MUSCLE ECHOGENICITY AND CHANGES RELATED TO AGE AND BODY MASS INDEX

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Clinical Relevance: Echogenicity, which is a practical, low-cost and specific method, can be used in clinical practice to assess muscle functionality.

ABSTRACT

INTRODUCTION: Muscle fibers are lost and replaced by fat and fibrous tissue infiltration during aging. This process decreases muscle quality and influences tissue appearance on ultrasound images over time. Increased muscle "echogenicity" represents changes caused by fat and fibrous tissue infiltration and can be quantified with recently developed software. **OBJECTIVE**: To investigate skeletal muscle quality through echogenicity estimates according to participant body mass index (BMI) and age. **METHODS**: This was a cross-sectional study performed at the Pennington Biomedical Research Center, Baton Rouge, Louisiana with 117 participants (57 men and 60 women), with mean age (X±SD) 38.9±17.0 years and BMI 28.6±6.2 kg/m². All participants were examined by ultrasound (GE LOGIC®), using a 5.0 MHz linear transducer. Participants had muscle thickness measured by ultrasound at 4 anatomic locations (biceps and triceps brachial, femoral quadriceps and calf triceps). Echogenicity was analyzed with specific software (Pixel Health®) that evaluated image gray scale. **RESULTS**: According to BMI, 41% of participants were obese. There was a positive correlation between age and thigh muscle echogenicity ($r_p = 0.534$; $p < 0.0001$) and a negative correlation between thigh muscle echogenicity and thickness ($r_p = -0.395$; $p < 0.0001$). In addition, there was high muscle echogenicity in participants with overweight and obesity age 50 years or older ($p<0.05$). **CONCLUSION**: Older age and higher BMI were associated with stronger echogenicity signals and smaller muscle thickness. People with overweight and obesity and/or people older than age 50 years have reduced muscle quality with smaller muscle thickness as observed with ultrasound.

INTRODUCTION

By the age of 80 healthy people lose about 30-40% of their fat-free mass and have a 20% of decline in cross-sectional area of their skeletal muscles; acute or chronic diseases can further increase this muscle loss.(1–3) Structural changes occur in skeletal muscles with some disease states and during the aging process, when muscle fibers, mainly type IIs, are lost and replaced by adipose and fibrous tissue infiltration.(2,4–7) The replacement of skeletal muscle fibers decreases muscle quality and increases density and thereby increases echogenicity on ultrasound images.(7–10)

In medical practice, ultrasound has been used since the early 1950s.(5) Ultrasound is based on echo reflections and represents a two-dimensional gray-scale image that ranges in echogenicity between relatively light strongly reflected echoes and dark non-reflected echoes that identify borders of the skin-subcutaneous fat, fat-muscle and muscle-bone interfaces.(5,11)

Ultrasound evaluations have high spatial resolution, include real-time evaluation with ability to compare to the contralateral site, and are non-invasive, portable, safe and an easy to use imaging method.(11–14) However, interpretation of ultrasound images are more difficult and subjective because of a lack of standardized procedures and measurements.(11)

Increased muscle density or echogenicity represents changes caused by adipose and fibrous tissue infiltration and these effects can be analyzed by pixel gray-scale.(1,2,7,9,15–17)

The objective of the present study was to investigate skeletal muscle quality through echogenicity estimates and its association with body mass index (BMI), age, and body composition.

MATERIALS AND METHODS

Study Design and Subjects

A cross-sectional study was performed in 117 healthy volunteer participants (57 men and 60 women) from August 2013 to January 2014 at Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, USA. The sample was recruited through advertisements in the local community and by word of mouth. Participants aged less than 18 years old and adults with chronic diseases were excluded from the study.

The study was exploratory with the aim of acquiring a sample >100 participants for echogenicity evaluation across the full adult lifespan. The study was approved by the institutional Ethics Committee and all the volunteers signed the written consent.

Anthropometric Measurements

In order to determine height (m), a stadiometer (with total height of 2.0 m and precision of 1.0 mm) was used, duly posted on the wall, with the patient standing, barefoot, with their heels together, with the back straight and arms outstretched at the sides of the body. The measurement of weight (kg) was performed with a calibrated scale, with the participant standing in the center of the scale base, barefoot and wearing light clothing. The magnitude of height and weight were measured twice; a third measurement was acquired only when the first two differed > 0.5 cm or > 0.5 kg, respectively. Results were averaged.

BMI was used to classify nutritional status of the adult participants as: < 16 kg/m^2: malnutrition grade III; 16 – 16,9 kg/m^2: malnutrition grade II; 17 – 18,4 kg/m^2: malnutrition grade I; 18,5 – 24,9 kg/m^2: normal; 25 – 29,9 kg/m^2: overweight;30 – 34,9 kg/m^2: obesity grade I; 35 – 39,9 kg/m^2: obesity grade II; ≥ 40 kg/m^2: obesity grade III.(18,19)

Body Composition

All participants were evaluated with dual X-ray absorptiometry (DXA) (iDXA, GE, USA) and bioelectrical impedance analysis (BIA) (MC980, Tanita Corp, Tokyo, Japan). Two DXA scans were made on each participant. The coefficients of variation (CVs) for appendicular lean mass and total lean mass were both <2%. Any DXA scans that had artefacts rendering them unreadable were excluded from the statistical analysis. The first scan was used for analysis unless it was excluded because of an artefact when the second scan was used instead. The MC980 BIA system had an 8-electrode configuration that separately captured each arm and leg along with trunk and right and left-body electrical properties. Appendicular lean mass, total lean mass, and total skeletal muscle mass were derived by the MC980 software.

Participants were classified as sarcopenic or non-sarcopenic by DXA criteria based on appendicular lean mass/ht^2, male <7.0 kg/m^2 and female ≤ 5.0 kg/m^2, and by BIA criteria based on total muscle mass/ht^2, male ≤ 8.5 kg/m^2 and female ≤ 5.75 kg/m^2. (20) Both DXA and BIA criteria were used to screen participants for sarcopenia.

Ultrasound

All participants were examined by ultrasound (GE LOGIC®), using a 5.0 MHz linear transducer. The quadriceps femoris and calf triceps, in the lower limb, and the biceps brachii and brachialis muscles in the upper limb, were the sites chosen for evaluation. The biceps brachii and brachialis muscles were measured in one thickness area only. A single reading was taken at each site and read in duplicate by the expert ultrasound technician; results were averaged unless the two readings differed at which point a third measurement was taken.

The right side was evaluated by a single experienced physician examiner, who obtained the mean of three ultrasound measurements performed in the same place in both the longitudinal and transverse planes. The subject rested in the supine position prone and relaxed, with arms and legs fully extended during the measurements. To standardize the measurements, the probe was held perpendicular to the corresponding bone. Water-soluble transmission gel provided acoustic contact without depression of the skin surface while the probe was placed perpendicular to the tissue interface under the marked sites.

The ultrasound probe was placed at the position of maximum circumference. In the upper limbs, the location of the muscle measurement was taken 15 cm from the humeral head, then distally on the ventral and intermediate area of the biceps muscle. In the lower limbs, the positions of muscle tissue measurements were performed 15 cm from the superior pole of the patella on the quadriceps muscle in the ventral, mid-line of the thigh. Measurement of calf triceps was taken in the position of maximum circumference in the mid-line.

Echogenicity was analyzed using a computer assisted gray-scale analysis (Pixel Health®, Uezima, Brazil). A region of interest was selected in the transverse ultrasound image in each muscle without any bone or surrounding fascia. The mean echo intensity of this region was calculated. (**Figure 1**) The ultrasound images were analyzed for echogenicity off-line and low-quality scans that were technically inadequate were excluded from further evaluation. The software generated a single echogenicity value for each image.

Statistical Methods

Categorical variables were described by absolute and relative frequencies. The quantitative variables were described using mean and standard deviation or median, minimum and maximum according to the distribution of normality of the data evaluated by the Kolmogorov-Smirnov test. We evaluated the correlations between quantitative variables using Spearman's test, Mann-Whitney`s and/or Kruskal-Wallis tests and Dunn's test were used for comparison between groups.

All analyses were carried out with SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and p-values less than 0.05 were considered statistically significant.

RESULTS

The sample included 117 participants, 51% of whom were female. The mean (±SD) sample age was 38.9±17.0 years; 11% (13/117) were over the age of 65 years. BMI distribution detected 1.6% with malnutrition, 26.4% as normal, 38% as overweight, and 34% were obese; the sample BMI was 28.6±6.2 kg/m^2. None of the participants had sarcopenia based on either DXA or BIA criteria.

Ultrasound measurements and echogenicity are presented in **Table 1**. Due to technical issues not all the images had echogenicity evaluated and thus the sample described in the following section have different participant numbers.

There was a significant difference in thigh muscle thickness in elderly (age >65 years) and non-elderly participants (30±9 mm and 39±10 mm respectively, $p=0.002$). BMI was similar between elderly and non-elderly participants.

Echogenicity was positively associated with age, being higher in elderly people. We found a significant difference between elderly and non-elderly people in thigh echogenicity ($p<0.001$; **Table 2**)

Related to BMI, using Dunn's multiple comparisons, we found a significant thigh echogenicity difference when we compared participants who were overweight/obese and normal weight ($p<0.05$). This higher BMI group had higher thigh echogenicity. This observation is also shown in **Table 3** according to BMI group.

We found a positive correlation between thigh echogenicity and age ($r_s=0.5$; $p<0.001$), but thigh echogenicity had a negative correlation with total lean mass evaluated by BIA and DXA ($r_s=-0.3$, $p<0.01$; -0.25, $p<0.01$) and with thigh muscle thickness (mm) ($r_s=-0.4$; $p<0.01$). Calf and biceps echogenicity were not significantly correlated with any of these measures.

The results of multiple linear regression and power calculations are presented **Table 4**. Thigh echogenicity lost power in relation to BMI. However, biceps and thigh echogenicity remained significant in association with age.

DISCUSSION

A muscle from a healthy young person has low echogenicity with a dark appearance on ultrasound images; on the other hand, muscle from an old or unhealthy person that has adipose tissue and fibrous tissue infiltration has high echogenicity with a lighter appearance on ultrasound images.(21) Several studies using ultrasound echogenicity and computed tomography density to evaluate muscle at different ages detected more fat and fewer muscle fibers in the elderly.(2,22–24) In our study, people who were elderly had higher echogenicity.(1)

Additionally, thigh echogenicity correlated negatively to total lean mass evaluated by BIA and DXA and with thigh muscle thickness evaluated by ultrasound that were higher in elderly participants. These results could be associated to low muscle fibers and high fat and fibrosis infiltration, as shown in other studies.(2,7,9,15,16)

Participants who were overweight and obese in this study had higher thigh echogenicity with no obvious clinical signs of sarcopenia. There are studies using computed tomography to evaluate the muscle area and muscle quality of people who are obese and sarcopenic and non-sarcopenic, nevertheless there are no studies using ultrasonography in this group of patients.(25–27)

In our study, measurement of easily identified thigh muscles showed the least variation for repeated measurements evaluating muscle and gray scale by ultrasound. Our observation is in agreement with other reports that chose thigh to evaluate muscle, sarcopenia, or muscle loss by ultrasound.(13,16,28–30) As well as in our clinical experience, the ultrasound transversal plane is more practical and convenient to evaluate muscle mass.(13)

The present study had several limitations, including ultrasound measurements by a single examiner in a relatively small sample and inclusion of healthy adults across a wide age range but none of whom had sarcopenia. Future studies need to address these limitations with focus on patients with both malnutrition and sarcopenia.

CONCLUSION

Echogenicity is a practical and safe method for evaluating the amount and quality (echogenicity) of skeletal muscle. The elderly, as compared to young adults, had more muscle loss and fat infiltration leading to higher echogenicity in the current study, an observation that reflects the well-known effects of senescence. Our findings suggest that ultrasound is a promising nutritional assessment tool as the instruments are widely available and are often operated by trained technicians in the clinical settings.

Statement of Authorship

Andrea Z Pereira, Steven Heymsfield and Maria Teresa Zanella equally contributed to the conception and design of the research; Jolene Zheng and Clarissa B Uezima contributed to the acquisition and analysis of the data; Andrea Z Pereira, Steven Heymsfield and Rogério Ruscitto contributed to the interpretation of the data; Maria Cristina Gonzalez, Maria Teresa Zanella, Andrea Z Pereira and Steven Heymsfield drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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Table 1. Biceps, calf, and thigh muscle thickness (mm) and echogenicity (pixel count).

Area	Echogenicity (pixels)				Thickness (mm)			
	N	Median	Min	Max	N	Median	Min	Max
Biceps	117	18,429	1,333	31,280	119	27	17	45
Calf	106	9,363	362	30,250	116	41	6	68
Thigh	105	19,426	4,028	31,045	117	38	19	62

Table 2: Biceps, calf, and thigh, muscle thickness (±SD).

Area	N	Mean
Biceps	119	28.0 ± 6.8
Thigh	117	38.0 ± 10.0
Calf	116	41.0 ± 14.0

Table 3: Echogenicity (pixels) in elderly (≥65 years-old) and non-elderly (<65 years-old) participants.

Echogenicity (pixel)	Elderly (yes or no)	Median (pixels)	Minimum	Maximum	N	p*
Biceps	No	17,896	1333	31,280	104	**0.006**
	Yes	24,997	13,905	30,061	13	

Calf	No	8,849	362	26,325	94	**0.026**
	Yes	12,827	4,864	30,250	12	
Thigh	No	19,140	4,028	30,319	94	**<0.001**
	Yes	26,590	22,109	31,045	11	
*Mann-Whitney's test						

Table 4. Multiple linear regression results.

Echogenicity (pixels)	Factors	Coefficient	Std. Error	Hypothesis Test (Wald)	df	Power observed	p
Biceps	Intercept	20,702	3284	39.7	1		**<0.001**
	Gender (female)	-670	1331	0.25	1	0.078	0.615
	Age (yrs)	172	41	18	1	0.985	**<0.001**
	BMI (kg/m^2)	-297	112	7	1	0.737	**0.008**
Thigh	Intercept	12,254	2,487	24	1		**<0.001**
	Gender (female)	-4,129	885	22	1	0.995	**<0.001**
	Age (yrs)	194	27	52	1	>0.999	**<0.001**
	BMI (kg/m^2)	74.6	88	0.7	1	0.131	0.394
BMI, body mass index; df, degrees of freedom.							

Figure1: Echogenicity(A) and ultrasound thickness measurements(B) of triceps in transverse plane

Figure 1: Echogenicity(A) and ultrasound thickness measurements(B) of triceps in transverse plane