Title: Influence of subcutaneous adipose tissue and skeletal muscle thickness on rectus femoris echo intensity in younger and older males and females

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Short running title: Adipose tissue on muscle echo intensity

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ABSTRACT

Objectives: Ultrasound measurements of muscle echo intensity are commonly used as a surrogate for muscle composition (e.g., intramuscular adipose tissue). However, given that soundwaves are increasingly attenuated with tissue depth, the interpretation of echo intensity may be confounded by adipose and skeletal muscle thickness. Our objectives are to compare the associations between adipose or muscle tissue thickness and rectus femoris echo intensity in younger and older males and females.

Methods: Participants included in this analysis were derived from 3 previously published cohorts of younger (<45 years) and older (≥60 years) males and females. Ultrasound images of the rectus femoris were evaluated for muscle thickness, echo intensity, and subcutaneous adipose tissue thickness.

Results: Older adults (n: 49 males, 19 females) had a higher body mass index (p=0.001) compared with younger adults (n: 37 males, 49 females). Muscle thickness was negatively associated with echo intensity in older males (r=-0.59) and females (r=-0.53), whereas no associations were observed in younger males (r=0.00) or females (r=-0.11). Subcutaneous adipose tissue thickness displayed no associations with echo intensity in any group.

Conclusions: Despite the known influence of subcutaneous adipose tissue thickness on beam attenuation, we observed no association with muscle echo intensity, indicating that adipose tissue correction may be required to better understand muscle echo intensity across differences in adiposity. The negative associations between muscle thickness and echo intensity in older, but not younger adults, suggests these associations may be related to the co-occurrence of skeletal muscle atrophy and intramuscular adipose tissue infiltration with advancing age.

Keywords: Ultrasound, muscle thickness, adipose tissue thickness, echo intensity, adipose tissue echo intensity

INTRODUCTION

Ultrasound is increasingly being utilized to assess skeletal muscle mass and composition (degree of intramuscular adipose tissue infiltration) in research and clinical settings.[1,2] While muscle thickness and cross-sectional area measured using ultrasound have been well-established as valid metrics of skeletal muscle mass,[3–5] the analysis of muscle composition is less well understood. Ultrasound provides surrogates of skeletal muscle composition through the analysis of echo intensity, which is the mean pixel intensity (pixel brightness) of a region of interest selected within the muscle fascia borders on an ultrasound image.[6] Typically, healthy young muscle appears hypoechoic (darker pixels), however, with infiltration of intramuscular adipose tissue, skeletal muscle presents as hyperechoic (brighter pixels), increasing the average echo intensity.[7,8] However, increased echo intensity can also relate to several other physiological features of the muscle (e.g., fibrotic tissue, inflammation) and should therefore be interpreted cautiously.[2]

Several publications have demonstrated that skeletal muscle echo intensity is positively associated with intramuscular adipose tissue using computed tomography and magnetic resonance imaging.[9–12] Furthermore, older adults typically display elevated skeletal muscle echo intensity compared to younger adults,[7,13,14] which has been associated with reduced muscle strength,[14,15] power,[16] and cardiorespiratory fitness.[17] While skeletal muscle echo intensity has the potential to be used as a surrogate of muscle composition, there are several factors that confound its interpretation. Well-established confounders of muscle echo intensity, such as gain, can be standardized within a single study, however, the influence of participant characteristics, such as muscle and subcutaneous adipose tissue thickness, are less well understood.[6] As the ultrasound beam travels through the underlying tissues, it becomes attenuated in deeper tissues due to absorption, scattering, and reflection of the soundwave.[18] Therefore, the attenuation of the ultrasound beam due to subcutaneous adipose tissue or skeletal muscle thickness may artificially shift pixel intensities, and therefore echo intensity, towards lower values. Recently, Varanoske et al. (2020)[19] demonstrated that the superficial region of the vastus lateralis muscle displayed higher echo intensity compared to the deeper regions in young males. Furthermore, vastus lateralis muscle thickness, but not the subcutaneous adipose tissue thickness, was negatively associated with muscle echo intensity.[19] These results suggest that the thickness of the muscle may confound interpretation of echo intensity; however, these analyses were limited to young, resistance trained males, limiting our understanding of these tissues in females and older adults.

The primary purpose of this study was to evaluate the associations between subcutaneous adipose tissue and skeletal muscle thickness and echo intensity of the rectus femoris in a cohort of younger and older males and females. As secondary objectives, we evaluated echo intensity differences in the superficial and deep regions of the rectus femoris and associations between adipose tissue thickness and echo intensity. We hypothesized that adipose tissue thickness would be positively associated with skeletal muscle echo intensity in all age groups, but skeletal muscle thickness would be negatively associated with echo intensity in only the older adult groups.

METHODS

Study design and participants

This study is a secondary analysis of participants from previously published work that aimed to 1) validate ultrasound to predict appendicular lean tissue mass,[3] 2) evaluate the influence of ultrasound image resolution on muscle composition,[20] and 3) examine site-specific differences in skeletal muscle thickness and echo intensity. Participants were stratified by sex and age (younger adults: <45 years of age; older adults: ≥60 years of age) and rectus femoris muscle thickness, rectus femoris echo intensity, and subcutaneous adipose tissue thickness were evaluated. Participants were excluded if they had: 1) a previous history of neuromuscular disorders, 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 cancer or cerebrovascular disease. Participants were instructed to refrain from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to laboratory visits. All studies were approved by a human research ethics committee at the University of Waterloo. Written informed consent was obtained from all participants in accordance with established protocols for human research.

Ultrasound landmarking, acquisition, and analysis

Landmarking was performed with participants laying supine on a table, with their feet secured in neutral rotation using a foot strap. A flexible tape measure was used to mark two-thirds the distance from the anterior superior iliac spine to superior pole of the patella. Landmarking was performed on the right side. Participants remained supine for 20 minutes prior to image acquisition to mitigate shifts in fluid distribution.[21]

Transverse images of the anterior upper leg were captured using a real-time B-mode ultrasound device (m-turbo, Sonosite, Markham, ON) equipped with a multi-frequency linear array transducer (L38xi: 5-10 MHz). Imaging mode was set to “resolution” and adjustable parameters gain, time-gain-compensation, and dynamic range (50%) were held constant across all participants. The ultrasound transducer was coated with a generous amount of water-soluble transmission gel to obtain minimal compression. Minimal compression was confirmed by ensuring that as: 1) a visible layer of ultrasound gel was maintained between the skin and probe surface, and 2) the natural curvature of the skin, adipose, and muscle tissue was maintained. Image depth was adjusted as needed to obtain a complete view of the muscle being analyzed. Ultrasound images were transferred to a personal computer for analysis.

Muscle thickness was measured by obtaining the perpendicular distance between the superior and inferior muscle fascia of the rectus femoris (Figure 1). Rectus femoris muscle echo intensity was evaluated by selecting the largest rectangular area within the fascia borders (Figure 1), as previously described.[22] The echo intensity derived from the largest rectangular box, denoted as full echo intensity, was further split into the superficial (top half) and deep (lower half) echo intensity. Subcutaneous adipose tissue thickness was evaluated as the perpendicular distance between the inferior border of the skin and the superior border of the rectus femoris muscle fascia (Figure 1). Subcutaneous adipose tissue echo intensity was measured by selecting the area of adipose tissue below the deep to the skin and superficial to the muscle fascia, using the polygon tool (Figure 1). Muscle thickness and echo intensity, and adipose tissue thickness and echo intensity were all measured a single time by a single trained investigator using ImageJ (NIH, Bethesda, MD, version 1.53e).

Statistical analysis

Normality of continuous variables was confirmed using Shapiro-Wilk test. Demographic and body composition differences between age and sex groups were evaluated using a two-way ANOVA. A paired samples t-test was used to evaluate differences in the superficial and deep echo intensity within the age and sex cohorts. Pearson correlation coefficient was used to examine the associations between full rectus femoris echo intensity and muscle or adipose tissue thickness. All statistical analyses were performed using SPSS (version 27, IBM, USA). Statistical significance was set as $p<0.05$.

RESULTS

Older adults (n: 49 males, 19 females) had a higher BMI ($p=0.001$) compared with younger adults (n: 37 males, 49 females) (Table 1). Males were significantly taller ($p<0.001$) and heavier ($p<0.001$) compared to females, but there were no differences in age ($p=0.358$) (Table 1).

The rectus femoris muscle was significantly thicker in the younger compared to older adults ($p<0.001$) and in males compared to females ($p<0.001$) (Table 2). Conversely, subcutaneous adipose tissue was thicker in the older compared to younger adults ($p<0.001$) and in females compared to males ($p<0.001$); however, given the significant age x sex interaction ($p<0.001$), the larger adipose thickness in older adults was driven by the female participants (Table 2). Full region, superficial, and deep muscle echo intensity were significantly higher in the older compared to younger adults ($p<0.001$), with no influence of sex ($p>0.05$) (Table 2). Echo intensity was significantly lower in the deep vs. superficial region in younger males ($p<0.001$) and females ($p<0.001$); however, no regional differences in echo intensity were present in older males or females (Table 2).

In younger adults, rectus femoris muscle thickness was not associated with full region echo intensity in males ($r=0.00$, $p=0.991$; Figure 2A) or females ($r=-0.11$, $p=0.451$; Figure 2B). Whereas in older adults, rectus femoris muscle thickness was negatively associated with full region echo intensity in males ($r=-0.59$, $p<0.001$; Figure 2C) and females ($r=-0.53$, $p=0.020$; Figure 2D). Subcutaneous adipose tissue thickness was not associated with full region echo intensity for younger males (r=0.01, p=0.951; Figure 3A), younger females (r=-0.10, p=0.491; Figure 3B), older males (r=0.09, p=0.541; Figure 3C), or older females (r=0.33, p=0.170; Figure 3D). Subcutaneous adipose tissue thickness was negative associated with adipose tissue echo intensity in younger females (r=-0.54, p<0.001; Figure 4B), older males (r=-0.55, p<0.001; Figure 4C), and older females (r=-0.63, p<0.001; Figure 4D), but not younger males (r=-0.25, p=0.136; Figure 4A).

DISCUSSION

Here, we observed that rectus femoris muscle echo intensity is negatively associated with muscle thickness in older, but not younger, adults. However, the subcutaneous adipose tissue thickness was not associated with rectus femoris muscle echo intensity in any adults, regardless of age or sex. Further, adipose tissue thickness was negatively associated with adipose tissue echo intensity in all groups, except for younger males. When we divided the echo intensity region of interest in half to delineate superficial and deep regions, we observed that echo intensity was significantly lower in the deep region compared to the superficial region in younger adults only.

Ultrasound has emerged as a potentially useful, non-invasive tool for evaluating muscle composition (i.e., the degree of non-muscle tissue infiltration),[1] but a more thorough understanding of its limitations is critical for accurate interpretation. Several groups have demonstrated that the subcutaneous adipose tissue layer attenuates the ultrasound beam, thereby lowering the average pixel intensity of deeper tissues and confounding measurements of skeletal muscle echo intensity.[6,9,23,24] Haberkorn et al. (1993)[23] first experimentally demonstrated that layering excised pig subcutaneous adipose tissue overtop of a phantom mimic resulted in a decrease in mean phantom echo intensity. Recently, Muller et al. (2020)[24] performed a muscle-focused follow up study examining the influence of increasing thickness of pig subcutaneous adipose tissue (0.4 to 3 cm) on tibialis anterior skeletal muscle echo intensity in younger males and females. Interestingly, they observed strong associations between increasing adipose tissue thickness and decreases in echo intensity at the tibialis anterior (r= -0.83), confirming that beam attenuation occurs to a large extent with increasing adipose tissue thickness. Furthermore, Young et al. (2015)[9] found that correcting raw muscle echo intensity values for the thickness of the subcutaneous adipose tissue (through an adjustment factor), provided stronger associations with magnetic resonance imaging derived measurements of intramuscular adipose tissue. Here, we further observed negative associations between adipose tissue thickness and subcutaneous adipose tissue echo intensity, indicating that thicker adipose tissue is attenuating the ultrasound energy in deeper tissues. Taken together, these data demonstrate that increased subcutaneous adipose tissue thickness can artificially decrease skeletal muscle echo intensity values due to beam attenuation in deeper tissues.

Despite the experimentally demonstrated attenuation of echo intensity in deeper tissues, we observed no associations between subcutaneous adipose tissue thickness and rectus femoris echo intensity across older and younger males and females. Several others have also observed a similar null or weak associations between muscle echo intensity and subcutaneous adipose tissue thickness across both younger and older males and females.[14,25–27] Given the clear demonstration of adipose tissue causing attenuation of the ultrasound beam in deeper tissues, the lack of associations with muscle echo intensity could be due to the poorer muscle composition in individuals with higher amounts of subcutaneous adipose tissue.[28] In other words, the beam attenuating influences of subcutaneous adipose tissue on muscle echo intensity may be offset by increased infiltration of intramuscular adipose tissue in obese individuals. Therefore, our hypothesized positive correlations between adipose tissue thickness and muscle echo intensity were likely not observed because of increased ultrasound beam attenuation in those individuals with thicker adipose tissue.

To better interpret skeletal muscle echo intensity across a wide range of adiposity, correcting this measure for the amount of subcutaneous adipose tissue may be necessary.[9,24] However, correcting muscle echo intensity for subcutaneous adipose tissue thickness may significantly alter the outcome of interest. For example, if the correlations between muscle echo intensity and glucose homeostasis were explored, correcting echo intensity using current approaches[9,24] may artificially alter the association, as it is well known that adipose tissue thickness is related to glucose control. In these instances, it may be more appropriate to perform multiple-linear regression, with both adipose tissue thickness and echo intensity as independent variables associated with glucose homeostasis. Yet, despite these potential limitations, several publications have observed associations between uncorrected echo intensity and muscle strength,[15,29] function,[16,30] and metabolism,[31] indicating that it may still be a valid metric of muscle composition, but future work exploring its correction is needed to better understand the ideal approaches for its measurement and analysis.

The influence of skeletal muscle thickness on muscle echo intensity is not well understood. In agreement with our observations, Akima et al. (2017)[27] observed that the quadriceps muscle thickness was negatively associated with muscle echo intensity (r= -0.438 to -0.736) in older males and females. Similarly, several others have observed negative associations between muscle echo intensity and muscle thickness in older adults.[26,30,32] However, negative associations between muscle thickness and echo intensity in older adults are not always observed (r= -0.10).[14] Interestingly, Chang et al. (2018)[33] observed a moderate negative association between rectus femoris muscle thickness and echo intensity (r= -0.48) in 140 community dwelling older adults, but weak associations for the biceps (r= -0.18), triceps (r= -0.07), and the gastrocnemius (r= -0.20) muscles. Given that we, and others, have observed negative correlations between muscle thickness and echo intensity in older adults, but not younger adults, suggests these associations may be due to the co-occurrence of skeletal muscle atrophy and intramuscular adipose tissue infiltration, rather than further beam attenuation due muscle thickness. However, Varanoske et al. (2020)[19] observed negative associations (r= -0.59) between the vastus lateralis muscle thickness and echo intensity in young, resistance trained males. While it is not entirely apparent why these discrepancies exist between our results and those of Varanoske et al. (2020),[19] they are potentially related to differences in probe orientation (transverse vs. longitudinal), training status, or muscles evaluated (vastus lateralis vs rectus femoris). However, in a smaller sample of 10 younger males and 10 younger females, Palmer et al. (2015)[34] also observed negative association between muscle thickness and echo intensity in the hamstring muscles (r= -0.63 to 0.11) using panoramic ultrasound. These correlations between skeletal muscle echo intensity and thickness require further clarification within younger adult populations to better understand if skeletal muscle thickness confounds the analysis of muscle echo intensity.

In alignment with the findings of Varanoske et al. (2020),[19] we observed that the superficial echo intensity was significantly greater than the deep echo intensity in the quadriceps muscles of younger adults. Similar results have been observed in the gastrocnemius muscles of younger adults.[35] These results align with the concept that beam attenuation in deeper tissues is altered. However, in older adults, muscle echo intensity was more homogenous, as we observed no differences in the superficial or deep echo intensity. Interestingly, despite the lack of differences in superficial and deep echo intensity in older adults, echo intensity displayed negative associations with muscle thickness, whereas in younger adults, no associations existed between muscle thickness and echo intensity despite significant differences in echo intensity of the superficial and deep regions. However, further studies clarifying these associations are needed, particularly given the discrepant findings in our cohort of younger adults compared to those of Varanoske et al. (2020).[19]

There are several limitations to our current investigation. While we recruited a relatively diverse cohort of older and younger males and females, there are no participants within the middle-aged group, which may further clarify the influence of depth on the associations between echo intensity and muscle composition. Furthermore, our cohort of older females was relatively small (n=19). Only the rectus femoris muscle was evaluated, as this was a common landmark across all participants, which limits the extrapolation of these results across other body parts. These differences may be particularly relevant to muscle groups such as the rectus abdominis, which typically present with much smaller muscle thicknesses and larger subcutaneous adipose thicknesses compared with other landmarks.[36] All of these analyses were performed by a single rater, which may ensure more consistent results across participants, however, it may limit the generalizability of these results to individuals performing these measures using multiple raters. Lastly, these results may be influenced by the ultrasound machine being utilized, as differences in machine hardware (e.g., processing power) and software (e.g., gain) create challenges when comparing results across different equipment.

In conclusion, the rectus femoris muscle thickness was negatively associated with muscle echo intensity in older males and females, but not in younger males or females. Whereas subcutaneous adipose tissue thickness overlying the rectus femoris displayed no associations with muscle echo intensity in either older or younger males and females. Given the influences of adipose tissue on beam attenuation in deeper tissues, the lack of associations between subcutaneous adipose tissue thickness and skeletal muscle echo intensity across both younger and older adults suggests that the beam attenuation may be offset by increased intramuscular adipose tissue. Therefore, correcting for subcutaneous adipose tissue may be necessary, however, future research is needed to understand how corrected echo intensity values relate to skeletal muscle function. Given the negative correlations between muscle thickness and echo intensity in older, but not younger adults, suggests this association may be related to the co-occurrence of skeletal muscle atrophy and intramuscular adipose tissue infiltration.

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Conflicts of interest: The authors declare no conflicts of interest.

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Table 1. Participant physical characteristics

	Males		Females				
	Younger (n=37)	Older (n=49)	Younger (n=49)	Older (n=19)	Age p-value	Sex p-value	Age x sex p-value
Age, y	27.0 (4.5)	74.4 (7.2)	27.5 (7.4)	71.8 (6.2)	<0.001	0.358	0.184
Height, m	1.75 (0.06)	1.74 (0.07)	1.66 (0.07)	1.60 (0.04)	0.003	<0.001	0.021
Weight, kg	78.4 (11.0)	80.8 (12.0)	64.3 (11.3)	67.4 (10.6)	0.160	<0.001	0.845
BMI, kg/m^2	25.5 (3.2)	26.5 (3.5)	23.3 (3.7)	26.3 (3.7)	0.001	0.051	0.111

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

Table 2. Ultrasound body composition characteristics

	Males		Females				
	Younger (n=37)	Older (n=49)	Younger (n=49)	Older (n=19)	Age p-value	Sex p-value	Age x sex p-value
Rectus femoris thickness, cm	1.64 (0.44)	1.37 (0.27)	1.44 (0.48)	0.93 (0.20)	<0.001	<0.001	0.095
Adipose tissue thickness, cm	0.58 (0.38)	0.58 (0.21)	0.94 (0.46)	1.44 (0.44)	<0.001	<0.001	<0.001
Full echo intensity, A.U.	39.7 (9.3)	52.6 (13.7)	37.7 (8.4)	52.5 (14.1)	<0.001	0.580	0.632
Superficial echo intensity, A.U.	45.2 (9.1)	53.4 (12.4)	40.9 (8.9)	52.0 (12.6)	<0.001	0.124	0.415
Deep echo intensity, A.U.	34.1 (11.3)*	51.6 (16.0)	34.4 (10.6)*	52.7 (17.2)	<0.001	0.768	0.863
Superficial – deep echo intensity, A.U.	11.1 (8.8)	1.7 (8.5)	6.4 (10.1)	-0.7 (10.4)	<0.001	0.030	0.498

Adipose tissue echo intensity, A.U.	76.5 (11.6)	72.1 (12.4)	63.3 (16.3)	48.4 (8.8)	<0.001	<0.001	0.023

Data are presented as mean (SD). * indicates a significant difference between deep and superficial echo intensity ($p<0.05$). A.U. arbitrary units.

Figure 1

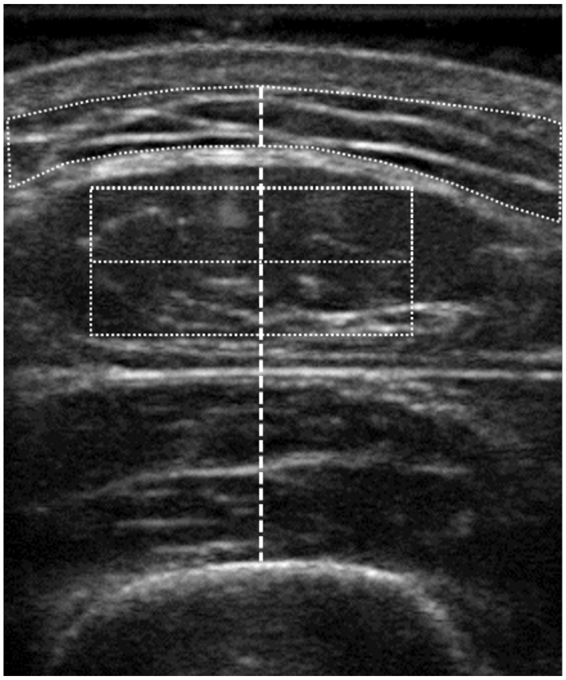

Figure 2

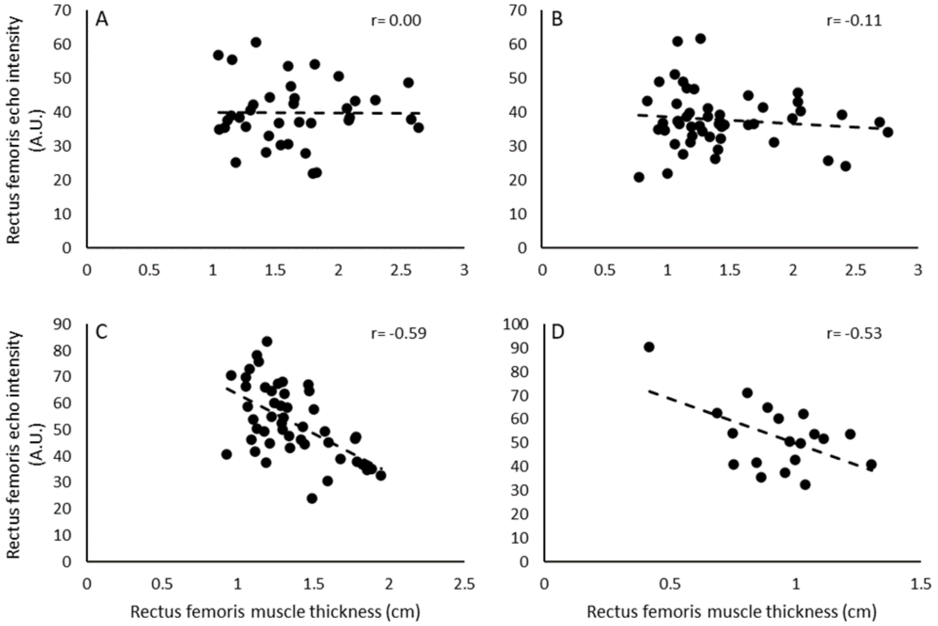
A
r= 0.00
B
r= -0.11
C
r= -0.59
D
r= -0.53
Rectus femoris echo intensity (A.U.)
Rectus femoris muscle thickness (cm)
0
10
20
30
40
50
60
70
80
90
100
0.5
1
1.5
2
2.5
3

Figure 3

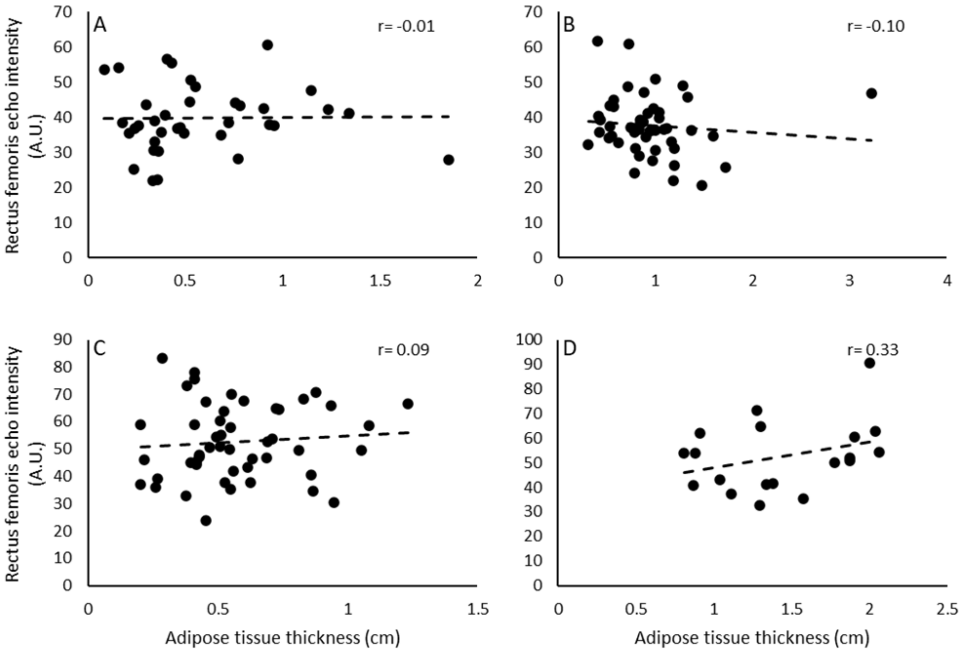
A
r= -0.01
B
r= -0.10
C
r= 0.09
D
r= 0.33
Rectus femoris echo intensity (A.U.)
Rectus femoris echo intensity (A.U.)
Adipose tissue thickness (cm)
Adipose tissue thickness (cm)
0
10
20
30
40
50
60
70
80
90
100
0
0.5
1
1.5
2
2.5
3
4

Figure 4

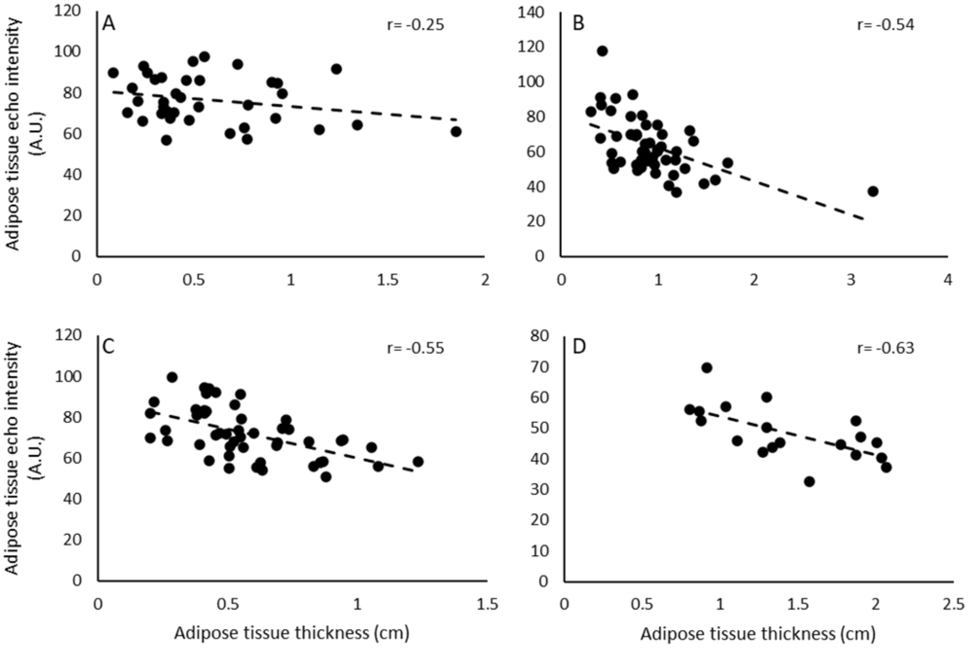
A
r= -0.25
B
r= -0.54
C
r= -0.55
D
r= -0.63
Adipose tissue echo intensity (A.U.)
Adipose tissue echo intensity (A.U.)
Adipose tissue thickness (cm)
Adipose tissue thickness (cm)
0
20
40
60
80
100
120
140
0
0.5
1
1.5
2
2.5
3
4

Figure legends

Figure 1. Analysis of muscle thickness, muscle echo intensity, adipose tissue thickness, adipose tissue echo intensity.

Figure 1. Pearson correlations between rectus femoris echo intensity and muscle thickness for A) younger males, B) younger females, C) older males, and D) older females. A) p=0.991, B) p=0.451, C) p<0.001, D) p=0.020.

Figure 2. Pearson correlations between rectus femoris echo intensity and adipose tissue thickness for A) younger males, B) younger females, C) older males, and D) older females. A) p=0.951, B) p=0.491, C) p=0.541, D) p=0.170.

Figure 4. Pearson correlations between adipose tissue echo intensity and adipose tissue thickness for A) younger males, B) younger females, C) older males, and D) older females. A) p=0.136, B) p<0.001, C) p<0.001, D) p<0.001.