

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

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ABSTRACT

Introduction: Age-related deterioration of muscle mass does not occur uniformly across the body. However, there is limited knowledge on the uniformity of age-related muscle composition changes across the body. Our primary objective was to evaluate muscle composition differences between younger and older adults across multiple muscle groups.

Methods: We re-analyzed data from a previously published cohort to evaluate differences in ultrasound muscle composition (echo intensity) between younger (<45 years) and older (>60 years) adults, when matched for adipose tissue mass at the anterior upper arm, anterior upper leg, and abdominal muscles. Analysis of echo intensity is confounded by subcutaneous adipose tissue (SAT) thickness overlaying the muscle; we accounted for these effects by matching older and younger adults (1:1), stratified by sex, for absolute SAT thickness at each landmark.

Results: From 96 adults (n=females), 58 (n=34) were SAT matched at the anterior upper arm, 52 (n=30) at the anterior upper leg, and 60 (n=30) at the abdominal region; thus, there were no age group differences in SAT thickness at each landmark. In comparison to younger adults, older adults presented with greater echo intensity at the anterior upper leg (females:40.3±6.8 vs. 52.4±7.6; males:35.7±8.0 vs. 54.3±9.8, p<0.01) and abdominal (females:38.7±27.6 vs. 73.4±31.0; males:18.7±15.2 vs. 60.9±23.4, p<0.01) muscles, but not anterior upper arm muscles (females:47.0±6.5 vs. 53.2±13.1; males:43.4±8.9 vs. 48.9±10.1, p=0.18).

Conclusions: Distinct age-related differences in trunk and lower limb muscle composition were evident compared to upper limb muscles; highlighting the importance of quantifying specific muscle groups when evaluating age-associated muscle characteristics.

Keywords: body composition, ultrasound, sites-specific sarcopenia, muscle composition, echo intensity

INTRODUCTION

Aging is associated with skeletal muscle atrophy and deterioration of muscle composition (e.g. increased intramuscular adipose tissue), which have implications for the health and disease of older adults (e.g. insulin resistance) (Addison et al. 2014, Reinders et al. 2015). While computed tomography (CT) and magnetic resonance imaging (MRI) are considered reference standards for assessing muscle mass and composition, these modalities are expensive, expose the patient to ionizing radiation in the case of CT, and are generally not available for prospective body composition assessments (Prado and Heymsfield 2014). Ultrasound has emerged as a portable, non-invasive, and cost-effective modality for measuring the quantity and composition of skeletal muscle (Paris and Mourtzakis 2016, Mourtzakis et al. 2017).

Ultrasound indices of muscle quantity (e.g. muscle thickness or cross-sectional area) are strongly associated with regional and whole-body skeletal muscle mass measured using MRI (Sanada et al. 2006) and dual-energy x-ray absorptiometry (DXA) (Takai et al. 2014, Paris et al. 2017); however, ultrasound measures of skeletal muscle composition, such as echo intensity, are less well established. Echo intensity is the mean pixel intensity from a region of interest outlined within the muscle fascia of the ultrasound scan (Pillen and van Alfen 2011). Skeletal muscle echo intensity of the quadriceps increases with age (Strasser et al. 2013, Wilhelm et al. 2014), and has been associated with elevated adipose and connective tissue infiltration (Reimers et al. 1993, Pillen et al. 2009, Young et al. 2015, Akima et al. 2016). While increased adiposity within skeletal muscle has been identified in the quadriceps of aged individuals (Marcus et al. 2010), there is a lack of literature evaluating the influence of age on other muscle groups across the body; limiting our understanding of the age-associated changes in muscle composition.

Furthermore, there may be sex-specific differences in the age-related degradation of muscle composition across the body, however, this has not been adequately explored.

While echo intensity provides an accessible and non-invasive surrogate of skeletal muscle composition, interpretation can be challenging. Since the ultrasound beam travels through the subcutaneous adipose tissue (SAT) before reaching the skeletal muscle layer, the sound waves will be attenuated (absorbed, scattered, or reflected); which may artificially reduce the mean muscle echo intensity, and thus may imply less adipose tissue infiltration (Haberkorn et al. 1993, Nijboer-Oosterveld et al. 2011, Young et al. 2015). However, in the evaluation of muscle echo intensity, SAT thickness is rarely accounted for when interpreting the results in obese and/or aged individuals.

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

METHODS

Study design and participants

We performed a secondary data analysis of ultrasound images collected from 96 participants who were involved in a study validating muscle thicknesses against DXA (Paris et al. 2017). The muscle size (thickness and DXA) and composition (echo intensity) features

presented here have been previously published (Paris et al. 2017); the primary purpose for this publication is to understand muscle composition differences between younger and older adults who are matched for SAT thickness. Participants were ≥ 18 years of age and refrained from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to data collection. For the secondary data analysis, we matched younger and older adults for absolute SAT thickness (see below for details) and evaluated differences in thickness and echo intensity of the anterior upper arm, abdomen and anterior upper leg muscles. This study was reviewed and cleared by a University of Waterloo Clinical Research Ethics Committee.

Dual-energy x-ray absorptiometry analysis

Certified Medical Radiation Technologists performed whole body DXA scans (Hologic Discovery QDR 4500, Hologic, Toronto, ON), as previously described (Paris et al. 2017). Scans were segmented into the head, trunk, left and right upper limbs, and left and right lower limbs by a single trained investigator according to a standardized protocol (Heymsfield et al. 1990). Total body fat (fat mass/ body weight, %) and appendicular lean tissue index (soft lean tissue in arms and legs/height², kg/m²) were calculated for all participants. Appendicular lean tissue index and body fat measures are used for descriptive purposes.

Ultrasound analysis

Ultrasound images of the anterior abdomen (rectus abdominis), anterior upper arm (biceps and brachialis), and anterior upper leg (rectus femoris and vastus intermedius) were utilized to represent the trunk, upper limbs, and lower limbs for body composition analysis, respectively (Figure 1). Images were obtained as previously described (Paris et al. 2017). Briefly, a B-mode ultrasound imaging (M-Turbo SonoSite, Markham, ON) device equipped with a multi-

frequency linear array transducer (L38xi: 5-10 MHz) was used for capturing transverse ultrasound images. The resolution mode was used for all images and gain, time-gain-compensation, and dynamic range (set to 0) were in the default setting. Images were taken on the right side of the body at the anterior upper arm (anterior surface, 60% distal from acromion to lateral epicondyle of the humerus), abdominal (3 cm right of the umbilicus), and anterior upper leg (anterior surface, midpoint between the greater trochanter and lateral epicondyle of the femur). Participants were supine for 20 minutes prior to imaging to mitigate potential influence of fluid shifts on muscle measures.

Muscle and subcutaneous adipose tissue thickness were measured using image analysis software (ImageJ: Version 1.51, National Institutes of Health, Bethesda, MD). Muscle thickness was quantified as the linear distance between the inferior border of the superior muscle fascia and the superior border of the underlying bone (for the upper and lower limbs) or deep muscle fascia (for the abdominal landmark) (Figure 1). Adipose tissue thickness was quantified as the linear distance between the deep border of the skin and the superior border of the superficial muscle fascia. All thicknesses were measured twice by a single investigator (in pixels using ImageJ), averaged, and converted to a linear distance using manufacturer conversion factors.

We quantified skeletal muscle echo intensity using image analysis software (ImageJ: Version 1.51, National Institutes of Health, Bethesda, MD). Echo intensity was quantified by manually placing a rectangular region of interest within the muscle (rectus femoris, rectus abdominus, and biceps brachii), that included as much of the muscle area as possible, excluding any fascia (Caresio et al. 2014). Mean muscle echo intensity (arbitrary units (A.U.)) was measured twice on the same image (same ROI placement criteria) by a single investigator and averaged.

Participant matching

Participants were first stratified by sex and age (young: <45 years of age and old: ≥ 60 years of age), and then matched for SAT thickness (older and younger SAT thickness was within 0.5 cm). SAT thickness may confound measures of skeletal muscle echo intensity due to beam attenuation in deeper tissues. For example, increased SAT thickness may result in reduced echo intensity due to beam scattering and reflection. By matching participants for SAT thickness at each landmark (anterior upper arm, anterior upper leg, and abdominal region), the confounding effects of the adipose tissue layer on muscle echo intensity would be normalized between younger and older groups. Matching occurred independently at each landmark; in other words, each cohort of participants for a given landmark may or may not contain the same participants. Participants who were not matched for absolute SAT thickness at given landmark were excluded from muscle thickness and echo intensity analysis.

Statistical analyses

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

RESULTS

Demographics and participant matching

At the anterior upper arm, abdominal, and anterior upper leg landmarks, 58 (24 males and 34 females), 52 (22 males and 30 females), and 60 (30 males and 30 females) younger and older participants were matched for SAT thickness, respectively (Table 1). The anterior upper arm and abdomen had 69% overlap of participants, the anterior upper arm and anterior upper leg had 67% overlap of participants, and the abdomen and anterior upper leg had 55% overlap of participants. SAT thickness was successfully matched between younger and older males, as no differences were observed for the anterior upper arm (younger: 0.35 ± 0.23 cm, older: 0.34 ± 0.20 cm, $p=0.867$), abdomen (younger: 1.96 ± 0.90 cm, older: 2.06 ± 0.86 cm, $p=0.740$), and anterior upper leg (younger: 0.67 ± 0.40 cm, older: 0.71 ± 0.39 cm, $p=0.851$) landmarks (Table 1). Similarly, no differences in SAT thickness were observed between younger and older females for the anterior upper arm (younger: 0.63 ± 0.30 cm, older: 0.65 ± 0.30 cm, $p=0.882$), abdomen (younger: 2.61 ± 0.90 cm, older: 2.62 ± 0.85 cm, $p=0.975$), and anterior upper leg (younger: 1.27 ± 0.63 cm, older: 1.27 ± 0.60 cm, $p=0.997$) (Table 1). Bland-Altman plots for the anterior upper arm, anterior upper leg, and abdominal landmarks depict the degree SAT thickness matching between older and young adults at the individual and group level (Figure 2).

Following SAT thickness matching, significant differences in age and body fat percent, but not BMI, were observed between younger and older adults across each landmark (Table 1). Across each landmark, older adults exhibited lower appendicular lean tissue index ($p<0.05$) compared with younger adults (Table 1). Importantly for evaluation of potential sex by age group interactions on muscle features, age was similar between males and females ($p>0.05$) for each landmark evaluated (Table 1).

Muscle echo intensity

Muscle echo intensity at the abdomen was higher for older males and females ($p < 0.01$) compared with younger males (younger: 18.7 ± 15.2 A.U., older: 60.9 ± 23.4 A.U.) and females (younger: 38.3 ± 27.7 A.U., older: 73.4 ± 31.0 A.U.) (Figure 3). Similarly, muscle echo intensity at the anterior upper leg was higher for older males and females ($p < 0.01$) compared with younger males (younger: 36.0 ± 8.03 A.U., older: 54.3 ± 9.79 A.U.) and females (younger: 40.3 ± 6.75 A.U., older: 52.4 ± 7.60 A.U.) (Figure 3). However, muscle echo intensity at the anterior upper arm was not different between older males and females ($p = 0.18$) compared with younger males (younger: 43.4 ± 8.92 A.U., older: 48.9 ± 10.1 A.U.) and females (younger: 47.0 ± 6.55 A.U., older: 53.2 ± 13.1 A.U.) (Figure 3).

Muscle thickness

Older males and females had significantly smaller muscle thicknesses ($p < 0.01$) compared with their corresponding younger male and female counterparts at the abdomen (younger males: 1.50 ± 0.29 cm, older males: 0.84 ± 0.24 cm; younger females: 1.08 ± 0.24 cm, older females: 0.77 ± 0.16 cm) and anterior upper leg (younger males: 4.31 ± 0.65 cm, older males: 3.03 ± 0.45 cm; younger females: 3.49 ± 0.68 cm, older females: 2.44 ± 0.49 cm) (Figure 4). However, mean muscle thickness of the anterior upper arm was not different between older males and females ($p = 0.13$) compared to younger males (younger: 3.72 ± 0.54 cm, older: 3.44 ± 0.44 cm) and females (younger: 2.52 ± 0.48 cm, older: 2.38 ± 0.33 cm) (Figure 4).

DISCUSSION

We observed that older adults, when compared with younger adults, exhibited elevated skeletal muscle echo intensity of the anterior upper leg and abdomen, but not the anterior upper

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

Aging is associated with increased adipose tissue infiltration of the thigh musculature (Marcus et al. 2010, Akima et al. 2016, Perkisas et al. 2018); which occurs regardless of changes in body weight (Delmonico et al. 2009). This age-associated deterioration of thigh muscle composition has implications for the development of insulin resistance and impaired functional capacity of older adults (Goodpaster et al. 2000). However, muscle composition shifts of other muscle groups due to advancing age is less commonly evaluated. Yoshiko et al. (2017) observed that the hamstrings muscle groups of older adults displayed a significantly higher intramuscular adipose tissue cross-sectional area compared to young adults, however, no differences were present in the quadriceps intramuscular adipose tissue between young and old adults. The lower trunk musculature (level of the 3rd lumbar vertebrae) exhibited a 67% reduction in CT muscle attenuation from 40 to 90 years of age (Graffy et al. 2019). This is important, given that CT attenuation of abdominal muscles is associated with metabolic impairments (Tanaka et al. 2019). Fukumoto et al. (2015) observed increased skeletal muscle echo intensity across the biceps, quadriceps, rectus abdominis, internal and external obliques, and the transverse abdominis in middle-aged (50-64 y), young-old (65-74 y), and old-old (≥ 75 y) females, compared to younger (19-30 y) females. While all these muscle groups demonstrated elevated echo intensity in older adults, the trunk muscle groups had the largest discrepancies (2-fold higher) in the old-old

compared to the younger cohort (Fukumoto et al. 2015); suggesting that the trunk muscles may display the largest shifts in composition with age. However, the evaluation of muscle echo intensity across different age groups may be influenced by differences in SAT thicknesses between younger and older adults.

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

Aging is also accompanied by a deterioration of skeletal muscle quantity. Skeletal muscle atrophy is typically cited to occur at a rate of 0.5 – 1.0 % per year beginning in the 5th decade of life (Mitchell et al. 2012). However, Janssen et al. (2000) used MRI to evaluate muscle volume across different age ranges and observed that the lower limb musculature exhibit a larger relative reduction compared with the upper limbs with increasing age, indicating that aging may be associated with regional skeletal muscle loss. Muscle group specific analyses have demonstrated that even within the trunk or an appendage, certain muscle groups may contribute more to age-related muscle atrophy (Overend et al. 1992, Abe et al. 2011, Ogawa et al. 2011, Ota et al. 2012). Abe et al. (2014) used ultrasound to compare 9 distinct muscle groups in older adults (70-79 years of age, n=139) with those of a younger adult cohort (20-29 years of age, n=227).

Compared to the younger cohort, the older adult cohort had lower muscle thickness in the anterior abdominal region (rectus abdominis, ~70% of younger cohort) and anterior upper leg (rectus femoris and vastus intermedius, ~70% of younger cohort); however, there were only marginal differences in the anterior upper arm (biceps brachii and brachialis, ~98% of younger cohort) (Abe et al. 2014). Our results (averaged across males and females) align well with the degree of muscle atrophy observed in the anterior upper leg (~70%), abdomen (~60%), and anterior upper arm (~92%) in our older cohort compared to our younger cohort.

Taken together, these results indicate that age-associated muscle impairments of the anterior abdomen and anterior thigh may occur to a greater extent than the anterior upper arm. These observed differences in muscle composition and thickness across the body agree with observations that muscle strength also appears to decrease earlier in the lower body compared to upper body (Lynch et al. 1999). While the reasons for these differences in age-related muscle impairments between the upper and lower body are not entirely understood, they may in part be related to reductions in physical activity. Reductions in physical activity would presumably impact the lower limb musculature to a greater extent compared with the upper limbs, due to their involvement in common movement activities (e.g. climbing stairs). Future work accounting for differences in physical activity are needed to better differentiate the influence of age and activity (or lack thereof). While these differences may exist, it will be critical to examine if these differences have implications for metabolic health or functional capacity of older adults. For example, Ido et al. (2015) observed that abdominal muscle thickness is a stronger indicator of metabolic syndrome in obese adults compared to DXA appendicular lean tissue index; suggesting that site-specific measures of muscle size may be advantageous over traditional measures of lean tissue (DXA) for metabolic impairments. Given the ~2-3 fold higher echo

intensity and ~0.6 fold lower thickness, the anterior abdominal muscle group may be a critical site to identify older adults with metabolic and functional impairments. However, these impairments will need to be evaluated against measures of muscle thickness and echo intensity at other common landmarks (i.e. quadriceps musculature), to determine if specific sites are more important than others for metabolic or functional impairments. Furthermore, the lack of observed sex by age interactions on muscle echo intensity or thickness need to be further explored, as a recent analysis of aggregated data demonstrated that age-related decline in dynamic muscle function of the knee extensors occurs earlier and to a greater extent in females, compared with males (Haynes et al. 2020).

There are several limitations within this secondary data analysis. The matching of absolute SAT thicknesses between older and younger adults at the three distinct landmarks resulted in overlapping participants across the groups, preventing statistical comparisons of muscle thickness and composition differences across each landmark. Furthermore, by matching participants on absolute SAT thickness, older adults on the upper spectrum of SAT thickness and younger adults on the lower end will have been excluded, due to fewer matches occurring in these opposite extremes. This may be particularly important for the abdominal landmark, as there will likely be differences between younger and older adults for both the deep and superficial SAT compartments (Marinou et al. 2014); which may confound echo intensity analysis of the rectus abdominus muscle. Lastly, the upper limb, trunk, and lower limbs are being represented by a single muscle group; however, emerging evidence is demonstrating that even within an appendage, age-associated differences exist in specific muscle groups (i.e. quadriceps display more muscle atrophy than the hamstrings) (Abe et al. 2014).

In conclusion, we observed age-associated differences in skeletal muscle thickness and echo intensity of the abdomen and anterior upper leg, but not the anterior upper arm of older adults compared to younger adults matched for SAT thickness. These non-uniform deteriorations across the upper limb, trunk, and lower muscles with age highlight the importance of quantifying these muscle groups separately when evaluating age-associated changes in body composition and their implications on strength, function, and metabolism. Future work accounting for potential differences in physical activity between age groups are needed to better understand why these site-specific differences in muscles tissue may be occurring with advancing age.

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CONFLICTS OF INTEREST

None declared.

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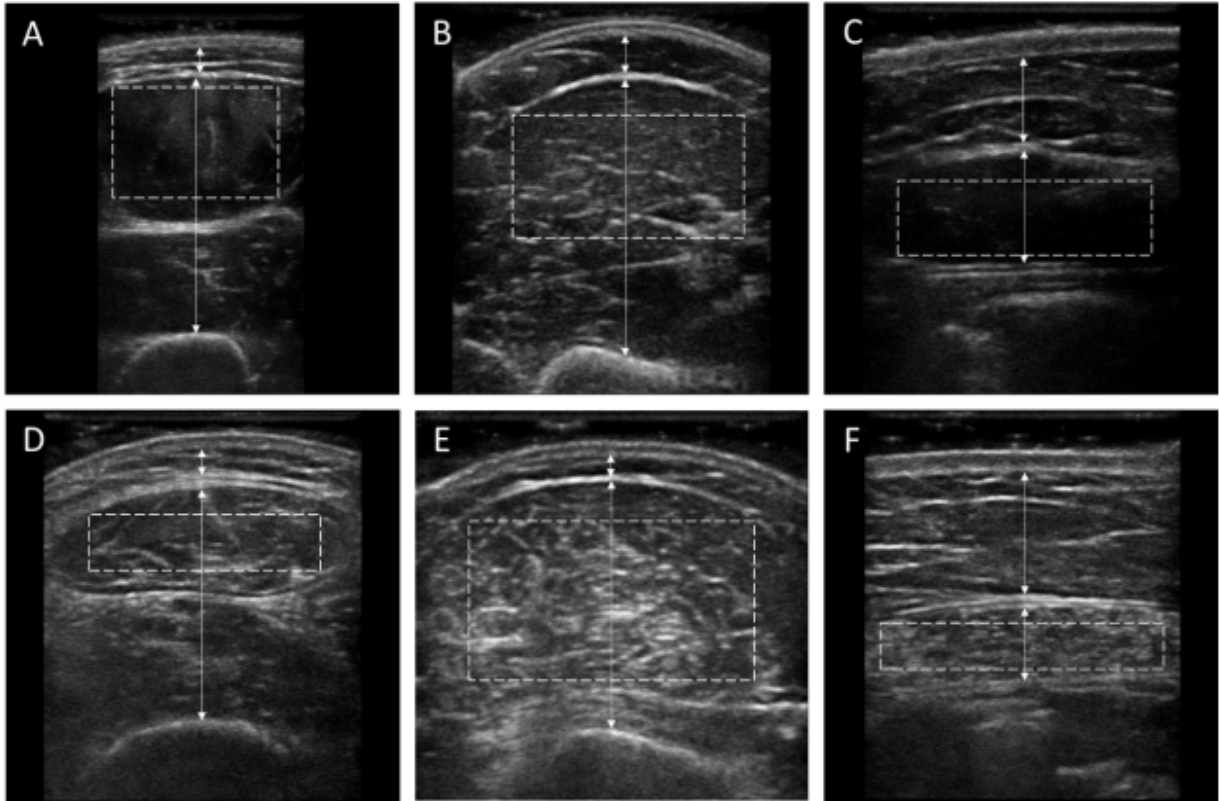
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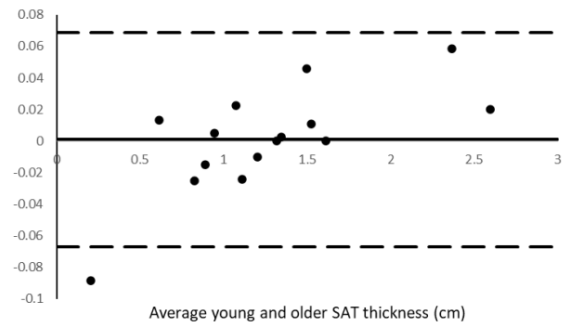
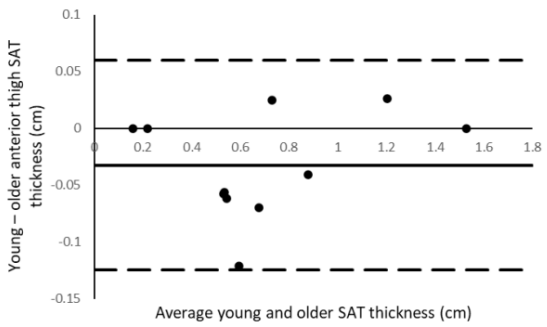
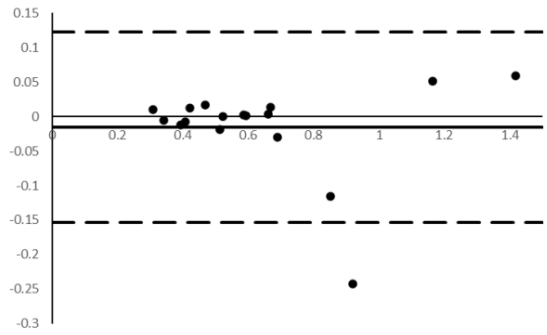
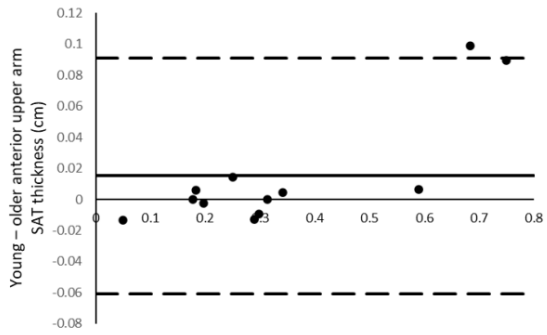
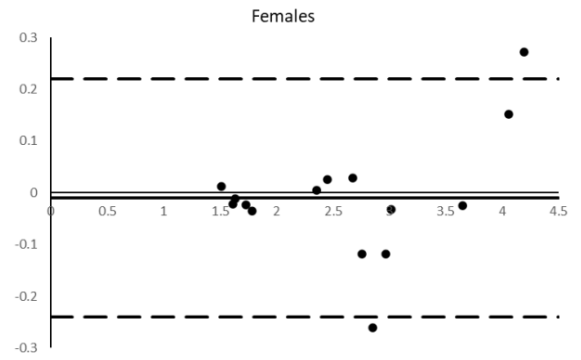
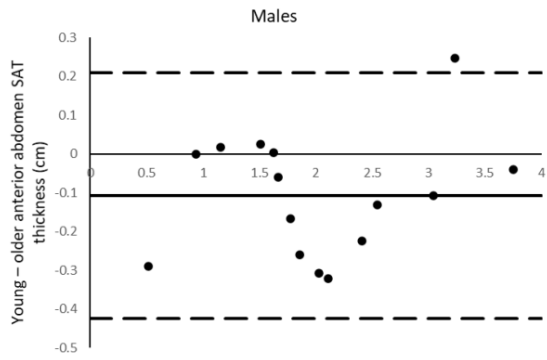
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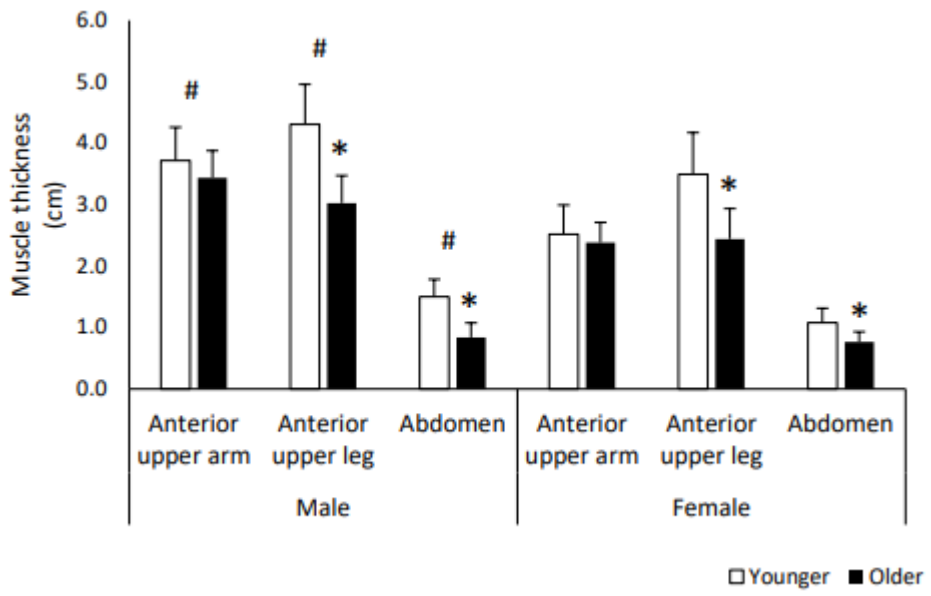
Table 1. Demographic and physical characteristics

	Males		Females		p-value age	p-value sex
	Younger (<45 y)	Older (≥60 y)	Younger (<45 y)	Older (≥60 y)		
Anterior upper arm, n	12	12	17	17	-	-
Age, years	27.4 (5.0)	71.6 (4.3)	28.8 (8.4)	71.8 (6.1)	<0.01	0.56
Height, m	1.74 (0.05)	1.78 (0.06)	1.68 (0.07)	1.60 (0.05)	0.04	<0.01
Weight, kg	80.0 (13.0)	87.3 (6.2)	68.7 (13.8)	67.7 (9.8)	0.10	0.01
BMI, kg/m ²	26.1 (3.1)	27.3 (2.1)	24.5 (5.3)	26.5 (3.6)	0.45	0.26
Body fat, %	22.9 (3.8)	29.3 (4.1)	34.0 (5.4)	40.8 (4.8)	<0.01	<0.01
ALTI, kg/m ²	8.75 (1.18)	7.57 (0.63)	6.28 (1.03)	5.69 (0.66)	<0.01	<0.01
SAT thickness, cm	0.35 (0.23)	0.34 (0.20)	0.63 (0.30)	0.65 (0.30)	0.89	<0.01
Anterior upper leg, n	11	11	15	15	-	-
Age, years	29.0 (6.8)	72.8 (5.5)	29.0 (6.8)	72.8 (5.5)	<0.01	0.85
Height, m	1.74 (0.07)	1.78 (0.07)	1.65 (0.08)	1.60 (0.05)	0.21	<0.01
Weight, kg	75.6 (10.2)	83 (10.2)	68.1 (15.6)	65.8 (10.2)	0.15	0.12
BMI, kg/m ²	24.9 (2.2)	26.3 (3.0)	24.9 (2.2)	26.3 (3.0)	0.41	0.91
Body fat, %	22.4 (4.1)	28.0 (5.5)	22.4 (4.1)	28.0 (5.5)	0.02	<0.01
ALTI, kg/m ²	8.16 (0.82)	7.36 (0.65)	6.54 (1.07)	5.71 (0.59)	0.03	<0.01
SAT thickness, cm	0.67 (0.40)	0.71 (0.39)	1.27 (0.63)	1.27 (0.60)	0.88	<0.01
Abdomen, n	15	15	15	15	-	-
Age, years	27.6 (6.5)	74.7 (6.3)	29.5 (8.7)	71.9 (5.9)	<0.01	0.46
Height, m	1.77 (0.06)	1.78 (0.06)	1.67 (0.06)	1.60 (0.04)	0.43	<0.01
Weight, kg	81.0 (10.3)	84.1 (12.2)	69.9 (13.7)	63.4 (7.2)	0.45	<0.01
BMI, kg/m ²	25.9 (3.0)	26.3 (2.8)	25.1 (5.4)	24.9 (2.8)	0.78	0.56
Body fat, %	21.7 (4.7)	28.4 (4.7)	34.1 (6.0)	39.2 (5.1)	<0.01	<0.01
ALTI, kg/m ²	8.83 (1.14)	7.32 (0.80)	6.47 (1.09)	5.42 (0.51)	<0.01	<0.01
SAT thickness, cm	1.96 (0.90)	2.06 (0.86)	2.61 (0.90)	2.62 (0.85)	0.73	0.05

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







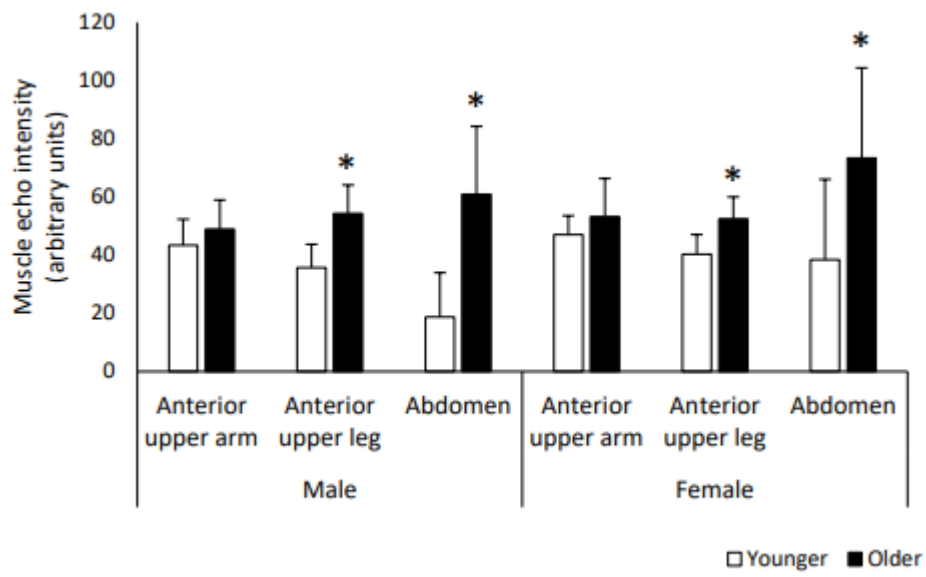


FIGURE CAPTIONS

Figure 1. Representative images depicting muscle thickness, adipose tissue thickness, and muscle echo intensity analysis of younger (upper panel) and older (lower panel) for the A/D) anterior thigh, B/E) anterior upper arm, and C/F) abdominal landmarks. Vertical lines indicate assessment of muscle and adipose tissue thickness. Dashed boxes indicate area analyzed for muscle echo intensity.

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

Figure 3. Muscle echo intensity of older adults and younger adults. Data and error bars are presented as mean and standard deviation. *main effects compared with younger adults. #main effects compared with females.

Figure 4. Muscle thickness of older adults and younger adults. Data and error bars are presented as mean and standard deviation. *main effects compared with younger adults. #main effects compared with females.