Relationship between quadriceps echo intensity and functional and morphological characteristics in older men and women

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Short title: muscle composition in older individuals

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Abstract

The age-related decrease in human skeletal muscle mass; i.e. sarcopenia, has received much attention, but an age-related increase in the ratio of adipose tissue to muscle tissue has received noticeably less. A few studies have shown that ultrasonographic echo intensity, an index of the adipose-to-muscle ratio, is negatively associated with functional capacity, but the best parameters by which to predict this ratio have not yet been established for older individuals. The purpose of this study was to assess the relationships between quadriceps femoris (QF) echo intensity and demographic, functional and morphological characteristics of older men and women. Sixty-four healthy men (n=27) and women (n=37) aged 62-88 years (72.0 ± 5.0 years) participated in this study. The echo intensity and muscle thickness of the QF were calculated using ultrasonography. Sit-up, supine-up, sit-to-stand, 5-m maximal walk and 6-min walk tests were performed. There were no significant differences in QF echo intensity between sexes, but QF echo intensity was significantly correlated with QF muscle thickness. Stepwise multiple regression analysis with QF echo intensity as a dependent variable revealed QF muscle thickness, sit-to-stand test in men and age, and QF muscle thickness and sit-to-stand test in women, to be significant variables. These results suggest that QF echo intensity can be explained by QF muscle thickness, sit-to-stand and/or age in older men and women; however, an "age" effect was present only in men.

(234 words/max 250 words)

Keywords: intramuscular adipose tissue, functional ability, muscle thickness

Introduction

Aging causes decreases in quantity and quality of skeletal muscle in humans. The age-related decrease in quantity of skeletal muscle is known as sarcopenia and has been widely investigated from the cellular to the organ level (Addison et al., 2014; Akima et al., 2001; Marcus et al., 2012; Sakuma et al., 2014; Trappe et al., 2001). Age-related decrease in the adipose-to-muscle ratio, such as the fatty infiltration of muscle (intramuscular adipose tissue, i.e. IntraMAT), has not been given adequate attention (Marcus et al., 2012). In research on aging, IntraMAT has been identified as a risk factor for metabolic abnormalities (Boettcher et al., 2009; Elder et al., 2004; Goodpaster et al., 2003; Marcus et al., 2010). The most notable negative impact of IntraMAT is insulin resistance, which carries a risk of type2 diabetes (Boettcher et al., 2009; Elder et al., 2004; Goodpaster et al., 2003; Marcus et al., 2010). Another characteristic of IntraMAT is its negative relationship to muscle function in older individuals. Several reports have demonstrated an inverse relationship between the adipose-to-muscle ratio, as evaluated by ultrasonographic echo intensity, and functional abilities in middle-aged and older individuals (Fukumoto et al., 2012; Lopez et al., 2016; Rech et al., 2014; Wilhelm et al., 2014). However, the mechanisms underlying the relationship between echo intensity and the functional, demographic, and morphological characteristics of older individuals are not well understood.

There may be a difference in age-related loss of muscle strength, i.e. dynapenia, between the sexes. According to our cross-sectional study of 164 men and women aged 20 to 84 years, isometric knee extension strength in men in their 70s and

80s was 40% lower than in young men, and women in their 70s and 80s had 23% lower isometric knee extension strength than young women (Akima et al., 2001). Sternäng et al. (Sternäng et al., 2015) also showed similar results for handgrip strength in a 22-year follow-up study. The results of both studies suggest that age-related impairment of muscle performance is larger in men than in women. We speculate that muscle quantity and quality are closely related to the age-related decline in muscle strength. According to Lynch et al. (Lynch et al., 1999), the age-associated change in muscle quantity, fat free mass, between the 50s and 70s is smaller in women than in men (0.5% vs. -4%, respectively). However, it is unknown how the adipose-to-muscle ratio is related to the difference in muscle function between the sexes in older individuals.

Medical imaging techniques such as magnetic resonance imaging (MRI), computed tomography, and ultrasonography have been used to measure muscle quantity and quality. Among these techniques, ultrasonography has the highest benefit cost ratio, and the information it provides is similar to that of MRI (Akima et al., 2016). Our recent study demonstrated that ultrasonographic echo intensity was significantly related to IntraMAT content as determined by MRI, in 30 young and older individuals ($r = 0.485\text{-}0.648$, $P < 0.05$ to 0.01), suggesting that ultrasonography can provide similar information about the adipose-to-muscle ratio as MRI.

The purpose of this study was to assess how ultrasonographic echo intensity can be characterized by demographic, functional, and morphological parameters in older men and women. We hypothesized that echo intensity could be explained by any combination of demographic, functional, and morphological parameters, and that those independent variables would differ between older men and older women.

Materials and methods

Subjects

Sixty-four older individuals (72.9 ± 4.5 years; 27 men; 71.5 ± 5.3 years; 37 women) participated in this study. Subjects' physical characteristics are shown in Table 1. The participants were healthy; habitually physically active; and free of neurological, cardiovascular, and lower-extremity diseases. Before the experiment, the procedure, purposes, risks, and benefits associated with the study were explained and written consent was obtained. This study was approved by the Institutional Review Board of the Research Center of Health, Physical Fitness & Sports at Nagoya University, and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Experimental procedures

Subjects visited the lab on 2 days separated by 1 week. At the first visit, they were interviewed about their daily life habits and physical activity level. The International Physical Activity Questionnaire was administered to gain information on the activity level of participants. At the second visit, demographic, functional and morphological tests were performed. Body composition and ultrasound measurements were performed first after taking rest on a chair for at least 15 min to avoid muscle contraction-induced fluid shifts and muscle blood flow during these measurements (Berg et al., 1993; Wilhelm et al., 2014). Therefore, the first measurement was body composition or echo intensity, subcutaneous adipose tissue and muscle thicknesses. After these measurements were completed, subjects performed functional tests. The order of functional tests was random, but the 6-min walk test was performed last because this test was sometimes expected to induce fatigue.

Clinical measurements

Body weight (kg), body fat mass (kg), %body fat, skeletal muscle mass (kg), and %skeletal muscle were measured using a bioelectrical-impedance device (ITO-InBody370, ITO Co., Ltd., Tokyo, Japan). Eight electrodes producing an electric microcurrent of 20 kHz and 100 kHz were applied to the right hand, left hand, right foot, and left foot. For accuracy result, examiners ensured that subjects' elbow and knee joints were fully extended, and subjects maintained the standing position with legs and arms straight throughout the test. Test-retest reliability was assessed by intra-class correlation coefficient (ICC). ICC (2,1) results for body fat mass, skeletal muscle mass, %body fat and %skeletal muscle were 0.988, 0.991, 0.998 and 0.988, respectively, indicating nearly perfect agreement.

Functional measurements

The subjects performed 5 functional tests in a gymnasium: sit-up, supine-up, sit-to-stand, 5-m maximal walking, and 6-min walk. For the sit-up test, each subject lay supine with knees bent to approximately 80°, feet flat on the floor, and arms crossed on the chest. With the examiner holding the subject's ankle joints, each subject performed as many sit-ups as possible within 30 s. The supine-up test timed each subject going as quickly as possible from the supine position to standing, with no restriction on the manner of getting to standing position. In the sit-to-stand test, subjects were timed performing, as quickly as possible, 10 repetitions of standing from a sitting position with arms crossed on the chest. The 5-m maximal walk test consisted of taping 4 parallel lines on the floor 1 m, 6 m, and 7 m (finish line) from the starting line (0 m). Subjects walked with maximal effort from the starting line toward the finish line, and the time it took to walk between the 1-m and 6-m lines was recorded by the examiner. The 6-min walk test measured the distance walked for 6 min with maximal effort on a 108-m round course. Markers were placed as landmarks every 6 m along the course, and examiners counted the number of laps. Final data were measured in tens of meters. The sit-up, sit-to-stand, and 6-min walks tests were conducted once. The supine-up and 5-m maximal walk tests were conducted twice, and the better result was used further analysis.

Morphological measurement

Real-time B-mode ultrasonography (LOGIQ e; GE Healthcare, Chicago, IL, USA) with an 8–12-MHz linear-array probe (width of probe, 3.8 cm) was used to assess the thicknesses of the muscle and subcutaneous adipose tissue and to measure echo intensity at the anterior and lateral regions of the right mid thigh (Akima et al., 2016; Ando et al., 2015). For the anterior region, thicknesses of the subcutaneous adipose tissue, rectus femoris (RF), and vastus intermedius (VI-ant) were measured. For the lateral region, the thicknesses of subcutaneous adipose tissue, vastus lateralis (VL), and VI (VI-lat) were measured. Ultrasound images were scanned using the following acquisition parameters: frequency 10 MHz; gain 70 dB; depth 4–8 cm; number of focal points 1 (at the top of the image). All measurements for the anterior and lateral mid thigh were carried out with subjects supine. The probe was coated with adequate water-soluble transmission gel to provide acoustic contact without depression of the dermal surface and was aligned perpendicular to the longitudinal axis of quadriceps femoris (QF) to obtain transverse images. Five images were obtained for each measurement site. Ultrasound images were stored in an ultrasound device with Digital Imaging and Communications in Medicine (DICOM) format for future analysis.

Image files were stored on a personal computer. The thicknesses of adipose tissue and muscle and echo intensity were analyzed using ImageJ software (version 1.44; National Institutes of Health, Bethesda, MD). Muscle thickness in the RF and VL was defined as the distance between the superior border of the subcutaneous fascia and the deep aponeurosis. In the VI, muscle thickness was defined as the distance between the inferior border of the superficial aponeurosis and the superior border of the femur (Ando et al., 2015).

We calculated QF muscle thickness and subcutaneous adipose tissue thickness using following equations:

QF MT = (RF MT + VI-ant MT + VL MT + VI-lat MT)/4, and

QF SCAT = (Anterior SCAT + Lateral SCAT)/2

where MT is muscle thickness and SCAT is subcutaneous adipose tissue thickness.

The first step in the analysis of echo intensity involved smoothing to convert the original grayscale of an ultrasound image to 256-value grayscale in ImageJ. The region of interest, which included as much muscle as possible but avoided visible bone and fascia, was determined using polygon selections to calculate echo intensity (Akima et al., 2016; Caresio et al., 2014). Mean grayscale level within a region of interest was used as echo intensity, which was expressed in arbitrary units as a value between 0 and 255 (0 = black and 255 = white). The mean value of echo intensity in 3 images for each muscle was used for analysis.

We calculated QF echo intensity using a following equation:

$$\text{QF echo intensity} = (\text{RF echo intensity} + \text{VL echo intensity})/2$$

Statistical analysis

All values are reported as means and standard deviation. The unpaired samples Student’s t test was used to compare variables for demographic, functional and morphological parameters between groups. Pearson product-moment correlation coefficient (r) was used to determine the association between variables. Stepwise multiple regression analysis was performed, with the QF echo intensity as a dependent variable and age, sex, body mass index (BMI), %body fat, skeletal muscle mass, sit-up, supine-up, sit-to-stand, 5-m maximal walk, 6-min walk, subcutaneous adipose tissue thickness, and QF muscle thickness as independent variables. Twelve independent variables were entered into a forward stepwise regression analysis if they significantly contributed to the explained variance (put in criteria, $P \leq 0.05$; put out criteria, ≤ 0.100; these were the default software settings). To avoid multicollinearity in the analysis, we checked that the variance inflation factor was lower than the criteria (i.e. 10) in all stepwise regression analyses. The level of significance was set at $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for MacOS (version 23.0J, IBM Japan Corp., Tokyo, Japan).

Results

Subjects' demographic, functional and morphological characteristics are shown in Table 1. The men were significantly taller (P < 0.001) and heavier (P < 0.001) than the women. The %body fat was significantly lower (P = 0.01), and skeletal muscle mass and %skeletal muscle was significantly greater (P = 0.001 and P = 0.001, respectively), in men than women. With regard to functional parameters, men performed significantly better than women in the sit-up, supine-up and 5-m maximal walk tests (P = 0.01, P = 0.001, P = 0.036, respectively). Morphological measurements revealed anterior and lateral SCAT thicknesses to be significantly smaller (P = 0.001 and P = 0.001, respectively), and VL, VI-lateral, VL & VI lateral and QF muscle thicknesses (P = 0.009, P = 0.01, P = 0.01, and P = 0.01, respectively) to be significantly greater, in men than in women.

The echo intensities of the RF, VL and QF are shown in Figure 1. We found no significant difference in echo intensity in any muscles between sexes.

Correlation coefficients between the RF, VL and QF echo intensities and demographic and functional parameters are shown in Table 2. For men and women, RF, VL and QF echo intensities were significantly correlated with age (r = 0.369 - 0.600, P = 0.025 to P = 0.001). RF echo intensity was significantly negatively correlated with the body weight (r = -0.357, P = 0.03), BMI (r = -0.382, P = 0.029), and skeletal muscle mass (r = -0.402, P = 0.014) in women.

We observed significant correlation coefficients between echo intensity and some functional tests. RF echo intensity was significantly correlated with the supine-up (r = 0.474, P = 0.013) and sit-to-stand tests in men (r = 0.398, P = 0.044), but only with the sit-to-stand test in women (r = 0.511, P = 0.001). We found VL echo intensity to be significantly correlated with supine-up, sit-to-stand, 5-m maximal walk and 6-min walk tests in men (r = -0.519 to 0.526, P = 0.006 to P = 0.005), but not in women. QF echo intensity was significantly correlated with the supine-up, sit-to-stand and 6-min walk tests for the older men (r = 0.535, 0.492, and -0.470, respectively, P = 0.013 to P = 0.004), but only with the sit-to-stand test in women (r = 0.385, P = 0.019).

Correlation coefficients between the RF, VL, and QF echo intensities and morphological parameters are shown in Table 3. For men and women, significant inverse relationships were found between RF, VL, and QF echo intensities and almost all muscle thicknesses in the anterior and lateral regions (r = -0.329 to -0.750, P = 0.045 to P = 0.001). We observed significant inverse relationships between VL (r = -0.452, P = 0.004) and QF echo intensities (r = -0.400, P = 0.014) and SCAT thickness in women.

Stepwise multiple regression analysis was performed QF echo intensity as the dependent variable and demographic, functional, and morphological variables as independent variables (Table 4). The QF echo intensity was explained by the combination of QF muscle thickness, age, and sit-to-stand test in men (R = 0.875, P = 0.001). In women, QF echo intensity was explained by the combination of QF muscle thickness and sit-to-stand test (R = 0.648, P = 0.001). When the same analysis was applied to all subjects, QF echo intensity was explained by the combination of sit-to-stand test, skeletal muscle mass, and age (R = 0.711, P =0.001).

Discussion

The purpose of this study was to assess the relationships between the adipose-to-muscle ratio, as evaluated by echo intensity, and demographic, functional, and morphological properties in older individuals. We found significant negative correlations between QF echo intensity and QF muscle thickness and sit-to-stand test in both men and women. In men, age was an additional parameter to explain QF echo intensity. These results suggest that QF muscle size and sit-to-stand test were the least explainable parameters of age-related the adipose-to-muscle ratio in both sexes. Furthermore, an age effect on the adipose-to-muscle ratio was limited to older men.

As expected, we found older men to have greater functional capacity, larger muscle mass, and less SCAT than older women, but no sex-related difference in QF echo intensity was observed (Fig. 1), suggesting that the adipose-to-muscle ratio was similar between age-matched older men and women. Wilhelm et al. (Wilhelm et al., 2014) and Rech et al. (Rech et al., 2014) found RF echo intensity in older individuals to be 78.3 ± 12.5 arbitrary units (a.u.) and 89.1 ± 18.0 a.u., respectively, and VL echo intensity to be 74.9 ± 14.8 a.u. and 78.8 ± 17.9 a.u., respectively. It is unclear whether echo intensity differed significantly between older men and older women because those data are reported in two different studies. However, there appears to be no difference in the echo intensity of any muscle between sexes, which is consist with the present study if our speculation was correct. Still, it is important to use caution when comparing echo intensity values between studies because these values are profoundly affected by setting (e.g. gain), scan modes, and by the specific ultrasound device used.

According to simple correlation analysis, the RF, VL and QF echo intensities were significantly correlated with 4 functional tests: the supine-up, sit-to-stand, 5-m maximal walk, and 6-min walk. It is important to note QF echo intensity was associated with decreased functional abilities in all tests. A greater echo intensity represents larger IntraMAT content (Akima et al., 2016), which leads to increased adipose-to-muscle ratio. A similar result was reported by Fukumoto et al. (Fukumoto et al., 2012), who showed the echo intensity of the QF to be negatively associated with muscle strength in middle-aged and older individuals ($N=92$, $r = -0.40$, $P < 0.01$). Similarly, negative correlations have been demonstrated between IntraMAT content, as determined by MRI or computed tomography, and sit-to-stand (Visser et al., 2002), walking speed (Visser et al., 2002) and 6-min walking tests (Marcus et al., 2012; Tuttle et al., 2012).

From a morphological stand point of view, the RF, VL and QF echo intensities were inversely related to muscle thickness. Akima et al. (Akima et al., 2015) used stepwise multiple regression analysis to demonstrate skeletal muscle cross-sectional area to be the only selected variable that was inversely related to the IntraMAT content of the thigh in both older and younger men and women using (older, $R = 0.672$; younger, $R = 0.733$). The present study supports the results of our previous study in terms of the close correlation between echo intensity and muscle size. Also of note is that, in almost all cases, echo intensity did not correlate with either %skeletal muscle or skeletal muscle mass of the whole body (Table 2). This result suggests that the level of echo intensity, i.e. an index of adipose-to-muscle ratio such as IntraMAT, might be related with local cellular circumstances, e.g. muscle contraction-induced fuel demand, contraction-induced heat, heat-induced elevation of mitochondrial-related enzyme activities or adipocytokine secretion from adipose tissue. In fact, faster-than-expected adaptations of echo intensity resulting from 10 weeks of daily walking training (unpublished observation) or 20-week resistance training (Radaelli et al., 2014) have been reported.

As a result of stepwise multiple regression analysis, 2 common parameters, QF muscle thickness and sit-to-stand, were found to predict QF echo intensity in older men and women. QF muscle thickness was inversely associated with the QF echo intensity, suggesting that a smaller QF muscle thickness represents higher echo intensity. In the present study, the sit-to-stand test was the only functional parameter of 5 that was found to be predictive of QF echo intensity. This was reasonable because there was repeated knee extension and flexion during the sit-to-stand test; thus, the QF muscle group contributed greatly to the performance of this test. Of the physical characteristics, age was the only selected parameter in older men and is known to be a factor influencing IntraMAT (Addison et al., 2014; Akima et al., 2015; Ryan and Nicklas, 1999). It is noteworthy that age was not selected in the stepwise multiple regression model in older women. As shown in this study and previous studies, muscle size is inversely related to IntraMAT content (Akima et al., 2015; Ryan and Nicklas, 1999), suggesting IntraMAT content could be influenced by an age-related decrease in skeletal muscle size. According to a cross-sectional study of 308 women and 346 men conducted by Lynch et al. (Lynch et al., 1999), age-associated change in fat free mass between the 50s and 70s was smaller in women (39 kg to 39 kg, 0%) than in men (56 kg to 54 kg, -4%), suggesting that women experienced less muscle atrophy with aging than men. This effect may explain our result of stepwise multiple regression for women. Age-related change in muscle strength was essentially similar to the change in fat-free mass.

When combining older men and women, the stepwise multiple regression analysis revealed that QF echo intensity could be explained by the sit-to-stand test, skeletal muscle thickness, and age. Among the independent variables, the sit-to-stand test contributed the most to explaining the variance of QF echo intensity, followed by skeletal muscle thickness and age, according to the standardized regression coefficients (Table 4). Previous studies have shown aging to increase IntraMAT (Addison et al., 2014; Akima et al., 2015; Rossi et al., 2010; Ryan and Nicklas, 1999), as discussed above; however, this factor is an inevitable biological process. In contrast, the sit-to-stand test and skeletal muscle thickness can be changed by increasing physical activity level or more vigorous physical training, thus preventing sarcopenia, metabolic disorders, or frailty in older individuals. Therefore, our results can greatly impact the direction of intervention for older individuals in future.

In a study of animal aging, the extramyocellular space around the type II fibers was shown to increase with age-related fiber atrophy or loss (Hatakenaka et al., 2001). This increased extramyocellular space around atrophied fibers can be filled with adipocytes as a result of aging and/or decreased physical activity, and the relative area of adipocytes in the extramyocellular space was significantly correlated with the area of adipose tissue, as determined by MRI (Rossi et al., 2010). These observations provide a reasonable explanation for the significant inverse relationship between echo intensity and muscle thickness in the QF muscle group observed in the present study; we previously demonstrated that the echo intensities of the VL and hamstrings are significantly related to extramyocellular lipids in 30 younger and older individuals (r = 0.458 and 0.648, respectively) (Akima et al., 2016), suggesting that a larger extramyocellular space filled with adipocytes results in higher echo intensity. This larger extramyocellular space could be induced by age-related muscle atrophy; in particular, type II fibers (Hatakenaka et al., 2001; Saltin and Gollnick, 1983), leading to smaller QF muscle thickness. Therefore, great deal of attention should be given to aging-induced increases in extramyocellular lipids. It is well known that IntraMAT is decreased with resistance training (Popadic Gacesa et al., 2011; Sipilä and Suominen, 1995; Taaffe et al., 2009). Resistance training may also induce a decrease in the extramyocellular space by hypertrophy of type II fibers, which may lead to decreased accumulation of adipose tissue in the extramyocellular space.

5. Conclusion

The results of this study suggest that QF echo intensity can be explained by QF muscle thickness, sit-to-stand test, and/or age in older men and women. It is important to note that age was predictive in men but not in women. As muscle echo intensity is associated with extramyocellular lipids (Akima et al., 2016), this result suggests extramyocellular lipids may have influence on these explainable variables. Effective interventions can be used to decrease extramyocellular lipids and increasing muscle mass as well as to prevent sarcopenia and dynapenia in older individuals.

Conflict of Interest

All authors declare no conflict of interest.

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Table 1 Demographic, functional, and morphological characteristics of older men and women

	Older men (N = 27)		Older women (N = 37)	
Demographic parameters	Mean ± SD	Range	Mean ± SD	Range
Age (years)	72.9 ± 4.5	65 - 82	71.5 ± 5.3	65 - 88
Height (cm)	164.1 ± 6.2	153.1 - 178.0	152.8 ± 4.8†	140.2 - 165.0
Body weight (kg)	60.6 ± 6.8	50.6 - 76.7	50.1 ± 6.8†	34.2 - 63.1
BMI (kg/m^2)	22.5 ± 1.9	18.7 - 27.0	21.4 ± 2.5	16.0 - 26.4
Body fat (kg)	14.8 ± 3.8	8.9 - 25.6	15.3 ± 4.6	5.3 - 23.4
%Body fat	24.4 ± 4.9	15.4 - 35.8	30.1 ± 6.1†	13.5 - 41.8
Skeletal muscle mass (kg)	25.0 ± 3.1	18.6 - 31.8	18.4 ± 2.1†	12.8 - 23.0
%Skeletal muscle	41.4 ± 2.9	33.5 - 46.6	36.9 ± 3.2†	30.3 - 45.0
Functional parameters				
Sit-up (reps)	10.5 ± 4.4	0 - 21	6.2 ± 5.2**	0 - 16
Supine up (s)	2.8 ± 0.5	2.0 - 4.6	3.5 ± 0.7†	2.3 - 5.3
Sit-to-stand (s)	12.0 ± 3.1	8.0 - 17.5	11.6 ± 3.3	7.9 - 19.6
5-m maximal walk (s)	2.3 ± 0.3	1.9 - 2.9	2.4 ± 0.3*	1.8 - 3.1
6-min walk (m)	617.3 ± 70.2	462 - 735	596.5 ± 48.5	474 - 714
Morphological parameters				
Anterior SCAT (cm)	0.6 ± 0.2	0.2 - 1.1	0.9 ± 0.2†	0.4 - 1.3
RF MT (cm)	1.5 ± 0.4	0.7 - 2.3	1.3 ± 0.4	0.8 - 1.8
VI-anterior MT (cm)	1.5 ± 0.5	0.9 - 2.4	1.3 ± 0.3	0.6 - 2.2
RF&VI-anterior MT (cm)	3.0 ± 0.8	1.7 - 4.6	2.7 ± 0.7	1.5 - 4.0
Lateral SCAT (cm)	0.5 ± 0.1	0.2 - 0.8	0.7 ± 0.3†	0.2 - 1.7
VL MT (cm)	1.8 ± 0.2	1.3 - 2.5	1.6 ± 0.4**	0.8 - 2.3
VI-lateral MT (cm)	1.5 ± 0.4	0.8 - 2.3	1.2 ± 0.4*	0.5 - 2.1
VL&VI-lateral MT (cm)	3.3 ± 0.5	2.2 - 4.3	2.8 ± 0.6**	1.3 - 4.2
QF SCAT (cm)	0.6 ± 0.2	0.2 - 0.9	0.8 ± 0.2**	0.3 - 1.4
QF MT (cm)	3.1 ± 0.6	2.2 - 4.1	2.7 ± 0.6**	1.4 - 3.8

* $P < 0.05$, ** $P < 0.01$, † $P < 0.001$. MT, muscle thickness; RF, rectus femoris; VL, vastus lateralis; QF, quadriceps femoris; SCAT, subcutaneous adipose tissue thickness; VI, vastus intermedius.

Table 2 Correlation coefficients between echo intensity and demographic and functional parameters in older men and women

	RF echo intensity		VL echo intensity		QF echo intensity	
	Older men	Older women	Older men	Older women	Older men	Older women
Demographic parameters						
Age	0.499 **	0.374 *	0.600 **	0.287	0.588 **	0.369 *
Height	0.177	-0.064	0.134	-0.231	0.168	-0.159
Body weight	0.077	-0.357 *	-0.036	-0.290	0.023	-0.359 *
BMI	-0.062	-0.382 *	-0.161	-0.209	-0.120	-0.331 *
Body fat	0.242	-0.235	0.126	-0.202	0.197	-0.242
%Body fat	0.305	-0.118	0.193	-0.110	0.267	-0.125
Skeletal muscle mass	-0.109	-0.402 *	-0.261	-0.318	-0.153	-0.402 *
%Skeletal muscle	-0.355	0.015	-0.176	0.023	-0.330	0.020
Functional parameters						
Sit-up	-0.072	-0.109	-0.245	-0.203	-0.169	-0.171
Supine up	0.474 *	0.058	0.526 *	-0.031	0.535 **	0.019
Sit-to-stand	0.398 *	0.511 **	0.518 *	0.168	0.492 *	0.385 *
5-m maximal walk	0.158	0.257	0.480 *	0.232	0.339	0.271
6-min walk	-0.363	-0.158	-0.519 **	0.009	-0.470 *	-0.086

* P < 0.05; *, P < 0.01. BMI, body mass index; RF, rectus femoris; VL, vastus lateralis; QF, quadriceps femoris.

Table 3 Correlation coefficients between echo intensity and morphological parameters in older men and women

	RF echo intensity		VL echo intensity		QF echo intensity	
	Older men	Older women	Older men	Older women	Older men	Older women
Anterior QF						
SCAT	0.200	-0.293	0.201	-0.379 *	0.215	-0.368 *
RF MT	-0.750 **	-0.550 **	-0.577 **	-0.363 *	-0.713 **	-0.511 **
VI MT	-0.472 *	-0.411 *	-0.586 **	-0.352 *	-0.565 **	-0.424 **
RF&VI MT	-0.643 **	-0.513 **	-0.631 **	-0.385 *	-0.682 **	-0.500 **
Lateral QF						
SCAT	0.083	-0.232	0.118	-0.459 **	0.108	-0.375 *
VL MT	-0.198	-0.294	-0.443 *	-0.303	-0.343	-0.329 *
VI MT	-0.488 **	-0.541 **	-0.457 *	-0.338 *	-0.505 **	-0.492 **
VL&VI MT	-0.498 **	-0.510 **	-0.592 **	-0.389 *	-0.583 **	-0.501 **
Total						
QF SCAT	0.164	-0.283	0.173	-0.452 **	0.180	-0.400 *
QF MT	-0.665 **	-0.576 **	-0.706 **	-0.438 **	-0.734 **	-0.565 **

* $P < 0.05$; ** $P < 0.01$. MT, muscle thickness; RF, rectus femora's; SCAT, subcutaneous adipose tissue; VI, vastus intermedius; VL, vastus lateralis.

Table 3 Stepwise multiple regression analysis as a dependent variable of echo intensity of the quadriceps femoris

Group	Dependent variable	Independent variables	Regression coefficient	SE	Standardized regression coefficients	P	R	Adjusted R2
Older men	QF echo intensity	QF MT	-6.854	1.857	-0.436	0.01	0.875	0.734
		Age	0.816	0.213	0.437	0.01		
		Sit-to-stand	1.016	0.300	0.364	0.01		
Older women	QF echo intensity	QF MT	-7.013	1.964	-0.495	0.01	0.648	0.383
		Sit-to-stand	0.845	0.337	0.347	0.05		
Total	QF echo intensity	Sit-to-stand	1.243	0.275	0.479	0.001	0.711	0.472
		Skeletal muscle mass	0.798	0.224	-0.379	0.01		
		Age	0.586	0.176	0.355	0.01		

Independent variables: age, BMI, % body fat, skeletal muscle, sit-up; supine-up, sit-to-stand, 5-m maximal wal,; 6-min walk; muscle thickness. BMI, body mass index; MT, muscle thickness; QF, quadriceps femoris, SE, standard error.

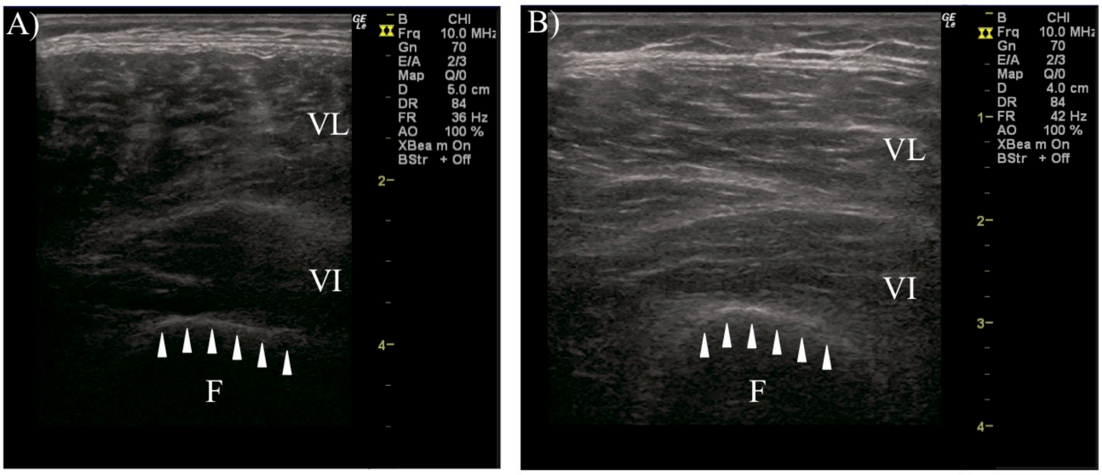
Legend

Fig. 1 Representative ultrasound images of lateral thigh in two older men who showed the lowest (left) and highest (right) echo intensities, respectively, among the older men.

F, femur; VI, vastus intermedius; VL, vastus lateralis.

White triangles represent the edge of femur.

Fig. 2 Echo intensity of the rectus femoris (RF), vastus lateralis (VL), and quadriceps femoris (QF) in the older men and women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Gn 70
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D 5.0 cm
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FR 36 Hz
AO 100 %
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BStr + Off
VL
VI
F
B)
B CHI
Frq 10.0 MHz
Gn 70
E/A 2/3
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AO 100 %
XBea m On
BStr + Off
VL
VI
F

Fig. 1

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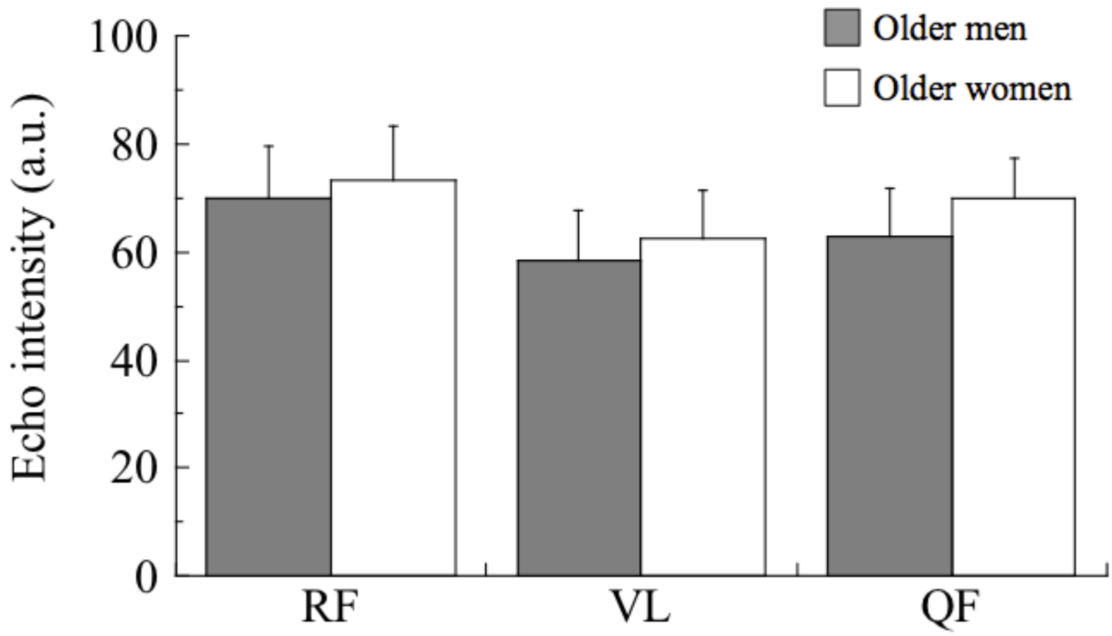

Older men
Older women
Echo intensity (a.u.)
100
80
60
40
20
0
RF
VL
QF