

Older males exhibit reduced anterior upper leg and anterior abdominal muscle thickness compared to younger males when matched for relative appendicular lean tissue

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## **ABSTRACT**

**Background:** Ageing-related muscle atrophy does not occur uniformly across the body; rather, atrophy occurs to a greater extent in specific muscle groups compared to others. However, site-specific comparisons of muscle mass between older and younger adults typically do not account for relative muscle mass (i.e., matched for age- and sex-specific percentiles), which may confound site-specific differences. Furthermore, the uniformity of ageing-related differences in muscle composition (e.g., intramuscular adipose tissue) across the body are not well characterized.

**Purpose:** To examine site-specific muscle mass and composition differences between younger and older males matched for relative muscle mass.

**Methods:** Younger (18-44 years old, n=19) and older ( $\geq 65$  years old, n=19) males were matched for relative appendicular lean tissue index (NHANES age- and sex-specific Z-scores) measured using dual-energy x-ray absorptiometry. Site-specific differences in skeletal muscle size (thickness) and composition (echo-intensity) were evaluated using ultrasound for 8 distinct landmarks across the body.

**Results:** Relative appendicular lean tissue mass was well matched between younger and older males (Z-score difference: -0.02,  $p=0.927$ ). Compared with younger males, older males had smaller muscle thickness for the anterior upper leg (difference: -1.08 cm,  $p<0.001$ ) and anterior abdomen (difference: -0.53 cm,  $p<0.001$ ). However, older adults displayed higher echo intensity across all muscles ( $p<0.05$ ), except for the posterior upper arm ( $p=0.377$ ), in comparison to the younger males.

**Conclusions:** When matched for relative appendicular lean tissue, muscle thickness differences between younger and older males are not-uniform across the body, whereas echo intensity was more uniformly higher in the older males.

**Keywords:** Muscle thickness, sarcopenia, appendicular lean tissue mass, dual-energy x-ray absorptiometry, echo intensity.

## 1.1 INTRODUCTION

Ageing is associated with skeletal muscle atrophy and degradation of muscle composition, which is often characterized by infiltration of non-contractile tissue (e.g., intramuscular adipose tissue, fibrotic tissue) (Mitchell et al., 2012). Measurement of low skeletal muscle mass for the identification of sarcopenia in older adults is frequently assessed by appendicular lean tissue mass measured using dual-energy x-ray absorptiometry (DXA), which consists of the lean soft tissue of the upper and lower limbs (Cruz-Jentoft et al., 2018; Heymsfield et al., 1990). However, accumulating evidence indicates that ageing-related muscle atrophy occurs to a greater extent in the lower limbs compared with the upper limbs (Janssen et al., 2000; Miyatani et al., 2003; Paris et al., 2020). More specifically within the lower limbs, several publications have demonstrated that the quadriceps and rectus abdominis muscles display the largest differences in muscle thickness and cross-sectional area between younger and older adults (Frontera et al., 2000; Kara et al., 2020; Loenneke et al., 2014; Paris et al., 2020). However, these studies are generally cross-sectional comparisons between older and younger adults, but they do not account for differences in relative muscle mass (i.e., age- and sex-specific percentiles). Matching older and younger adults for relative muscle mass would allow more equitable comparisons of site-specific muscle size by reducing the likelihood that differences occur due to disparities in relative muscle mass (e.g., comparing younger adult in 75<sup>th</sup> percentile to older adult in 25<sup>th</sup> percentile). A more thorough understanding of ageing-related differences in skeletal muscle mass across the body is critical for establishing sensitive protocols for the identification of sarcopenia in older adults.

For skeletal muscle composition, substantial evidence supports that thigh intramuscular adipose tissue is positively correlated with age (Correa-de-Araujo et al., 2020; Marcus et al., 2010). However, the ageing-related degradation of composition of other muscle groups is less well characterized. While several body composition modalities can measure the infiltration of non-contractile tissue, ultrasound is a non-invasive and widely-available tool that can be easily applied across multiple muscle groups to quantify echo intensity (Paris & Mourtzakis, 2016). Echo intensity is the average pixel intensity of skeletal muscle from ultrasound images, which is associated with several components of skeletal muscle composition (e.g., intramuscular adipose tissue, fibrotic tissue, etc.) (Akima et al., 2016; Pillen et al., 2009; Stock & Thompson, 2020; Young et al., 2015), however, it may be confounded by other physiological aspects of skeletal muscle tissue (e.g., infiltration of inflammatory cells) (Wong et al., 2020). While some groups have observed that older adults have a higher echo intensity in trunk, upper limb, and lower limb muscles compared with younger adults (Arts et al., 2010; Fukumoto et al., 2015; Paris et al., 2020; Yoshiko et al., 2018), comprehensive comparisons across multiple muscle groups within the same group of participants is lacking.

Focusing sarcopenia assessments on specific muscle groups that are more susceptible to ageing-related muscle atrophy or deleterious shifts in muscle composition may provide earlier and more sensitive markers of impaired functional capacity in older adults compared to traditional whole-body approaches (e.g. appendicular lean tissue). Here, our primary objective was to evaluate differences in muscle thickness and echo intensity across multiple landmarks (upper limbs, lower limbs, and trunk) between older ( $\geq 65$  years of age) and younger (18-44

years of age) males who were matched for relative appendicular lean tissue mass (age- and sex-specific NHANES reference (Kelly et al., 2009)).

## **2.1 METHODS**

### 2.1.1 Study design and participants

We performed a cross-sectional study which evaluated community dwelling older (n=32,  $\geq 65$  years of age) and younger (n=22, 18-44 years of age) males from the Kitchener-Waterloo area. Younger males (18-44 years of age) were from a previously collected cohort (Paris et al., 2017). From the original participant pool, younger and older males were individually matched for relative appendicular lean tissue index (see section 2.1.3 for further details). Participants were excluded if they had: 1) a neuromuscular disorder, 2) undergone administration of oral or intra-venous contrast for nuclear medicine scans within the past 3 weeks, 3) a prosthetic joint replacement, or 4) a history of diabetes, cancer, or cerebrovascular disease. Participants were instructed to refrain from moderate to vigorous physical activity for 48 hours, and alcohol consumption for 24 hours, prior to all laboratory visits. This study was approved by a human clinical research ethics committee at the University of Waterloo. Written informed consent was obtained from all participants in accordance with established protocols for human research.

### 2.1.2 Dual-energy x-ray absorptiometry

Height and weight were measured in lightweight clothing or cloth hospital gowns using a balance beam and stadiometer, respectively. Whole body DXA scans (Hologic discovery QDR4500, Hologic, Toronto, ON) were performed by certified medical radiation therapists for each participant. Quality control and calibration procedures were performed as outlined by

manufacturer specifications prior to all scans. Participants were placed supine on the scanning bed, with their legs fully extended and toes internally rotated and held in place with masking tape (to prevent movement during the scan). Hologic software (version 13.2) was used to segment the body into the head, torso, left and right arms, and left and right legs, as previously described (Heymsfield et al., 1990). If a participant required two scans (i.e., did not fit within the lateral limits of a single scan), scans were analyzed by excluding the missing limb(s) and averaging all other segments across the two scans.

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

### 2.1.3 Participant matching

To examine the relative difference in appendicular lean tissue mass index between the original cohort of younger ( $n=22$ ) and older ( $n=32$ ) males, age- and sex- specific z-scores were calculated using normative NHANES data (Kelly et al., 2009). When comparing all older and

younger males, significant differences were observed for appendicular lean tissue index z-score ( $p=0.005$ ) (Table 1). Due to these relative differences in appendicular lean tissue mass, younger and older males were matched on an individual basis within  $\pm 0.5$  z-score units (i.e., younger and older adults with closest z-scores were matched until no z-score differences occurred within 0.5 units). Matching for relative appendicular lean tissue using normative data (i.e. older male in the 50<sup>th</sup> percentile for their age is matched with a younger male in the 50<sup>th</sup> percentile for their age) reduces the likelihood that differences between age groups occur due to disparities in relative muscle mass. From the original sample of younger ( $n=21$ ) and older ( $n=32$ ) males, 19 matches were made within 0.5 z-score units.

Table 1. All participant characteristics.

	All younger males ( $n=22$ )	All older males ( $n=32$ )	Unadjusted p-value
Age, y	27.2 (5.6)	75.4 (7.9)	<0.001
Height, m	1.76 (0.06)	1.73 (0.08)	0.208
Weight, kg	78.5 (11.4)	80.0 (13.3)	0.680
BMI, $\text{kg}/\text{m}^2$	25.4 (3.2)	26.6 (4.0)	0.233
Appendicular lean tissue index z score	-0.31 (0.78)	-0.93 (0.74)	0.005

Data are presented as mean (standard deviation [SD]). BMI, body mass index.

#### 2.1.4 Landmarking for ultrasound imaging

During landmarking, participants lay supine (for anterior sites) or prone (for posterior sites) on a table, with their feet secured in neutral rotation using a foot strap to prevent



internal or external hip rotation. A flexible tape measure and pen were used to mark sites for ultrasound imaging. Sites for ultrasound imaging included the anterior and posterior upper arm, anterior forearm, anterior abdomen, anterior and posterior upper leg, and anterior and posterior lower leg, as previously described (Abe et al., 1994; Paris et al., 2017). Upper arm images were taken on the anterior and posterior surface, 60% distal from the acromial process to the lateral epicondyle of the humerus. Anterior forearm images were taken on the anterior surface, 30% distal from the radial head to the styloid process of the ulna. Anterior abdomen images were taken 3 cm right of the umbilicus. Anterior thigh images were taken two-thirds the distance from the anterior superior iliac spine to the superior pole of the patella. The posterior upper leg image was taken on the posterior surface, 50% of the distance between the greater trochanter and lateral epicondyle of the femur. The anterior and posterior lower leg images were taken on the anterior and posterior surface, 30% distal from the head of the fibula to the lateral malleolus. All landmarking, and subsequent imaging, was performed on the right side of the body. Landmarking took approximately 20 minutes, during which participants remained supine or prone to mitigate shifts in fluid distribution during ultrasound imaging.

#### 2.1.5 Ultrasound image acquisition

Transverse images were taken using a real time B-mode ultrasound imaging device (M-turbo, Sonosite, Markham, ON), equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz). During image acquisition, imaging mode was set to “resolution”, and adjustable parameters (gain, time-gain-compensation, and dynamic range [50%]) were held constant across all study participants. The ultrasound transducer was generously coated with water-soluble transmission gel. To ensure the landmark aligned with the middle of the muscle bulk,

medial-lateral movement of the ultrasound transducer was allowed for all landmarks to centre the muscle within the field of view. Minimal compression of the underlying tissues during imaging was achieved by: 1) maintaining a visible layer of ultrasound gel between the skin and transducer, and 2) ensuring the natural curvature of the skin, subcutaneous adipose tissue, and skeletal muscle was maintained, as previously described (Paris et al., 2017). Imaging depth was adjusted to the minimum depth required to obtain a complete view of the muscles being analyzed. All ultrasound images were saved in the Digital Imaging and Communications in Medicine (DICOM) format and transferred to a computer for analysis.

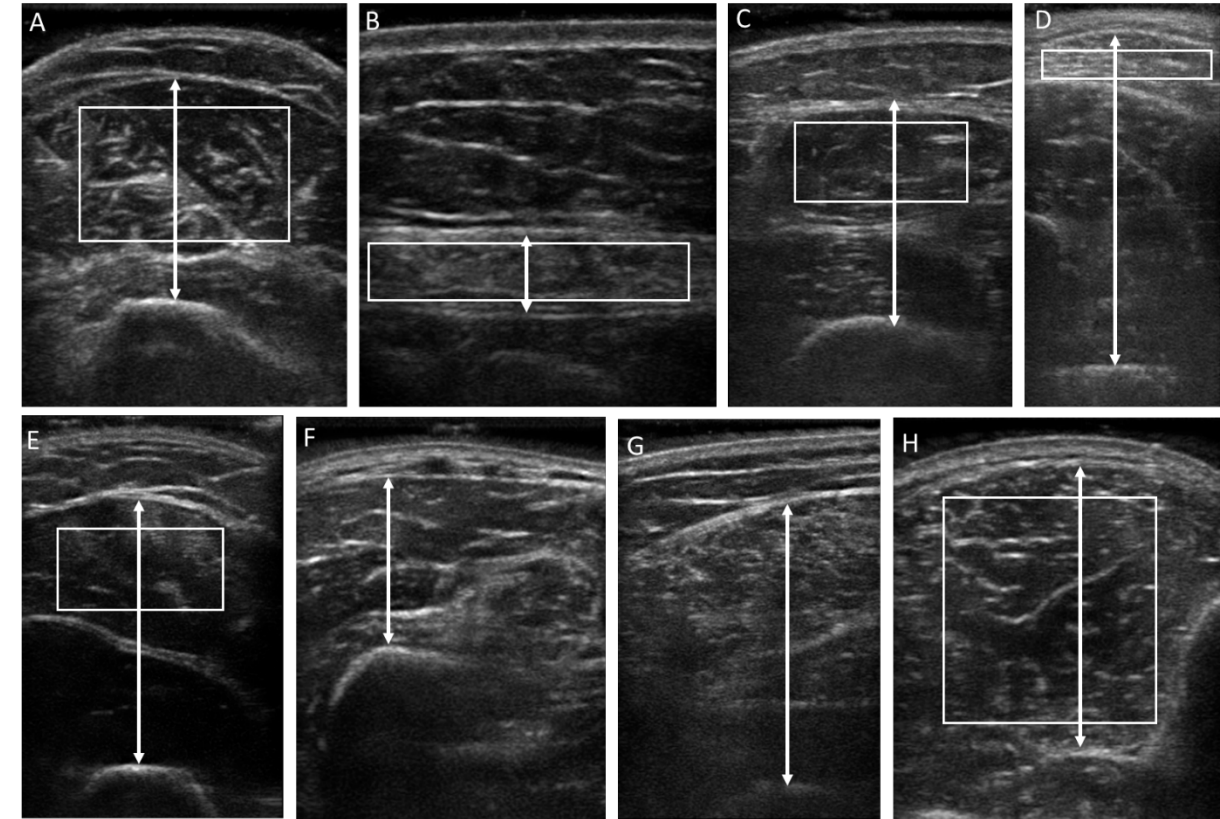
#### 2.1.6 Muscle thickness analysis

For each muscle group, we measured the vertical distance between the superior muscle fascia and either the upper margin of the underlying bone or the inferior muscle fascia (anterior abdomen and anterior lower leg) (Figure 1). Muscle thickness was measured once for each landmark by a single trained investigator. All measurements were performed using ImageJ software (NIH, Bethesda, MD, version 1.52e) and converted to a linear distance using manufacturer provide image resolutions.

#### 2.1.7 Echo intensity analysis

Muscle echo intensity was evaluated by selecting the largest rectangular area within the muscle fascia borders (ImageJ, NIH, Bethesda, MD, version 1.52e), as previously described (Caresio et al., 2014). Specific muscles analyzed were the rectus abdominis (anterior abdomen), tibialis anterior (anterior lower leg), biceps brachii (anterior upper arm), rectus femoris (anterior upper leg), lateral gastrocnemius (posterior lower leg), and lateral triceps brachii

(posterior upper arm) (Figure 1). Echo intensity is expressed as an arbitrary unit (A.U.) between 0 (black) and 255 (white).



**Figure 1.** Muscle thickness and echo intensity analysis for A) anterior upper arm, B) anterior abdominal, C) anterior upper leg, D) posterior lower leg, E) posterior upper arm, F) anterior forearm, G) posterior upper leg, H) anterior lower leg. White lines indicate muscle thickness assessment and white boxes indicate area selected for analysis of echo intensity.

### 2.1.8 Statistical analysis

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

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

### 3.1 RESULTS

After matching, no differences in appendicular lean tissue index z-scores were observed between the older and younger males ( $p=0.927$ ). The matched older male cohort had a higher BMI ( $p=0.011$ ) and body fat percent ( $p<0.001$ ) compared with the matched younger males (Table 2). Prior to matching, significant differences were observed for appendicular lean tissue ( $p<0.001$ ), upper arm lean tissue ( $p<0.001$ ), and upper leg lean tissue ( $p<0.001$ ), after correcting for multiple comparisons (Table S1). Between matched older and younger males, after correction for multiple comparisons, there were no differences in appendicular or limb-specific lean tissue (Table 3).

Table 2. Characteristics for matched participants.

	Matched young males (n=19)	Matched older males (n=19)	Unadjusted p-value
Age, y	27.3 (5.8)	72.2 (6.8)	<0.001
Height, m	1.76 (0.06)	1.74 (0.06)	0.336
Weight, kg	77.3 (11.6)	88.2 (15.4)	0.032
BMI, kg/m <sup>2</sup>	25.1 (3.4)	29.2 (4.5)	0.011

Appendicular lean tissue index z score	-0.45 (0.75)	-0.43 (0.66)	0.927
DXA body fat, %	21.9 (4.8)	30.8 (4.9)	<0.001

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

Table 3. Dual-energy x-ray absorptiometry lean tissue characteristics for matched participants.

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

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

Prior to matching, older males displayed significantly smaller muscle thickness compared with younger males for the anterior upper arm (15% lower, p=0.001), posterior upper arm (20% lower, p=0.002), anterior abdomen (42% lower, p<0.001), anterior upper leg (37% lower, p<0.001), and posterior upper leg (24% lower, p<0.001) (Table S2). However, after matching for relative appendicular lean tissue index, compared with younger males, older males displayed significantly lower muscle thickness for the anterior abdomen (36% lower,

p<0.001) and anterior upper leg (26% lower, p<0.001) landmarks after correcting for multiple comparisons (Table 4). The posterior upper leg trended towards a lower muscle thickness in the matched older males compared with matched younger males (13% lower, p=0.055); however, no differences existed for the anterior forearm, anterior upper arm, posterior lower leg, or posterior upper arm. Unexpectedly, matched older males displayed a larger muscle thickness for the anterior lower leg (11% larger, p=0.001) compared to the matched younger male cohort (Table 4).

Table 4. Muscle thickness characteristics for matched participants.

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

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

Prior to matching, all muscle groups displayed higher muscle echo intensity in the older compared with younger males (Table S3). Similarly, after matching, all muscle groups, except for the posterior upper arm ( $p=0.377$ ), displayed higher muscle echo intensity in the older males compared to the younger males (Table 5).

Table 5. Muscle echo intensity characteristics for matched participants.

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

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

AU, arbitrary values.

## 4.1 DISCUSSION

We observed that site-specific differences in muscle thickness are not uniformly distributed across the body between older and younger adults matched for relative appendicular lean tissue index. Older males had significantly smaller muscle thicknesses for the anterior upper leg and anterior abdomen, with a trend for the posterior upper leg; however, no differences were observed for the anterior or posterior upper arm, anterior forearm, or the posterior lower leg. Unexpectedly, the anterior lower leg muscle thickness was larger in older males compared to younger males. Despite the non-uniform differences in muscle thickness, muscle echo intensity was consistently elevated in older males across all landmarks, except for the posterior upper arm, when compared with younger males.

Ageing-related skeletal muscle atrophy occurs due to losses in muscle fibre number and atrophy of remaining fibres (Lexell et al., 1988). The process of ageing-related muscle atrophy begins around the 5<sup>th</sup> decade of life and proceeds at a rate of 0.6 – 1.2% per year; however, the extent of atrophy and age at which it begins is variable across studies (Mitchell et al., 2012). These discrepancies in ageing-related muscle atrophy may be associated with several factors which include differences in cohort characteristics, body composition modalities used for assessing muscle size, or the muscle groups selected to characterize muscle atrophy.

Several cross-sectional comparisons have demonstrated that compared with younger adults, older adults present with proportionally smaller musculature of the lower limbs than the upper limbs (Abe, Kawakami, et al., 2011; Janssen et al., 2000; Loenneke et al., 2014). More specific analyses indicate that the quadriceps muscles may account for a larger proportion of



lower limb muscle mass differences between younger and older adults (Abe, Ogawa, et al., 2014; Abe, Thiebaud, et al., 2014; Miyatani et al., 2003; Overend, Cunningham, Paterson, et al., 1992). A review by Abe et al. (2014) observed across 8 independent publications, that compared with the younger adults (n=584), older adults (n=466) consistently presented with relatively lower cross-sectional area and thickness of the quadriceps (on average, ~28% lower than young adults) compared with the hamstrings muscle groups (on average ~8% lower than young adults). In the present study across all participants, we observed significantly smaller muscle thickness for the anterior and posterior upper leg in the older compared with younger males; however, these differences may be related to relative differences in muscularity between the age groups. After matching for relative appendicular lean tissue, we observed that the anterior thigh muscle thickness is significantly smaller (~26%, p adjusted <0.001) in the older compared with younger adult, but only a tendency for smaller hamstring thickness (~13% smaller, p adjusted = 0.055). These findings are further substantiated in several larger cohort studies demonstrating negative correlations between anterior thigh thickness and age, but weak or null association between posterior upper leg muscle thickness and age (Abe, Sakamaki, et al., 2011; Kara et al., 2020). However, Frontera et al. (2000) observed similar reductions in quadriceps (-16.1%) and hamstrings (-14.9%) cross-sectional area over a 12 year follow-up in older men (n=9, baseline age: 65.4 years). Furthermore, while there is a disconnect between muscle mass and strength with advancing age (Newman et al., 2006), several studies are in concordance with Frontera et al. (2000), which have observed similar differences in knee extensors and flexors isometric and isokinetic torque production between younger and older adults (Abe, Loenneke, et al., 2014; Candow & Chilibeck, 2005; Frontera et al., 2008; Overend,

Cunningham, Kramer, et al., 1992), suggesting that muscle function of the quadriceps and hamstring muscles decrease to a similar extent with advancing age. Taken together, these data demonstrate that ageing is associated with declines in function of both the quadriceps and hamstring muscle groups. However, the aetiologies of these impairments may not be similar, as the declines in specific strength (i.e. strength/mass) of the quadriceps may be more related to reductions in muscle mass (Abe, Loenneke, et al., 2014) whereas the hamstring impairments may be more related to neuromuscular degradation (Kirk et al., 2018).

In agreement with our results in the matched older and younger males, others have observed no age-related differences in muscle thickness of the upper limbs and lower leg (Abe, Patterson, et al., 2014; Arts et al., 2010; Ata et al., 2019), however, these lack of differences are not always noted (Abe, Thiebaud, et al., 2014; Miyatani et al., 2003). We also observed that the anterior abdominal muscles (rectus abdominis) demonstrated the largest difference between the older and younger males, which is in agreement with several other reports (Abe, Thiebaud, et al., 2014; Fukumoto et al., 2015; Kara et al., 2020; Miyatani et al., 2003; Ota et al., 2020), indicating the trunk musculature may be particularly prone to ageing-related muscle atrophy. However, it should be noted that the trunk musculature was not considered when matching for appendicular lean tissue, which may add additional confounding factors when comparing abdominal muscle thicknesses between our older and younger males.

The emerging findings that ageing-related skeletal muscle atrophy is primarily localized to specific muscle groups, such as the anterior upper leg and anterior abdomen, has important implications for identification of older adults with low skeletal muscle mass. DXA measured appendicular lean tissue mass is the most common metric of ageing-related muscle atrophy;

however, given the relative preservation of mass for certain muscle groups (e.g., upper limbs), appendicular lean tissue may limit the sensitivity to detect skeletal muscle atrophy. Even the use of the upper thigh lean tissue, which encompasses the entirety of the quadriceps and hamstrings, may not be the ideal approach to assess ageing-related muscle loss, as we only observed an ~10% difference between older and younger males. Whereas the anterior thigh and anterior abdomen exhibited ~26% and ~36% smaller muscle thickness in older males. Furthermore, understanding these regional differences in skeletal muscle mass across the upper and lower limbs with advancing age has implications for mobility and independence of older adults, as the lower limb muscle function is essential for activities of daily living (e.g., standing from a chair). Take together, these data highlight the potential use of ultrasound as a clinically applicable tool to quantify the anterior upper leg and abdomen for the characterization of low muscle mass in the identification of sarcopenia. However, the ease of access, cost, and training required to perform these measurements should be taken into consideration. Furthermore, there is a wealth of normative data available for DXA measures of lean tissue (Kelly et al., 2009), whereas ultrasound normal values are lacking (due in large part to differences in acquisition protocols) (Mourtzakis et al., 2017).

While we and others have observed that differences in skeletal muscle thickness between older and younger adults do not occur uniformly across the body, less is known about differences in muscle echo intensity. Increased echo intensity in older compared to younger adults has been observed in biceps brachii (Arts et al., 2010), all of the quadriceps muscles (Fukumoto et al., 2015; Paris et al., 2020; Strasser et al., 2013; Watanabe et al., 2017), hamstrings muscle groups (Palmer & Thompson, 2017), and several trunk muscle groups,

including rectus abdominus, internal and external obliques, and transverse abdominus (Fukumoto et al., 2015; Ota et al., 2012; Paris et al., 2020). The rectus abdominus typically displays the largest differences in echo intensity between younger and older adults (Fukumoto et al., 2015; Paris et al., 2020), which we also observed (~3 fold greater in older compared with younger adults). Given the substantial differences in body fat between the older and younger adults, the differences in subcutaneous adipose tissue, which confound the analysis of echo intensity (Young et al., 2015), may require correction when interpreting differences in aged and/or obese individuals. However, the ideal approaches for correcting for subcutaneous adipose tissue thickness are not entirely clear, but a few approaches have been proposed (Neto Müller et al., 2020; Young et al., 2015). While several publications, including the present one, demonstrate that certain muscles appear to be relatively “spared” from ageing-related decline in mass, it is likely that many of these muscles exhibit impairments in muscle composition or function, highlighting that caution may be necessary in discussions around the relative importance of different muscles when assessing ageing-related degradation of the skeletal muscle system. However, these indices of muscle composition were derived using echo intensity, which is not a direct evaluation of intramuscular adipose tissue (Wong et al., 2020).

#### 4.1.1 Limitations

An important limitation of the present study is the use of muscle thickness as the primary metric of site-specific muscle size. While thickness of several limb muscles are strongly associated with MRI measured muscle volume (Miyatani et al., 2004), skeletal muscle mass may not be adequately represented through a linear dimension, which may contribute to an inability to detect differences (e.g. posterior upper leg) or perhaps even overestimate ageing-related

declines (e.g. rectus abdominus). Furthermore, ultrasound muscle thickness or cross-sectional area is unable to differentiate between intramuscular adipose or connective tissue and functional contractile tissue, which would mask the true degree of muscle atrophy. While we observed non-uniform differences in muscle thickness between older and younger males, our analyses were limited to a single sex, limiting our understanding of site-specific differences in muscle thickness and echo intensity in females. While we successfully matched for appendicular lean tissue to minimize the differences in relative muscularity, on average, the older adults in this cohort did not have clinically low appendicular lean tissue mass (only one participant was below recent guidelines (Cruz-Jentoft et al., 2019) of  $<7.0 \text{ kg/m}^2$  for low appendicular lean tissue). Older adults with low appendicular lean tissue may demonstrate differences in the specific sites that exhibit skeletal muscle atrophy or intramuscular adipose tissue deposition in comparison to those older adults with normal muscle mass (Chang et al., 2018). Further, differences in adiposity were not accounted for between older and younger males, which may confound interpretation for changes in skeletal muscle thickness and echo intensity.

#### 4.1.2 Conclusions

In the present study, we show that, when matched for relative appendicular lean tissue index, older males display reduced muscle thickness at the anterior upper leg and anterior abdominal muscle groups compared with younger males. Whereas no differences, or even larger thicknesses, were observed for several other landmarks across the body. These results highlight the need to better understand these differences in muscle mass across the lifespan to identify how best to characterize skeletal muscle mass in aged adults. We further observed that

muscle echo intensity is elevated in most landmarks evaluated, suggesting a more uniform degradation of muscle composition across the body.

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