 ± 3.7	0.6 ± 3.5	0.631
Height (m)	1.70 ± 0.09	1.75 ± 0.06	1.64 ± 0.07	-0.12 ± 0.07	<**0.001**
Weight (kg)	66.2 ± 10.4	73.0 ± 6.8	59.4 ± 9.0	-13.5 ± 8.0	<**0.001**
BMI (kg/m^2)	23.0 ± 2.7	23.8 ± 2.5	22.1 ± 2.7	-1.7 ± 2.6	0.069
IPAQ: Total METs	3606.9 ± 2283.9	4059.6 ± 1972.6	3154.3 ± 2536.1	-905.3 ± 2271.9	0.254
IPAQ: Leisure Activity (mins)	252.5 ± 213.5	287.6 ± 191.6	217.3 ± 233.9	-70.3 ± 213.8	0.345

Values are mean ± sd. Independent samples t-tests were used to determine statistical significance when comparing men to women. BMI - body mass index; IPAQ - international physical activity questionnaire.

Table 8.2. Ultrasound-based muscle characteristics

	All	Men	Women	Difference	P-value
Thickness: Anterior (cm)	4.23 ± 0.75	4.69 ± 0.67	3.78 ± 0.52	-0.9 ± 0.60	<**0.001**
Thickness: Lateral (cm)	4.66 ± 0.84	5.25 ± 0.62	4.08 ± 0.59	-1.17 ± 0.60	<**0.001**
Echo: Anterior (AU)	46.11 ± 9.76	41.91 ± 6.72	50.31 ± 10.66	8.39 ± 8.91	**0.011**
Echo: Lateral (AU)	43.37 ± 7.22	40.78 ± 6.92	45.96 ± 6.74	5.18 ± 6.83	**0.034**
Pennation Angle (degrees)	17.51 ± 4.08	19.2 ± 4.44	15.82 ± 2.91	-3.38 ± 3.76	**0.014**
Fascicle Length (cm)	8.32 ± 2.04	8.73 ± 2.00	7.91 ± 2.05	-0.81 ± 2.03	0.251

Values are mean ± sd. Independent samples t-tests (men vs. women) were used to determine statistical significance. AU - arbitrary units.

Table 8.3. Associations between ultrasound-based muscle characteristics

	Thickness: Anterior	Thickness: Lateral	Echo: Anterior	Echo: Lateral	Pennation Angle	Fascicle Length
Thickness: Anterior	-	0.199 (-0.155 to 0.508)	**-0.556 (-0.755 to -0.263)**	-0.190 (-0.500 to 0.164)	0.175 (-0.179 to 0.489)	-0.043 (-0.380 to 0.305)
Thickness: Lateral	0.199 (-0.155 to 0.508)	-	-0.249 (-0.546 to 0.103	**-0.433 (-0.676 to -0.105)**	0.274 (-0.077 to 0.564)	0.243 (-0.110 to 0.541)
Echo: Anterior	**-0.556 (-0.755 to -0.263)**	-0.249 (-0.546 to 0.103)	-	**0.399 (0.064 to 0.653)**	-0.008 (-0.35 to 0.336)	-0.227 (-0.529 to 0.126)
Echo: Lateral	-0.190 (-0.500 to 0.164)	**-0.433 (-0.676 to -0.105)**	**0.399 (0.064 to 0.653)**	-	-0.202 (-0.510 to 0.152)	0.037 (-0.311 to 0.375)
Pennation Angle	0.175 (-0.179 to 0.489)	0.274 (-0.077 to 0.564)	-0.008 (-0.350 to 0.336)	-0.202 (-0.51 to 0.152)	-	**-0.772 (-0.882 to -0.584)**
Fascicle Length	-0.043 (-0.380 to 0.305)	0.243 (-0.11 to 0.541)	-0.227 (-0.529 to 0.126)	0.037 (-0.311 to 0.375)	**-0.772 (-0.882 to -0.584)**	-

Values are listed as partial correlation coefficient (95% CI), significant correlations are in bold. CI - confidence interval.

Table 8.4. Partial correlations of ultrasound-based muscle characteristics to DXA measures

	Leg Lean Mass	Leg Fat Mass	Leg Total Mass	Leg Fat-to-Lean Ratio
Thickness: Anterior	0.398 (0.064 to 0.652)	-0.195 (-0.504 to 0.159)	0.180 (-0.174 to 0.493)	-0.426 (-0.671 to -0.096)
Thickness: Lateral	**0.632 (0.368 to 0.801)**	0.159 (-0.195 to 0.476)	**0.558 (0.265 to 0.756)**	-0.137 (-0.459 to 0.216)
Echo: Anterior	-0.220 (-0.524 to 0.133)	0.059 (-0.29 to 0.395)	-0.12 (-0.445 to 0.233)	0.183 (-0.171 to 0.495)
Echo: Lateral	-0.200 (-0.508 to 0.154)	0.173 (-0.181 to 0.487)	-0.042 (-0.379 to 0.306)	0.129 (-0.225 to 0.452)
Pennation Angle	0.128 (-0.225 to 0.451)	-0.121 (-0.446 to 0.232)	0.033 (-0.314 to 0.372)	-0.147 (-0.466 to 0.207)
Fascicle Length	0.237 (-0.115 to 0.537)	0.186 (-0.168 to 0.498)	0.277 (-0.074 to 0.566)	-0.017 (-0.358 to 0.328)

Values are listed as partial correlation coefficient (95% CI), significant correlations are in bold. CI - confidence interval.

Table 8.5. Partial correlations of ultrasound-based muscle characteristics in relation to strength and power

	Isometric Torque	Isotonic Power	Isotonic Velocity
Thickness: Anterior	0.053 (-0.296 to 0.389)	0.384 (0.047 to 0.643)	0.364 (0.024 to 0.629)
Thickness: Lateral	0.394 (0.059 to 0.65)	-0.077 (-0.409 to 0.274)	-0.345 (-0.616 to -0.002)
Echo: Anterior	-0.076 (-0.408 to 0.275)	-0.150 (-0.469 to 0.204)	-0.100 (-0.428 to 0.252)
Echo: Lateral	-0.103 (-0.431 to 0.249)	0.184 (-0.17 to 0.496)	0.309 (-0.038 to 0.59)
Pennation Angle	-0.075 (-0.408 to 0.276)	-0.203 (-0.511 to 0.15)	-0.114 (-0.44 to 0.238)
Fascicle Length	0.272 (-0.079 to 0.563)	0.279 (-0.071 to 0.568)	0.073 (-0.277 to 0.406)

Values are listed as partial correlation coefficient (95% CI), significant correlations are in bold. CI - confidence interval.

Table 8.6. DXA-based body composition characteristics

	All	Men	Women	Difference	P-value
Whole Body					
Total Weight (kg)	64.90 ± 10.20	71.54 ± 6.72	58.26 ± 8.70	-13.28 ± 7.77	<0.001
Lean Mass (kg)	46.78 ± 9.68	54.92 ± 4.64	38.65 ± 5.57	-16.27 ± 5.13	<0.001
Fat Mass (kg)	15.62 ± 5.01	13.72 ± 4.99	17.52 ± 4.38	3.80 ± 4.69	0.025
Fat-to-Lean Ratio	0.35 ± 0.14	0.25 ± 0.10	0.46 ± 0.10	0.20 ± 0.10	<0.001
ALTI (kg/m^2)	7.17 ± 1.41	8.28 ± 0.82	6.07 ± 0.91	-2.21 ± 0.86	<0.001
Legs					
Total Weight (kg)	22.77 ± 3.65	24.88 ± 2.20	20.66 ± 3.62	-4.22 ± 3.00	<0.001
Lean Mass (kg)	15.85 ± 3.84	19.01 ± 1.93	12.70 ± 2.36	-6.31 ± 2.16	<0.001
Fat Mass (kg)	6.00 ± 2.07	4.77 ± 1.70	7.23 ± 1.66	2.45 ± 1.68	<0.001
Fat-to-Lean Ratio	0.42 ± 0.20	0.26 ± 0.11	0.58 ± 0.13	0.32 ± 0.12	<0.001
Arms					
Total Weight (kg)	6.97 ± 1.68	8.23 ± 1.12	5.71 ± 1.07	-2.53 ± 1.10	<0.001
Lean Mass (kg)	5.03 ± 1.62	6.4 ± 0.91	3.65 ± 0.76	-2.75 ± 0.84	<0.001
Fat Mass (kg)	1.61 ± 0.58	1.42 ± 0.54	1.79 ± 0.56	0.37 ± 0.55	0.060
Fat-to-Lean Ratio	0.36 ± 0.19	0.23 ± 0.10	0.50 ± 0.17	0.28 ± 0.14	<0.001

Values are listed as mean ± sd. Independent samples t-tests (men vs. women) were used to determine statistical significance. ALTI - appendicular lean tissue index.

Table 8.7. Leg extension strength and power

	All	Men	Women	Difference	P-value
Isometric (Nm)	389.59 ± 110.15	464.52 ± 84.04	314.66 ± 77.65	-149.86 ± 80.91	<0.001
Isotonic Power (Watts)	477.78 ± 154.14	575.63 ± 131.88	379.93 ± 106.14	-195.70 ± 119.7	<0.001
Isotonic Velocity (°/sec)	231.88 ± 47.72	239.65 ± 50.23	224.10 ± 45.23	-15.55 ± 47.80	0.350
Isokinetic 60°/sec (Nm)	330.69 ± 77.46	378.72 ± 60.98	282.65 ± 61.26	-96.07 ± 61.12	<0.001
Isokinetic 180°/sec (Nm)	231.57 ± 74.40	283.65 ± 50.92	179.50 ± 55.31	-104.15 ± 53.16	<0.001

Values are listed as mean ± sd. Independent samples t-tests (men vs. women) were used to determine statistical significance.

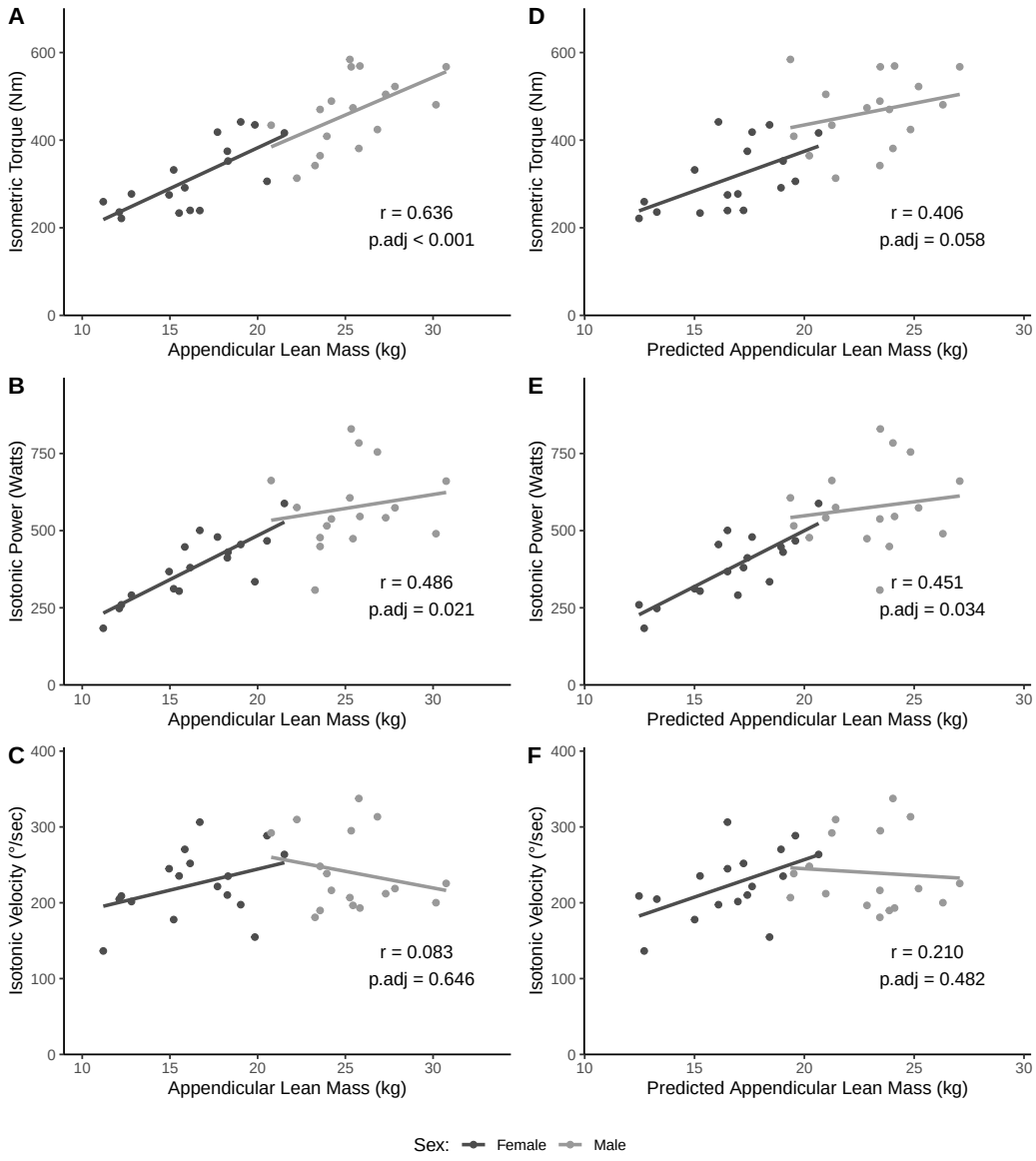

Figure 8.1. Partial correlations between DXA-based appendicular lean mass (left column) or appendicular lean mass predicted using ultrasound-based muscle thickness measures (right column) with respect to isometric strength (A, D), isotonic power (B, E) or isotonic velocity (C, F).

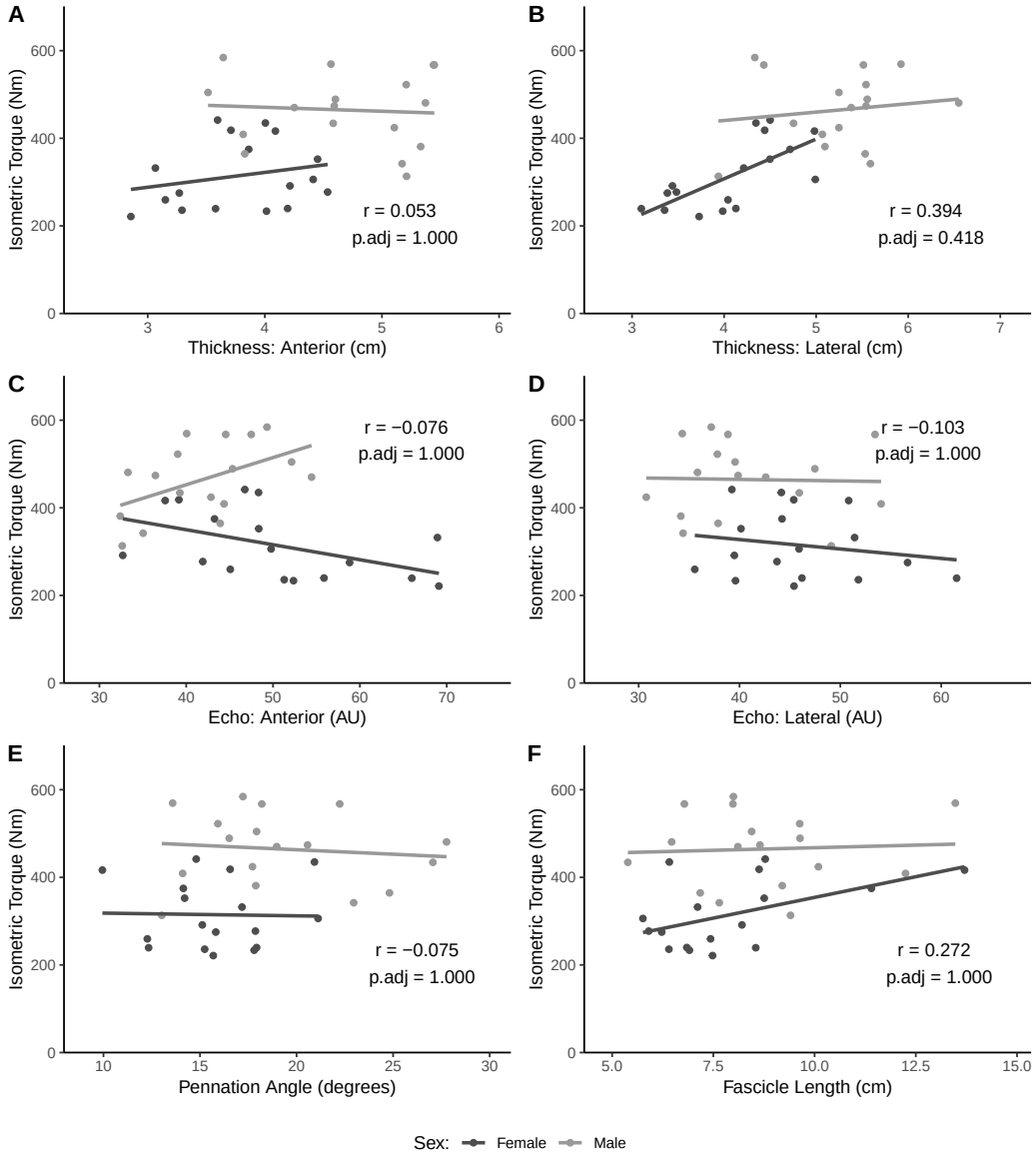

Figure 8.2. Partial correlations between ultrasound-based muscle thickness at the anterior (A) and lateral (B), muscle echogenicity at the anterior (C) and lateral (D), as well as pennation angle (E) and fascicle length (F) with respect to peak isometric strength values.

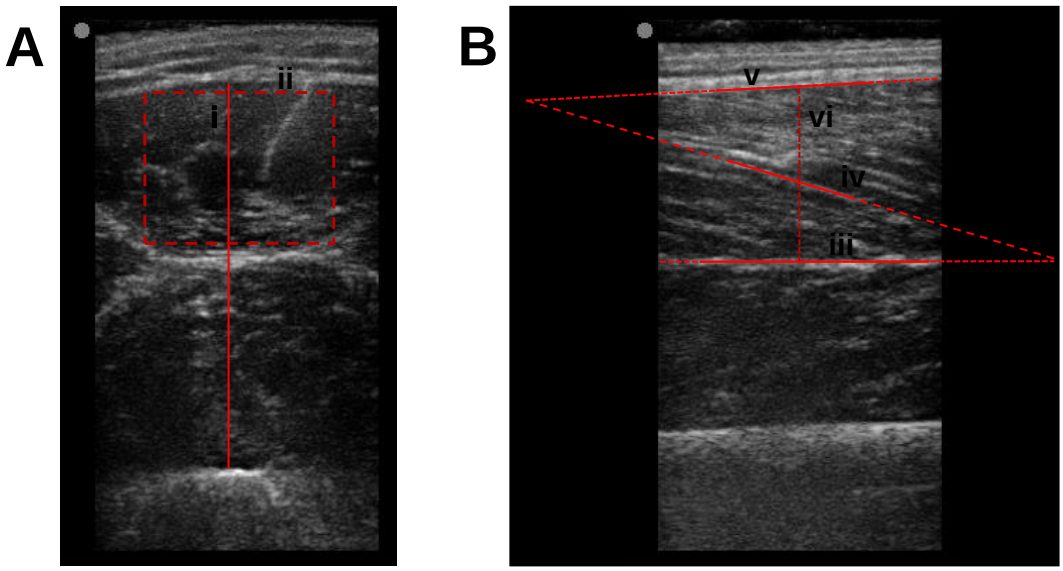

Figure 8.3. Segmentation of ultrasound images to determine (A) muscle thickness and echogenicity, and (B) pennation angle and fascicle length.

(i) Muscle thickness was measured as the perpendicular distance between the upper margin of the femur and the superior boundary of the superficial muscle group. (ii) Mean echogenicity was calculated by averaging pixel intensities in a rectangular region of interest placed within the superficial muscle such as to maximize the area of the region without including any of the surrounding fascia. The visible section of the deep and superficial fascia and a representative fascicle was identified (represented as solid lines). Since images often do not contain the entire length of the fascicle, the angle of each line relative to horizontal was determined, the angle between (iii) the deep fascia and (iv) the representative fascicle was calculated to compute pennation angle. To calculate fascicle length (vi) the distance between (v) the superficial and (iii) deep fascia at the center of the image was determined, this distance was then divided by the sine of pennation angle.

Chapter 9

Discussion

Multiple studies and meta-analyses identify that lower than normal muscle mass is linked with poor clinical and secondary outcomes, including mortality, treatment effectiveness and quality of life in patients who are undergoing cancer treatment and further into survivorship [34, 85, 138, 145]. Conversely, there is less information available on the links between derangements in muscle features that occur over the cancer trajectory with respect to muscle strength or muscle function; this was a major aim of the studies in this thesis. Currently, CT and DXA measures of muscle characteristics are primarily used to examine muscle health in clinical populations [30, 45, 49]. These methods have been held as reference body composition assessment modalities, and have been extensively validated in both healthy and clinical cohorts [49, 76, 128]. Chapter 6 explores the use of lean tissue mass measurements obtained using DXA, and their association with muscle strength in breast cancer patients in- and post-treatment. In turn, chapters 7 and 8 investigate the use of ultrasound imaging to assess muscle features and evaluate the links between muscle size, composition and architecture with respect to contractile function. Ultrasonography is an emerging modality that is portable, relatively accessible, and does not expose the patient to radiation. Thus, it may be a promising, practical alternative to CT and DXA, particularly for prospective studies [106, 155].

This current chapter highlights the main results from each of the studies and key themes that emerge from the findings. These include considerations for strategies aimed at classify-

ing breast cancer patients into lower than normal versus adequate muscle mass categories. Evidence to inform and highlight the role of further development of protocols that use ultrasound-based muscle thickness measurements to predict regional or whole-body muscle mass. Moreover, the results of chapters 7 and 8 also discuss shortfalls in current methods aimed at evaluating muscle composition and architecture using ultrasonography, which may need to be addressed for successful future clinical studies.

9.1 Summary of key findings

9.1.1 Study 1

Detrimental changes in muscle size are not the only type of tissue level alterations that are reported to take place over the breast cancer treatment trajectory. It has been widely reported that gain in fat over the treatment trajectory, and even the links between excess adiposity and breast cancer risk at the time of diagnosis, may mask muscle losses [23, 147, 159]. Simple body size measurements such as weight, or BMI, are ineffective for the purposes of identifying those with lower than normal muscle mass [21, 49, 140]. Modalities that determine tissue-specific weight are needed. However, quantifying muscle or fat mass separately may only yield a partial picture. Several reports highlight the need to use thresholds for skeletal muscle index (cross-sectional area or mass relative to height) that are stratified by BMI or include fat mass as a covariate to more effectively classify patients according to their risk of mortality [23, 30, 97]. Chapter 6 of this thesis provides further evidence that adjustments based on fat mass are also needed when the association between low lean tissue mass (calculated as ALTI where <5.45 kg/m^2 is considered low) and poor muscle strength is examined in breast cancer patients in- and post-treatment.

There is a strong positive correlation between fat mass and lean mass measured by DXA, whereby 55% of the variability in whole body lean tissue mass was explained by fat mass in this cohort. Importantly, when lean mass was expressed as percent of total tissue weight there was no difference between those classified as having low appendicular lean tissue index (ALTI) when compared to those classified above the low ALTI threshold.

Normalization strategies to account for the relationship between adiposity and lean mass include a ratio between lean mass and body weight (i.e. percent lean mass), a ratio between fat mass to lean mass, and a ratio between appendicular lean mass to BMI [29, 50, 79, 101, 150]. Although the use of these normalization methods has been previously demonstrated in multiple large cohort studies in a healthy older adult population, they have not been evaluated in breast cancer patients. Due to simultaneous fat gain and loss of muscle that occur during the breast cancer treatment trajectory, such normalization strategies may be critical for effectively classifying patients at risk of poor physical function. Our results show that women in the low ALTI category had lower muscle strength. Surprisingly, there was no correlation between any of the other normalized lean tissue measures with respect to strength.

Evaluating the residual values of the regression between lean mass and fat mass has been previously suggested as another potential strategy to assess muscle depletion [46, 114, 166]. Similar to the normalization strategies mentioned previously, this approach has been validated in a healthy cohort and has not been tested in breast cancer patients in- and post-treatment, or any other cancer cohort. The residual values represent a measure of the observed lean mass value compared to what would be expected at a given fat mass value [46, 114, 166]. In other words, participants whose values are negative have less lean mass than what would be predicted for an average person of their body size. In chapter 6, we assess the association between the residual values and muscle strength to demonstrate that this metric accounted for approximately 25% of the overall variability, a value that is similar to the correlation coefficient observed between unadjusted appendicular lean tissue index to strength.

The results of study 1 only represent an initial step in uncovering the links between muscle size and contractile function. Isometric strength, which was the outcome evaluated in the study, needs to be further corroborated by examining functional outcomes that are a more direct surrogate of physical abilities and potential mobility limitations. Importantly, determining muscle composition and architecture, in addition to muscle size, may be another avenue that helps explain the links between muscle features and strength in this cohort. These results further supported the rationale of chapters 7 and 8, the portion of this thesis that evaluates the use of ultrasonography to study muscle characteristics.

When compared to DXA-based measures of lean tissue mass, ultrasound evaluates muscle size through image segmentation. A targeted approach that examines the size of muscle groups that are most relevant for functional tasks (such as mobility or activities of daily living), may advance our understanding of the nature of changes in muscle characteristics throughout the cancer trajectory. Modalities, such as DXA, are often used as reference measurements of muscle mass. However, our findings demonstrate the further examination of DXA-based lower than normal muscle mass classification methods is warranted in breast cancer patients and survivors.

9.1.2 Study 2

Ultrasound imaging is increasingly used in research studies examining elderly and some clinical cohorts to study skeletal muscle features [106, 156]. However, this modality has not been used in body composition assessment of breast cancer patients in- and post-treatment, or any other cancer patient cohort. Pilot studies are needed in order to provide the quantitative evidence to help guide large scale studies. These are especially important in clinical research, which focus on a narrow subset of the population and may face participant recruitment challenges. Currently, there are multiple gaps in the understanding of changes in muscle size, composition and architecture that take place over the cancer treatment trajectory. In chapter 7, we pilot ultrasound-based measures of muscle features and examine a number of practical considerations such as the reliability of the measurements and if protocols used to estimate DXA-based appendicular lean mass developed in the general cohort are effective in breast cancer patients.

Practical assessment of body composition in a clinical setting needs to consider several unique factors, which may be incorporated into any ultrasound protocol [51, 106]. For example, when used in ICU patients access to some landmarks may be challenging due to the need to re-position the individual. Treatment-related tissue damage or swelling, may be factors which confound ultrasound-based muscle measurements in individuals treated for cancer. Thus, the selection of landmarks at which the images are obtained needs to be optimized to accommodate the practical aspects, while simultaneously effectively capturing the end-point of interest, such as whole body lean tissue mass. Comprehensive ultrasound

protocols currently used to evaluate muscle mass incorporate up to 9 measurements across both the anterior and posterior aspects, of the upper body, torso and lower body [1, 2]. The 5-site protocol previously developed in our lab, is accurate and precise while using sites that are easily accessible on the anterior aspects of the upper arm and thighs [119]. In study 2, we were able to successfully acquire all the images necessary to evaluate muscle thickness according to the 5-site protocol. Bland-Altman analysis revealed that when used in breast cancer patients the 5-site protocol yielded a nonbiased estimate of appendicular lean mass with limits of agreement corresponding to -1.47 kg to 3.63 kg. This range is similar to limits of agreements observed in the healthy reference cohort, and in other studies in our lab.

Ultrasound measurements of muscle features are highly operator-dependent and involve multiple steps, including landmarking, participant positioning, image acquisition and image analysis. Reliability studies are important to help establish the contribution of each individual step to error in the final estimate. Since landmarking and participant positioning were not deemed as problematic with the breast cancer cohort, we evaluated the reliability of image analysis. At this time there is no consensus regarding which landmarks or protocols are best suited for evaluating muscle features, which would need to be established prior to examining the reliability of the complete assessment. We compared the reliability of image segmentation for muscle thickness, echogenicity, pennation angle and fascicle length between 2 raters and within a single rater who performed the measurements twice. These variables were assessed in the breast cancer cohort as well as the healthy young cohort to help gauge if any disease or treatment specific factors may influence the image analysis step. Percent coefficient of variation (%CV) and intra-rater reliability coefficients (ICC) observed in analysis performed by a single rater were similar when compared with measurements from a second analyst. Also, the reliability was similar in the breast cancer group and the healthy reference group. In agreement with results previously observed in our lab [119], there was a high level of agreement between repeated analyses of muscle thickness, and this was also the case for measurement of muscle echogenicity. Conversely, measurements of muscle architecture (i.e. fascicle length, pennation angle) had a %CV that was greater the 10%, on average, which suggests that perhaps alternative approaches need to be explored to help increase confidence in ultrasound-based muscle architecture

features.

Ultrasound measurements of thickness have been previously demonstrated to effectively predict DXA-based lean mass [2, 119]. Interestingly, as uncovered in chapter 6, the use DXA based lean mass values to study the links between muscle mass with muscle function may be confounded due to adiposity in breast cancer patients. Although a larger sample size is needed to make statistical inferences on the data, we observed differences in both muscle strength and ultrasound-based muscle thickness when comparing breast cancer patients to a reference cohort of young healthy women. The mean of the breast cancer group was smaller when compared to values observed in the reference group. Conversely, the average DXA-based ALTI value of the breast cancer group was greater than the mean of the reference group. These discrepancies perhaps suggest that it is important to establish an adequate unbiased metric of muscle depletion, prior to its use to calibrate ultrasound-based predictive equations.

In contrast to measurements of muscle size, measurements of muscle composition assessed as mean echogenicity was not different between the breast cancer and the reference cohorts. These results are surprising, a number of studies suggest that echogenicity is increased in older adults and that there are links between strength and muscle composition [41, 156]. In the cohort examined in study 2, the differences in age and muscle strength between the breast cancer patient and the reference cohorts were not accompanied with an increase in echogenicity. This led us to examine fat thickness as a potential confounding factor, as it was previously reported to influence echogenicity values [171]. We observed a negative association between fat thickness and echogenicity values in the study cohort. Concurrently to the studies of this thesis our group further evaluated the effect of adjusting image depth and resolution, which influences ultrasound-based muscle composition metrics even when images are compared within the same individual and with the probe in a fixed position [118]. Depth and resolution of the image are commonly adjusted to help capture the features of muscle groups that would be deeper in individuals with more subcutaneous fat, and further compounded due to the inverse relationship between fat thickness and echogenicity values. Protocols used to assess ultrasound-based muscle composition in breast cancer patients need to either better control or otherwise implements statistical adjustments in order to measure echogenicity accurately. The results of study 3 further

supplement these findings since they examine the relationship between ultrasound-based measurements of muscle composition with respect to muscle strength and power in a cohort of healthy non-obese young participants, thus minimizing any potential bias resulting from excess adiposity.

9.1.3 Study 3

Muscle mass or muscle size are linked with contractile function, measurements of these characteristics enable the identification of individuals at risk of poor physical function and mobility limitations [60, 102]. Chapter 8 characterizes ultrasound-based muscle features and their associations with DXA-based body composition profile as well as muscle strength and power. Factors such as adiposity, age or disease states may confound ultrasound-based muscle characteristics. Study 3 expands on the results of the previous studies by providing further evidence about the relationship between muscle features and muscle contractile function in a non-obese, healthy, young reference cohort, to help minimize these possible confounding effects.

For both the predicted ALM using the 5-site protocol with ultrasound as well as actual ALM observed with DXA, there were positive associations with respect to muscle isometric torque and peak isotonic power, but not isotonic velocity. These results indicate that ultrasound measurements of muscle thickness can represent whole body or regional body composition information as observed with DXA. In circumstances where DXA is not accessible, ultrasonography may serve as an alternative approach to determine lean tissue mass. This is encouraging for future clinical studies aimed at elucidating the links between muscle wasting and poor physical function or mobility limitations. However, when we proceeded to evaluate the associations between strength and power versus the muscle thickness values observed at the anterior or the lateral aspects of the thigh, the correlation coefficients were not as robust. Although the results were significant when evaluated in isolation, after adjusting for multiple comparisons the observed coefficients were no longer at a 0.05 p value threshold. These findings highlight the need for additional evidence the elaborate the potential associations between ultrasound-based muscle size measures and contractile function.

In contrast to ultrasound measurements of muscle size, measurements of echogenicity, pennation angle and fascicle length were not correlated with any DXA-based measurements or with muscle strength and power. Although, other researchers have previously reported that high values echogenicity values correspond to an increase in fatty infiltration [41, 164, 171]. Our results did not identify any links between mean echogenicity values with fat mass or fat to lean mass ratio. Echogenicity was inversely correlated with muscle thickness at both the anterior and lateral thigh landmarks, but the association between echogenicity and DXA based lean tissue was null. Echogenicity values are influenced by multiple image acquisition presets on ultrasound equipment and may vary between device models and manufacturers. Although all the settings were standardized throughout all the data collection sessions, further standardization of image acquisition protocols across studies may be needed to help replicate findings across different studies. The null correlations between mean echogenicity in relation to DXA-based fat mass or with any strength and power measurements, provides additional supporting evidence to the results observed in study 2. The lack of differences in echogenicity between breast cancer and reference cohorts could be explained by confounding effects such as differences in fat thickness or image depth. Yet, the cohort evaluated in this study contained only participants in the 18.5-29.9 kg/m^2 BMI range, and also controlled for factors such as age and disease states. Thus, the findings from both studies 2 and 3 suggest that ultrasound-based measurements of muscle composition require additional validation and possibly method development.

The reliability of pennation angle and fascicle length, which are both measurements of muscle architecture, were evaluated as part of chapter 7. Chapter 7 of this document and other studies suggest that the agreement between values obtained by 2 different analysts, or when the segmentation was repeated twice by the same analyst, was poor. This may help explain the null findings that pertain to measurements of muscle architecture performed in this study. Although, some reviews suggest that the reliability of these measurements is acceptable, there may be heterogeneity between the different muscle groups or landmarks that are used across studies [88, 116]. Determination of pennation angle and fascicle length involves several steps, these include landmarking, subject positioning, image acquisition, segmentation and training. Each of these steps may contribute as a source of error in the measurements and future research is needed to help evaluate each distinct protocol com-

ponent. The methodological challenges may be partially addressed by establishing better protocols to help minimize error due to landmarking, positioning or image acquisition. Recently, a few automated segmentation algorithms have been developed to help automate image analysis, and these may be used to further improve on the current approaches [98, 144]. Overall, unlike muscle size measurements, ultrasound-based muscle composition and architecture measures have been shown through studies 2 and 3 to require further method development.

9.2 Integrative discussion

9.2.1 Classification of low muscle mass may be biased due to body size and adiposity differences

Identifying individuals with low muscle mass within a clinical population is important because low muscle mass is associated with poor clinical outcomes. Thus, classifying patients who are at risk or exhibit low muscle mass is important because they may require targeted nutrition and exercise interventions to maintain skeletal muscle health, and ultimately improve their clinical outcomes. CT, DXA and MRI are commonly considered reference modalities for examining various facets of body composition. Despite DXA being considered a reference method, the findings of chapter 6 in breast cancer patients along with findings from earlier studies performed in older adults show that caution is required when interpreting lean mass measurements from DXA. Specifically, due to the positive correlation between lean mass and fat mass those classified at risk of poor physical function are also likely to have smaller body size. These shortfalls are underscored within the consensus of defining sarcopenia, which highlight that muscle strength and function are important and independent predictors of outcomes such as mortality and physical dysfunction. Current recommendations suggest that measurements of physical function are needed alongside measurements of muscle mass [44].

Different modalities often rely on distinct strategies to identify the tissue of interest. For example, image-based analysis of tissue size in contrast to approaches that evaluate

molecular properties, such as density, to distinguish different tissues. Modalities like CT and MRI are used to acquire cross-sectional area or volume, while DXA partitions tissue mass into one of 3 compartments (bone, lean, fat) based on assumptions about the makeup of these tissues at the molecular level [76, 128]. The lean tissue compartment derived from DXA includes skeletal muscle, but it also includes visceral organs (such as liver) and connective tissue; this modality is unable to distinguish the exact type of lean tissue. Thus, appendicular lean mass is used as a surrogate of muscle mass, since lean tissue in the arms and legs predominantly consists of muscle [76, 128]. While some studies show that there is good agreement between DXA-based appendicular lean tissue index (sum of lean tissue in the appendices relative to height squared) when compared with CT-based muscle index [108], emerging evidence suggests that excess adiposity may be a potential confounding factor [114, 166]. Connective tissue within adipose tissue depots may partially account for this bias. Approximately 15% of total adipose tissue mass is comprised of connective tissue [74]. In individuals with excess adiposity, increased connective tissue may result in appendicular lean tissue mass that overestimates muscle mass.

Variables related to body size, such as weight and/or height, are commonly used to normalize data. For example, BMI recognizes that body weight is proportional to the height of the individual, and the ratio of weight to height squared was empirically determined to provide a value for body mass that is not biased due to the covariance between the 2 properties [74]. Similarly, DXA-based appendicular lean tissue index and CT-based muscle index implemented this concept to adjust for the confounding effects of height on lean mass or muscle mass, respectively. The interrelationship between tissue weight to height may not be the only factor that needs to be accounted for when evaluating muscle characteristics. There is plenty of evidence suggesting that individuals with greater adiposity, or body size, also have more muscle mass [94, 114, 130]. It has been hypothesized that this interrelationship may be a consequence of increased demand on skeletal muscle tissue because it is moving a larger body mass or muscle may be responding to greater metabolic demands [130, 150]. As a result, strategies that express muscularity in relative terms to total weight, fat mass or BMI have been developed [29, 50, 79, 101, 150]. However, as suggested by the findings of chapter 6, the use of fat-to-lean mass or ALM-to-BMI ratios may not increase the effectiveness of muscle measurements in identifying cancer patients

with weaker scores of muscle strength. These findings need to be confirmed by future, large-scale studies.

Results from chapter 6, that are supported by previous work as well, propose an alternative approach to quantifying lean tissue. One may predict the lean mass that would be anticipated for a person of a certain level of adiposity, and then determine the difference between the observed and the predicted values [46, 114, 166]. This approach would provide the magnitude by which adiposity confounds lean mass measurement. In contrast to this approach, chapter 8 focused on evaluating non-obese young and healthy participants, to help minimize the bias in appendicular lean tissue index related to adiposity. These results show that appendicular lean mass and appendicular lean tissue index values are correlated with both muscle strength and power. Thus, it provides the evidence against relying solely on residual values when evaluating the links between muscle size and muscle contractile function. Perhaps both the absolute muscle mass and its quantity relative to the amount of non-contractile tissue carried by the individual are important determinants of muscle function. Further evidence is needed to contrast the 2 strategies and help inform approaches aimed at classifying individuals who have muscle depletion, and who may be at risk of mobility limitations and poor function.

9.2.2 Protocols incorporating several landmarks along with covariates are more effective than site-specific ultrasound-based muscle thickness measures

A number of predictive equations and measurement protocols have been suggested to help generalize from the localized site-specific ultrasound-based muscle thickness measures to whole body or regional muscle size equivalents [2, 107]. Often these involve a combination of measures from multiple sites and common covariates such as age and height. However, the choice of landmarks for imaging may vary depending on the participant cohort or the objectives of the study. Abe *et al.* developed a comprehensive 9-site protocol, with many of the landmarks corresponding to sites where caliper-based body fat measurements are evaluated [1]. These landmarks evaluate the upper body, torso and lower body at both the anterior and posterior aspects. This approach has been demonstrated to strongly correlate

with MRI and DXA measurements of muscle mass [2]. Utilizing protocols that include landmarks that are easily attainable is important because these protocols may be more practical in clinical cohorts such as cancer and critically ill patients. Some landmarks may be difficult to assess due to edema, local tissue damage due to surgery or radiation, location of illness/injury, the need to re-position a patient to access a specific landmark, or positioning of intravenous lines or other medical tools. It is also important to assess time and extent of cooperation that is needed from the participant to perform an assessment. These practical considerations served as a motivation for the development and validation of expedited protocols [107]. The 5-site protocol has been developed as an alternative to the comprehensive 9-site protocol, by narrowing the number of sites assessed and performing the assessment in supine (to accommodate immobile participants), while maintaining good level of agreement with DXA-based lean tissue mass [119].

Examination of specific skeletal muscle groups as surrogates of total muscle tissue in the body is important for efficiency and practicality. Nonetheless, changes in muscle size may be perhaps heterogeneous across different muscle groups. For example, selective wasting of lower body muscles may occur at a higher rate than upper body muscles over the course of aging [102]. Bedrest studies have also helped identify that changes in the size of abdominal muscle groups also vary, the cross-sectional area of the paraspinal muscles decreased, while the psoas muscle group did not change or even increased [10]. As mentioned previously, another example is localized cancer treatment, such as radiation and surgery, which affect a specific area of the body and cause functional impairments [34].

Better characterization of the links between muscle features and muscle function is one of the main goals behind the studies in this thesis. Currently, there are few studies that have been focused on developing ultrasound-based protocols to optimize approaches that identify individuals with poor muscle strength and power, and none specifically that evaluate these relationships in cancer. The results presented in chapters 7 and 8 are early efforts in this domain, that may help inform future work. Chapter 8 demonstrated that muscle thickness measurements at the anterior or lateral aspects of the thigh relates to DXA-based lean mass. Yet, the confidence in the association between muscle mass measurements (represented as the confidence intervals and the multiple comparison adjusted p values) with respect to muscle strength and power is greater when a combination of landmarks

is used as compared to each single site. These findings highlight the significance of using protocols that evaluate multiple landmarks to more effectively estimate the links between muscle features and muscle function. Conversely, the results of chapter 7, may also be further corroborated by examining if rate of muscle wasting is heterogeneous, and whether any muscle groups are better surrogates of lower body muscle function.

9.2.3 Ultrasound-based echogenicity and architecture measures require further method development

Since metrics of muscle mass or size only partially explain differences in strength that may be observed in older adults or some clinical cohorts, accounting for muscle features such as muscle composition and muscle architecture is important [41, 156]. There is currently no evidence linking muscle composition or architecture features to muscle function in breast cancer patients in- and post-treatment. However, CT-based muscle attenuation has been shown to be associated with poor clinical outcomes in this cohort [4, 6]. Thus, examining muscle composition along with muscle size is important for enhancing our understanding of the role that skeletal muscle plays in overall health.

Visceral and subcutaneous adipose tissues are considered to be the primary sites of fat storage [110, 149]. Ectopic accumulation of fat in tissues like skeletal muscle also occurs. Since skeletal muscle constitutes a large portion of total body weight, inter-muscular adipocytes and intra-myocellular lipids, may account for a significant fraction of total fat storage in individuals with excess adiposity [3, 12, 65]. Ectopic accumulation of fat within skeletal muscle has been reported in studies investigating individuals with type 2 diabetes. Goodpaster *et al.* observed that participants who are diagnosed with type 2 diabetes when compared with those without the condition, surprisingly, had greater muscle cross sectional area, but also intermuscular adipose tissue [64]. This highlights that fatty infiltration into skeletal muscle may be linked with metabolic derangements and that greater cross-sectional area may be confounded by adiposity.

More recently, ultrasound-based measurements of echogenicity have been supported by studies that measure inter-muscular fat via MRI [164, 171]. Although a few reports suggest

that echogenicity is inversely correlated with muscle strength in older adults [2, 164], there are also a number of possible confounding factors that have been identified. For example, subcutaneous fat thickness is shown to be positively related to muscle echogenicity [171], and therefore echogenicity may not only reflect the composition of the muscle but also the depth at which the features are evaluated. Chapter 8 assesses the correlation between echogenicity and leg fat tissue mass in healthy, non-obese, young adults, our results do not support earlier evidence that echogenicity is related to fat mass. Mean echogenicity was also not associated with muscle strength or power. Furthermore, the preliminary findings of study 2 also indicate that although the differences in DXA-based fat mass between breast cancer patients and women from the reference cohort corresponded to a large effect size, there were no differences in echogenicity between the 2 groups. As discussed earlier, in part these null results can be explained due to the confounding effects caused by subcutaneous fat thickness, or methodological inconsistencies related to image acquisition and segmentation. Perhaps, MRI-based intermuscular adipose tissue measurements or analysis of tissue samples may better relate to muscle echogenicity, as compared to DXA-based measurements of fat mass, since DXA is unable to differentiate between the different adipose tissue depots.

Muscle architecture is an important determinant of contractile properties. Examining pennation angle and fascicle length, along with muscle size, may help evaluate the quantity of sarcomeres in parallel and in series, respectively [91, 111]. At the myofibre level, sarcomere structures are the force-generating component, and therefore inclusion of these measurements is hypothesized to better reflect the contractile capacity [112]. Some initial evidence, albeit in a small sample, which evaluated MRI-based inter-muscular fat alongside muscle architecture, suggests that there may be links between fatty infiltration and muscle architecture in obese women [136], but further evidence is needed to confirm and corroborate these results. Both chapters 7 and 8 of this thesis used ultrasound-based measurements of pennation angle and fascicle length. Feasibility and reliability of these measures were investigated in cancer patients, and the association between muscle architecture with respect to muscle strength and power was also evaluated in healthy young adults. Our results indicate that when the image segmentation step (which is only a portion of the complete examination) is considered, the reliability is worse when compared to

values observed with measures of size and echogenicity. Also, muscle architecture characteristics were not correlated with muscle strength or power, nor with any other DXA-based or ultrasound-based characteristics. Further refining the methods used to evaluate muscle echogenicity and architecture may help uncover the potential links between these properties and muscle contractile function. Also, measurements at multiple sites and incorporating easily obtained co-variables, strengthens the relationship between muscle size and muscle strength or power. Echogenicity, pennation angle and fascicle length are commonly examined as stand-alone values, single-site measurements, and further research is needed to determine if their effectiveness may be increased by combining multiple approaches.

9.3 Limitations and future directions

There are several considerations related to study design and methods that influence the outcomes of this thesis. As with many clinical cohorts, there are numerous confounding factors that are introduced, including physiological alterations due to the disease state, the heterogeneity of treatments, their timing and side effects, as well as lifestyle changes in survivorship. To account for these confounding factors, multivariate models would be utilized, which would necessitate a greater sample size than what was used in the present thesis. Recruitment of breast cancer patients in- and post-treatment is also relatively challenging when compared with the non-cancer or other non-clinical cohorts. Thus, sample size is an important limitation of the studies in this thesis. In particular in chapter 7, where a number of the conclusions would be strengthened with the inclusion of additional participants.

The studies of this thesis are cross-sectional, observational studies and findings could be strengthened by further characterizing the cohorts or through including additional control groups. Breast cancer cohorts are highly heterogeneous in terms of demographic and disease-related factors, such as cancer stage and treatment types. More detailed characterizations of these various demographic- and disease-related factors and their relationship with respect to body composition would bring considerable evidence to help understand the derangements that occur in this cohort. The use of the healthy young cohort as the

reference group could be supplemented by also using an age- and BMI-matched group of non-breast cancer participants. A healthy matched group could provide a reference point for distinguishing the effects of age and body size on muscle characteristics in this clinical cohort.

In addition, longitudinal studies could help provide further corroborative results. One of the main applications of body composition measurements, and specifically the assessment of muscle features, is to better understand the alterations that occur during cancer treatment. Understanding how deleterious changes in body composition can be mitigated with exercise and diet interventions is imperative. It is challenging to address these research questions using a cross sectional-study design. A longitudinal, randomized control trial that employs a usual care arm compared with a strength exercise intervention group would perhaps be the most comprehensive approach to study muscle characteristics in this patient cohort.

There are also several limitations related to the methods used to assess body composition and muscle characteristics. Ultrasonography is a versatile tool, and there are multiple different types of features and ultrasound devices. Only a subset of ultrasound-based measurements have been used in this thesis. In light of the relatively complex, logistical considerations involved with the use of MRI, CT, or DXA in clinical settings, practical and expedited measurements using ultrasound are prioritized in chapters 7-8. Thus, further testing of methods that are perhaps more sophisticated while not as practical, may help better elucidate muscle features and their relationship with muscle strength. For example, extended field of view ultrasonography enables the assessment of cross-sectional area which may improve the accuracy and precision of muscle size measurements [143]. Some ultrasound devises, such as the SonoSite M-Turbo used in chapters 7 and 8, are designed for field uses and prioritize ease of use and practicality. Other, more advanced models may allow direct control of various image acquisition settings and thus enable better control and standardization of image acquisition settings for a research environment. These features are helpful for further investigating ultrasound-based measurements of echogenicity and muscle composition.

The studies in this thesis use DXA to assess whole body and regional lean tissue mass. Either MRI or CT could be alternatively used to acquire measurements of muscle volume and muscle attenuation, which are not available from DXA scans. Although DXA-based

lean tissue mass is an established and commonly used measure to help identify older adults at risk of poor physical function [15, 44], it is not clear if lean tissue mass or muscle volume is a stronger predictor of muscle strength or power. Furthermore, the opportunity to measure subcutaneous adipose tissue area and muscle fat infiltration could be useful in further corroborating ultrasound-based echogenicity measures.

There are also multiple limitations pertaining to methods used to assess the contractile function of skeletal muscle in this thesis. In chapter 6, maximal voluntary isometric torque was measured using a load cell, which is less precise and standardized when compared to an isokinetic dynamometer (Biodex) that was used in the subsequent 2 chapters. Measurements of peak isometric contractile force may not be optimal at capturing the full scope of muscle contractile function. Assessment of physical function using tests such as the SPPB, 6-minute walk, or 30s sit-to-stand tests, among others could further enhance the applicability study 1 finding. Also, fatigue and familiarity are areas that need further evaluation with maximal strength testing to more comprehensively understand muscle contractile function. The regulation of movement is highly complex and involves not only muscle tissue, but also other structures such the neural and vascular systems. Importantly, these potential factors require future research to better understand their involvement throughout the cancer trajectory and into survivorship.

9.4 Conclusions

In summary, the studies of this thesis highlight multiple methodological considerations that help guide the development of body composition measurement modalities. These methods may be used to gain an understanding of the changes that occur over the cancer treatment trajectory and in turn how those may impact health and quality of life outcomes in survivorship. Muscle wasting beyond the rate anticipated due to healthy aging, or increased risk of adverse clinical outcomes in those patients classified to have lower than normal muscle mass, both underscore the important role of this tissue over the cancer treatment trajectory and in survivorship. With improvements in treatments and diagnostic methods, cancers such as breast cancer, are typically associated with relatively good survival rates;

thus, with a growing survivor population more research into areas beyond the immediate biology of the tumour is warranted. The functional consequences of lower than normal muscle mass is an area that has been sparsely explored to date in this cohort, and advances in methodology may help future studies.

Body composition methods that are currently employed to classify lower than normal muscle mass may only detect a subset of patients who are at risk of poor physical function. Adiposity may confound these measures and mask muscle atrophy. One potential strategy to optimize the assessment of muscle characteristics in cancer patients, such as the breast cancer patients and survivor cohort examined in this thesis, may be to better understand the links between muscle features and contractile function.

Ultrasonography is a modality that has been used in populations like older adults and ICU patients to evaluate muscle characteristics. This modality also helps provide an alternative to commonplace modalities like CT and DXA, since it is relatively more accessible, easy to use and do not expose the patients to radiation. These practical considerations coupled with the use of this tool to assess muscle size, composition and architecture, make it a desirable method. At this time, few studies evaluated the use of musculoskeletal ultrasound in any cancer cohort. To address the uncertainty related to the effectiveness of this modality in determining muscle features in cancer patients, chapters 7 and 8 of this thesis focused on building the evidence needed to inform a large-scale investigation into this modality. We were unable to generate evidence supporting the usefulness of ultrasound-based measurements of echogenicity, pennation angle and fascicle length, which contradicts the original hypothesis that muscle composition and architecture are important alongside muscle size. Further method development may offer the additional clarity needed to inform the use of this modality to study alterations in muscle characteristics over the cancer trajectory. In contrast to measurements of muscle composition and architecture, ultrasound-based measures of muscle size are shown to be related to muscle strength and muscle power in chapter 8. As indicated in chapter 7, muscle size measurements may allow researchers to identify differences in breast cancer patients in- and post-treatment when compared to a reference cohort.

The studies in this thesis identified a number of methodological aspects that require closer examination. Based on the results of chapter 8, aggregate measurements of multiple

sites and predictive equations may help increase the confidence of the inferences about the links between muscle size and contractile strength. A reference modality is a crucial consideration in calibrating predictive equations. In light of the confounding effect of excess adiposity when using tools which assess body composition at the molecular level, such as DXA. Development of methods to assess body composition in breast cancer patients in- and post-treatment requires further examination of practical modalities such as ultrasound.

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