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MUSCULOSKELETAL

# Speed of sound ultrasound: a pilot study on a novel technique to identify sarcopenia in seniors

Sergio J. Sanabria[1] · Katharina Martini[2] · Gregor Freystätter[3] · Lisa Ruby[2] · Orcun Goksel[1] · Thomas Frauenfelder[2] · Marga B. Rominger[2]

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## Abstract

**Objectives** To measure speed of sound (SoS) with a novel hand-held ultrasound technique as a quantitative indicator for muscle loss and fatty muscular degeneration.

**Methods** Both calf muscles of 11 healthy, young females (mean age 29 years), and 10 elderly females (mean age 82 years) were prospectively examined with a standard ultrasound machine. A flat Plexiglas® reflector, on the opposite side of the probe with the calf in between, was used as timing reference for SoS (m/s) and ΔSoS (variation of SoS, m/s). Handgrip strength (kPA), Tegner activity scores, and 5-point comfort score (1 = comfortable to 5 = never again) were also assessed. Ultrasound parameters (muscle/adipose thickness, echo intensity) were measured for comparison.

**Results** Both calves were assessed in less than two minutes. All measurements were successful. The elderly females showed significantly lower SoS (1516 m/s, SD17) compared to the young adults (1545 m/s, SD10; $p < 0.01$). The ΔSoS of elderly females was significantly higher (12.2 m/s, SD3.6) than for young females (6.4 m/s, SD1.5; $p < 0.01$). Significant correlations of SoS with hand grip strength ($r = 0.644$) and Tegner activity score ($r_s = 0.709$) were found, of similar magnitude as the correlation of hand grip strength with Tegner activity score ($r_s = 0.794$). The average comfort score of the elderly was 1.1 and for the young adults 1.4. SoS senior/young classification (AUC = 0.936) was superior to conventional US parameters.

**Conclusions** There were significant differences of SoS and ΔSoS between young and elderly females. Measurements were fast and well tolerated. The novel technique shows potential for sarcopenia quantification using a standard ultrasound machine.

**Key Points**

• *Speed of sound ultrasound: a novel technique to identify sarcopenia in seniors.*
• *Measurements were fast and well tolerated using a standard ultrasound machine.*
• *The novel technique shows potential for sarcopenia quantification.*


**Keywords** Skeletal muscle · Ultrasonography · Aging · Sarcopenia · Adipose tissue

**Abbreviations**

| | |
|---|---|
| 2D | 2-dimensional |
| 3D | 3-dimensional |
| ACR | American College of Radiology |
| AUC | Area under curve |
| BMI | Body mass index |
| CT | Computed tomography |
| MRI | Magnetic resonance imaging |
| ROC | Receiver operating characteristic |
| SoS | Speed of sound |
| US | Ultrasonography |

✉ Marga B. Rominger
Marga.Rominger@usz.ch

1 Computer Assisted Applications in Medicine, ETH Zurich, Sternwartstrasse 7, 8092 Zurich, Switzerland

2 Institute of Diagnostic and Interventional Radiology, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

3 Department of Geriatrics and Aging Research, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

## Introduction

A decrease in muscle mass (sarcopenia), and an increase in subcutaneous and intermuscular (myosteatosis) adipose tissue, are both linked to age-, cancer-, and chronic disease-related changes [1–5]. In the last years, the sarcopenia definition has expanded to not only include low muscle mass but also reduced physical function [6, 7]. Sarcopenia leads to frailty, falls, fractures, and ultimately mortality [8, 9]. In the United States, the direct health care cost attributable to sarcopenia in 2000 was estimated at $18.5 billion [10]. In the Netherlands, low skeletal muscle mass was independently associated with increased hospital costs of about €4000 per patient [4].

Non-imaging diagnostic techniques for sarcopenia include questionnaires, physical performance (e.g., grip strength), anthropometry (e.g., body mass index), bioelectrical impedance analysis, as well as serum and urinary biomarkers. The most common imaging diagnostic techniques to evaluate body composition are Dual X-Ray Absorptiometry, CT, MRI and ultrasound (US). Well-known attributes of US include low cost, ease of use, and accessibility. Thus far, regional US assessment of muscle size, echo intensity and architecture has been investigated for sarcopenia assessment [11].

3D speed of sound (SoS) US using a dedicated ultrasound machine with a water bath has been used for breast density and tumor evaluation [12–20] based on SoS differences of tumor, glandular, and adipose tissue. Hand-held 2D SoS-US is a novel technique, which uses a conventional ultrasound system to measure SoS in breast tissue [21]. A passive reflector is positioned in opposite of a hand-held ultrasound probe with the breast in between (Fig. 1). The reflector serves as a reference tool to measure the time of the ultrasound echo transmission through the muscle and reflection to the probe [22, 23]. The results are visualized as a quantitative SoS profile along the probe width (Fig. 2). Using this method, a significant correlation and accordance between SoS-US and the ACR mammographic breast density categories have been observed [22].

Because muscular tissue has a different SoS than adipose tissues, there is potential of this novel technique for regional quantification of sarcopenia. The nominal value of SoS in muscle is 1585 m/s and for fat 1440 m/s, a difference of around 10% [24]. Therefore, SoS could be potentially used to quantify muscle and fat content.

The aim of this pilot study was to assess calf muscles of young and elderly females with a novel hand-held ultrasound technique measuring SoS, thus quantifying muscle loss and fatty muscular degeneration.

## Materials and methods

### Study design

This prospective, single-institution pilot study, was approved by the institutional review board and local ethics committee. Both calf muscles of 11 healthy, young, and female volunteers, as well as 10 elderly females (4 community dwelling women and 6 currently hospitalized women), were

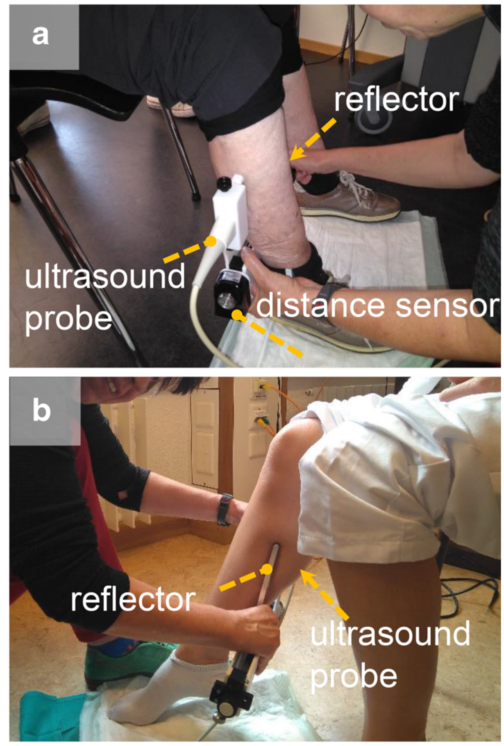



**Fig. 1** Concept of hand-held speed-of-sound (SoS)-US [23]. A positioning frame connects the probe with the reflector and allows distance measurements via a digital sensor. Ultrasound gel was applied on both the US probe and reflector surfaces to assure sufficient coupling with the calf. **a** Demonstrates the setup for an elderly female and (**b**) for a young female

prospectively examined with a standard ultrasound machine (SonixTouch, Ultrasonix) by one radiologist (M.B.R.). A flat Plexiglas® reflector was used as timing reference for SoS (m/s, probe segment-average, "overall muscle content") and ΔSoS (variation of SoS, m/s, "muscle heterogeneity") measurement. The reflector was positioned on the opposite side of the probe with the superficial posterior compartment of the calf longitudinally in between at the level of largest calve circumference (Fig. 1). SoS, ΔSoS, size/echo intensity parameters from conventional ultrasound images (B-mode), handgrip strength (kPA), 5-point comfort score (1 = comfortable to 5 = never again) and Tegner activity scores were assessed.

### Participants

The mean age of the young women was 28.9 years, ranging from 25 to 34 years. The mean age of the elderly women was 82.7 years, ranging from 73 to 92 years. Probands were selected through advertisement at the local black board. Inclusion criteria were a young, healthy female state as well as an age between 18 and 35. Patients were referred from the local geriatric clinic. Excluding criteria were severe dementia, congestive heart failure (NYHA III), leg edema, or paralysis. On the day of study, no participant underwent sports activities

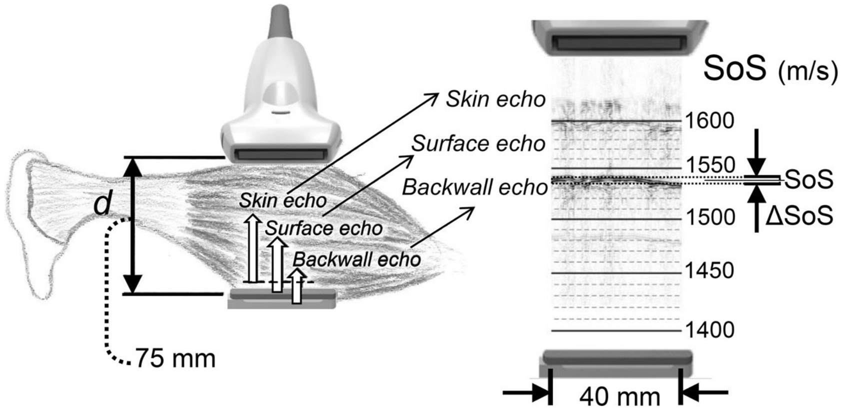



**Fig. 2** Measurements of the superficial posterior calf compartment in longitudinal direction. Whereas the vertical axis displays the arrival time t of the US echoes, the horizontal axis in the image illustrates the probe width. Our method is based on the reflection from the back wall of the reflector [23]. The speed of sound is then calculated with the formula SoS = 2 * $d$ / $t$ with d representing the distance read from the digital sensor and t the arrival time of the echoes. The factor 2 is used as the echo travels from the transmitter element to the reflector and back (there is a typo in the formula of [23], where a 0.5 factor is used instead). Three US echoes are visible, with the first representing the reflection from the calf skin layer and the second originating from the top reflector surface, with a possible overlap over time between the first and the second echoes. The third echo represents the reflector from the back wall of the reflector, and is well separated from the other two echoes, being used as a reference for SoS reading. The average SoS represents a measuring method of density of the muscle, and the SoS variation range (ΔSoS) one for the heterogeneity of the tissue

prior measurements. All participants rested at least 15 min prior to the examinations. The characteristics of the young and elderly women were analyzed and compared to the SoS measurements. These characteristics are listed in Table 1.

## Hand-held 2D SoS ultrasound measurements and comfort scores

We used a commercially available US system (SonixTouch, Ultrasonix) to acquire radiofrequency US data. As timing reference for the US signals transmitted through the calf, we used a flat passive Plexiglas® reflector positioned opposite to the calf with respect to a hand-held 5–14 MHz linear US probe (L14–5/38) at the maximum calf perimeter (Fig. 1). We attached both probe and reflector to a positioning frame that allowed controlling the distance between the probe and reflector (calf thickness). The frame was adjusted to achieve contact between ultrasound probe, calf, and reflector. The measurements were performed in a sitting position, with the calf muscle in rest state.

**Table 1** Participant data

| | Young females | Elderly females |
|---|---|---|
| Age (yrs.)** | 28.1 (3.9) | 82.7 (7) |
| BMI * | 19.8 (1.4) | 23.9 (3.5) |
| Tegner activity score** | 4.2 (1.1) | 0.8 (1.0) |
| Hand grip strength dominant (kPA)** | 79.5 (8.3) | 34.3 (12.3) |
| Hand grip strength non-dominant (kPA) ** | 83.8 (12.4) | 43.0 (14.6) |
| Hang grip mean dominant + non-dominant (kPA)** | 83.8 (11.6) | 40.8 (16.2) |
| Calf perimeter (mm) | 368 (21) | 340 (36) |
| Reflector distance (mm)* | 76.4 (2.3) | 65.3 (9.6) |
| SoS (m/s)** | 1542 (9) | 1515 (20) |
| ΔSoS (m/s)** | 5.5 (2.2) | 11.6 (4.6) |
| Muscle thickness** (mm) | 64.4 (1.4) | 52.6 (6.7) |
| Subcutaneous fat thickness (mm) | 6.0 (1.4) | 7.4 (2.1) |
| Subcutaneous fat/muscle ratio* | 0.08 (0.02) | 0.11 (0.03) |
| Muscle echo intensity (0…1) | 0.28 (0.03) | 0.29 (0.04) |
| Musche echo std. deviation (0…1) | 0.11 (0.01) | 0.12 (0.02) |

*,** Significant differences (*$p < 0.05$, **$p < 0.001$) between young and elderly females. In parenthesis standard deviation

A single ultrasound (M.B.R.) operator read the calf thickness from a digital distance sensor and manually noted the distance for SoS quantification. Two readers (L.R. and S.J.S.) blinded to the clinical data performed the SoS and ΔSoS readings within the 40-mm wide probe window in m/s of the examined calf (Fig. 2). Interoperator and interreader values of the SoS imaging technique have been previously assessed for breast tissues with different densities [23]. We use a Likert-like 5-point scale for convenience assessment, which ranged from 1 = agreeable; 2 = slightly uncomfortable; 3 = inconvenient, unsure whether to repeat the examination; 4 = inconvenient, I would favor not to repeat the examination; and to 5 = very inconvenient, I never want to have the examination done again.

### Analysis of SoS data

A specifically developed software and interface enabled raw ultrasound data acquisition and SoS reading [23]. Three recorded echoes were identified in the ultrasound image as a function of echo time and probe width. The echo representing the reflection from the back wall of the reflector served as a reference for SoS reading (Fig. 2). A conversion into velocity units for SoS was performed using both calf thickness and the time of flight of the reflector echo. The highest SoS value of both calves and the average ΔSoS value of both calves were used for further evaluation. SoS values in the muscular region ($SoS_{muscle}$) were calculated by subtracting the influence of the subcutaneous fat layers with the following equation:

$$\frac{d_{muscle}}{SoS_{muscle}} = \frac{d_{reflector}}{SoS} - \frac{2d_{adipose}}{SoS_{fat}} \quad (1)$$

where $d_{adispose}$ is the subcutaneous fat layer thickness, $d_{muscle}$ is the calf muscle thickness, and $d_{reflector} = 2d_{adispose} + d_{muscle}$ is the reflector-probe distance. SoS are the measured speed-of-sound values and $SoS_{fat}$ the nominal speed of sound in adipose tissue (1440 m/s) [23].

### Measurement of size/echo intensity/architecture ultrasound parameters

Based on the acquired raw ultrasound data, conventional B-mode ultrasound images were generated at the SoS measurement positions. The calf muscle thickness and thickness of subcutaneous fat layer were assessed, together with the echo intensity in the muscle region. For echo intensity, the average and std. deviation of the grayscale pixels in the muscular regions were measured in a scale between 0 and 1 (maximum grayscale value). All images were acquired with the same gain settings. Fascia regions and adipose layers were excluded from the echo intensity calculations. The average values of both calves were used for further evaluation.

### Handgrip strength assessment

Handgrip strength is a convenient and reliable measure of overall muscle strength. Martin Vigorimeter [25] and the local standard operating procedure were used for handgrip strength assessment (N.N). The Martin Vigorimeter is composed of a rubber bulb that is grasped by the hand. The pressure in the bulb is registered on a manometer via a rubber junction tube. Three grip strength measurements were performed for each hand with a rest period of 30 s between successive attempts. Both hands were tested, the dominant first. Hand size circumference was determined for the choice of bulb (big or middle). The highest value of both hands was used for further evaluation. Normal values were based on age, gender, and dominant side based on normative data from Desrosiers et al [26].

### Tegner activity score

The validated Tegner activity score form was used for activity assessment of all participants [27].

### Statistical analysis

We performed the statistical analysis with Matlab® (2014a, The MathWorks Inc.). Whereas categorical values were displayed as frequencies or percentages, continuous values were shown as the means ± SD (standard deviation). Interreader agreement was assessed using the interclass correlation coefficient (ICC) and the Bland-Altmann method [28].

The Student $t$ test was used for comparison of means between young and elderly females. Correlation coefficients between SoS and ΔSoS of dominant and non-dominant leg, and also of SoS and ΔSoS with handgrip strengths and Tegner activity scores were calculated. Spearman's rank correlation coefficient rs was used to assess correlations with the score variables, while Pearson correlation coefficient $r$ was used for continuous variables. The strength of the correlation was evaluated following [29], with correlation coefficient between 0 to 0.19 considered as very weak, 0.20 to 0.39 as weak, 0.40 to 0.59 as moderate, 0.60 to 0.79 as strong and 0.80 to 1.00 as very strong. In order to evaluate the diagnostic accuracy of our method, a ROC analysis was carried out. A $p$ value < 0.05 was regarded to indicate statistical significance.

## Results

### Correlation of SoS and ΔSoS with participant characteristics

The mean examination time of SoS-US for both calf measurements was 1 min and 30 s (ranging from 1 to 2 min). All measurements were successful. The examination time of handgrip strength measurements was at least 3 min. The mean calf thickness was 71 mm, ranging from 46 to 92 mm. There was a moderate negative correlation between SoS and patient age ($r = -0.590$, $p = 0.004$) and a strong negative correlation between SoS and BMI ($r = -0.656$, $p = 0.001$), while SoS did not show a statistically significant association with calf perimeter ($r = 0.267$, $p = 0.242$). ΔSoS showed a strong positive correlation with patient age ($r = 0.647$, $p = 0.002$) and BMI ($r = 0.498$, $p = 0.021$), and no significant association with calf perimeter ($r = 0.020$, $p = 0.932$).

## Comparison of SoS and ΔSoS of young and elderly females

The elderly females showed a significant lower SoS (1523 m/s, SD 16) compared to the young adults (1550 m/s, SD 8; $p < 0.001$). The ΔSoS of the elderly females was significantly higher (12.2 m/s, SD 3.6) than the ΔSoS of young females (6.4 m/s, SD 1.6; $p < 0.001$). (Figs. 3, 4 and 5). The $SoS_{muscle}$ in muscle (calculated with Eq. 1) was significantly lower in elderly females (1550 m/s, SD 22) than in young adults (1573 m/s, SD 12; $p = 0.008$). The AUC for elderly/young diagnosis was 0.936 for SoS, 0.927 for ΔSoS and 0.855 for $SoS_{\text{muscle}}$.

There was a significant correlation between SoS in dominant and non-dominant calves ($r = 0.789$; $p < 0.001$) and between ΔSoS in dominant and non-dominant calves ($r = 0.567$; $p = 0.007$). A statistically significant difference of means between dominant and non-dominant calves was not observed for either SoS ($p = 0.636$) and ΔSoS ($p = 0.304$).

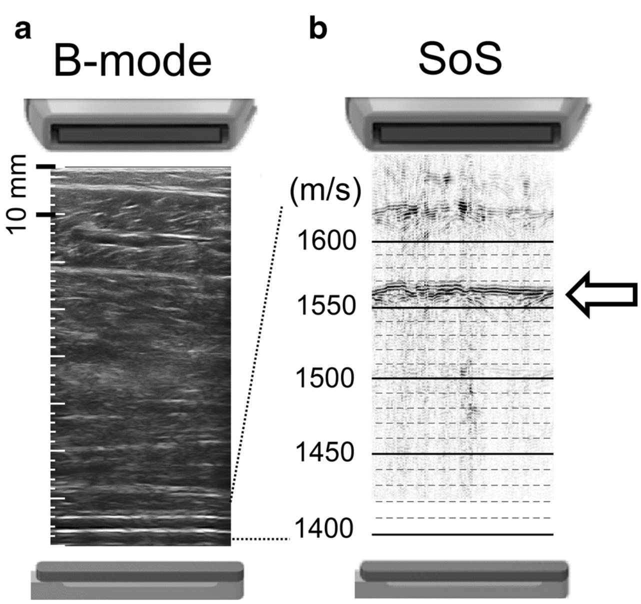



**Fig. 3** Young female. **a** B-mode ultrasound; **b** hand-held SoS-US measurement with an average SoS of 1560 m/s. Notably, there is little segment variability, namely, ΔSoS = 5 m/s ("flat" profile of SoS)

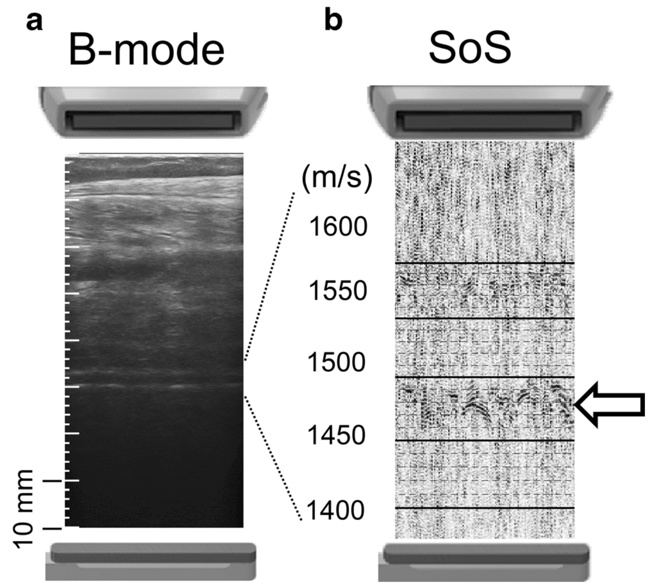



**Fig. 4** Elderly female. **a** B-mode ultrasound; **b** hand-held SoS-US measurement with an average SoS of 1483 m/s. Note that ΔSoS = 15 m/s is higher than ΔSoS in Fig. 3

## Interreader agreement

We compared the SoS and ΔSoS values acquired by two readers using the 42 calf measurements available. We found a correlation of $r = 0.976$ ($p < 0.001$) and an ICC of 0.988 for SoS, and a correlation of $r = 0.744$ ($p < 0.001$) and an ICC of 0.844 for ΔSoS. The average SoS difference ± 1.96 SD (Bland-Altmann) between readers was 0.0 m/s, ranging from -9.1 to 9.1 m/s. For ΔSoS, the average difference was -2.9 m/s, with a range of -10.0 m/s to 4.1 m/s.

## Comparison of SoS and ΔSoS with handgrip strength and Tegner activity score

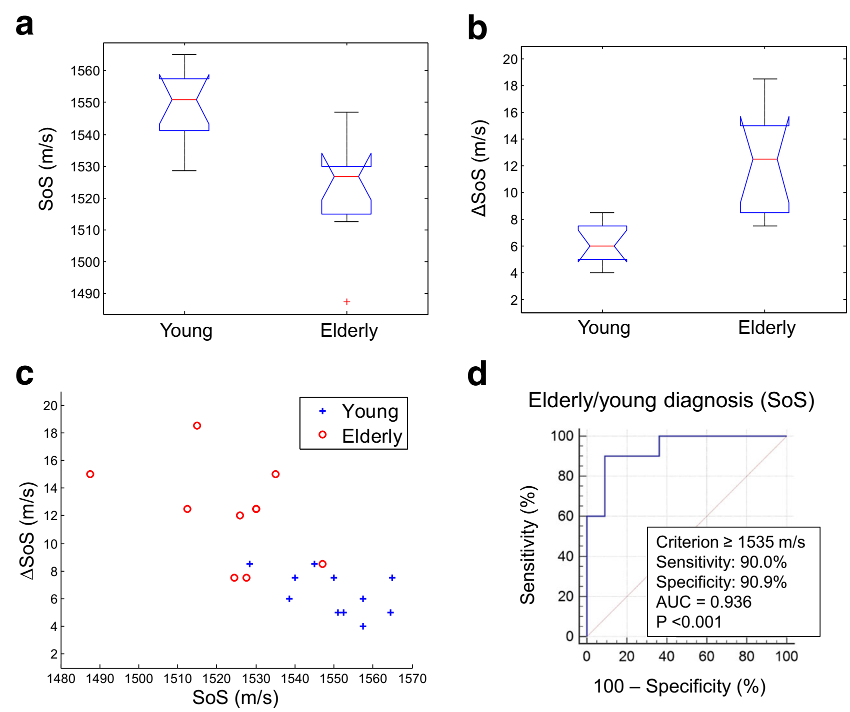
The mean handgrip strength of the young females was significantly higher than the elderly females concerning the right, left, and both hands (83.8 versus 40.8, $p < 0.001$). The mean Tegner activity score of the young females was significantly higher than the elderly females (4.3 versus 0.8, $p < 0.001$) (Table 1).

Considering all test subjects, there was a strong positive correlation between SoS and handgrip strength ($r = 0.644$, $p = 0.002$) and between SoS and Tegner activity score ($rs = 0.709$, $p < 0.001$). On the other hand, ΔSoS showed a negative correlation with both handgrip strength ($r = -0.492$, $p = 0.02$) and Tegner activity score ($rs = -0.568$, $p = 0.009$). In comparison, the correlation between handgrip strength and Tegner activity score was ($rs = 0.794$, $p > 0.001$) (Fig. 6).

## Size/echo intensity ultrasound parameters

The elderly females did not show a significantly larger subcutaneous fat layer thickness (7.4 m/s, SD 2.2) compared to the

**Fig. 5** Classification results. (**a**) and (**b**) show boxplots of muscle density and speed of sound (SoS) and ΔSoS of all test subjects ($N = 21$). **c** Scatter plots of young and elderly female categories compared to the combined SoS and ΔSoS values. Young females showed high SoS and low ΔSoS ("flat" echo line), whereas elderly females had low SoS and high ΔSoS ("mountainous" echo line). Thus, the addition of ΔSoS can be used as an indicator of false low SoS values. **d** ROC curves for young/elderly female diagnosis (positive for elderly female)




young adults (6.0 mm, SD 1.4; $p = 0.089$). The calf muscle thickness of the elderly patients was significantly lower (52.6 mm, SD 7.0) than the young females (64.4 mm, SD 1.4, $p < 0.001$). The subcutaneous fat layer thickness to calf muscle thickness ratio (fat/muscle ratio) of elderly patients was significantly larger (0.11, SD 0.03) than for young females (0.08, 0.02, $p = 0.05$). No significant difference was found between elderly and young patients for the echo intensity ($p = 0.265$) and echo std. deviation ($p = 0.314$). The AUC for elderly/young diagnosis was 0.927 for calf muscle thickness and 0.827 for fat/muscle thickness ratio (Fig. 7).

There was a strong positive correlation between SoS and calf muscle thickness ($r = 0.681$, $p < 0.001$) and a strong negative correlation between SoS and fat/muscle ratio ($r = -0.627$, $p = 0.002$). However, a significant correlation was not found between SoS and subcutaneous fat layer thickness ($r = -0.345$, $p = 0.126$), SoS and echo intensity ($r = -0.1559$, $p = 0.531$) and SoS and echo std. deviation ($r = -0.267$, $p = 0.242$). ΔSoS showed a moderate correlation with subcutaneous fat layer thickness ($r = 0.460$, $p = 0.036$), a strong negative correlation with muscle thickness ($r = -0.619$, $p = 0.003$), and a strong positive correlation with fat/muscle ratio ($r = 0.644$, $p = 0.002$), with no significant correlations with echo intensity ($r = 0.249$, $p = 0.277$) and echo std. deviation ($r = 0.249$, $p = 0.277$).

Tegner activity showed a strong correlation with muscle thickness ($rs = 0.794$, $p < 0.001$), while the correlations with subcutaneous fat thickness ($rs = -0.102$, $p = 0.667$), fat muscle ratio ($rs = -0.442$, $p = 0.05$), echo intensity ($rs = -0.183$, $p = 0.439$), and echo std. deviation ($rs = -0.028$, $p = 0.905$) were not significant. Similarly, there was a strong positive correlation of handgrip strength with muscle thickness ($rs = 0.667$, $p < 0.001$), while the correlations with subcutaneous fat thickness ($rs = -0.035$, $p = 0.882$), fat muscle ratio ($rs = -0.357$, $p = 0.112$), echo intensity ($rs = -0.191$, $p = 0.407$), and echo std. deviation ($rs = -0.176$, $p = 0.447$) were not significant.

As for the participant characteristics, significant correlations were found between muscle thickness and patient age ($rs = -0.541$, $p = 0.01$), muscle thickness and BMI ($r = -0.593$, $p = 0.005$), subcutaneous fat thickness and BMI ($r = 0.474$, $p = 0.03$), and fat muscle ratio and BMI ($r = 0.596$, $p = 0.005$), while other combinations did not show significant correlations. Calf perimeter did not significantly correlate with any size/echo intensity ultrasound parameters. Echo intensity and std. deviation did not significantly correlate with patient age and BMI.

### Comfort scores of handheld SoS-US

The comfort score of handheld SoS-US for calf density assessment was 1.1 (ranging from 1 to 2) for elderly women, and that of young women was 1.4 (ranging from 1 to 2). (Table 1).

## Discussion

This study showed that SoS of calf tissue can be measured with hand-held US using a simple sound wave reflector at the opposite side of the probe and calf. The women acceptance rate was high. The SoS software can be implemented as an

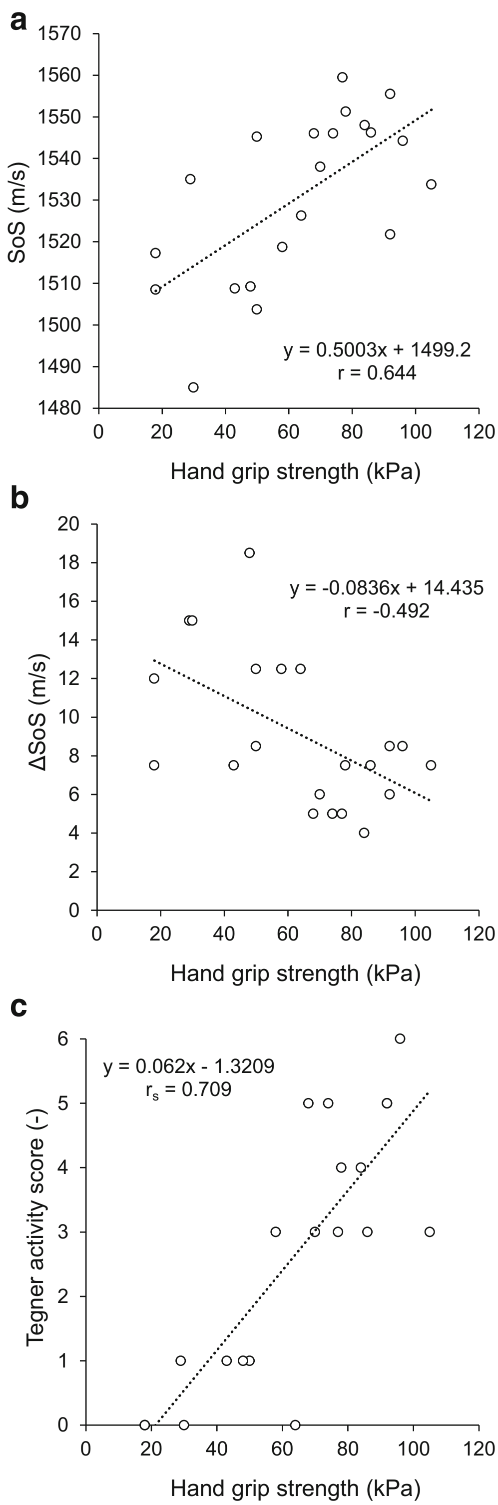



**Fig. 6** Correlation of (**a**) SoS (m/s), (**b**) ΔSoS (m/s) and (**c**) Tegner activity score with handgrip strength (kPa)

add-on to conventional ultrasound systems [23, 30]. Our results showed that SoS and ΔSoS of young and elderly females were significantly different, indicating muscle loss, and fatty muscular degeneration in elderly females. SoS was significantly lower for elderly females.

The role of skeletal muscle US for screening and diagnosing sarcopenia in elderly individuals is a field for research [30]. None of the current definitions of sarcopenia includes US in diagnostic algorithms [6, 7, 31]. Some studies acknowledge the potential usefulness of muscle ultrasound for sarcopenia assessment, mainly based on pilot studies assessing muscle size, echogenicity, and architecture [32–42].

Ultrasound B-mode can be used to obtain anatomic muscle characteristics such as muscle thickness or fascicle length, which show some degree of alteration with patient age, however no conclusive recommendation is presently available about its use in geriatric practice and ultrasound is presently poorly utilized [30, 43–45].

In this pilot study, the classification of elderly and young adults showed a superior classification with SoS parameters than with conventional thickness and echo intensity parameters. Echo intensity and subcutaneous fat tissue thickness did not show significant differences between both groups, while muscle thickness provided better classification results. These results suggest that the SoS measurements are mostly influenced by changes in the constitution of muscular region. This is also confirmed by subtracting the influence of the subcutaneous fat tissue layers and calculating SoS values only for the muscular region with Eq. 1.

Since SoS is known to be lower in pure fat than in muscular tissue, this may be an indication that fatty muscular degeneration processes occur in the senior females. Simple echo intensity parameters (average intensity and standard deviation) do not allow to separate elderly and young patients, and more sophisticated texture analysis methods may be required to achieve an improvement in this direction [46]. On the contrary to conventional US parameters, SoS provides a quantitative method to evaluate muscular constitution, which is less subjective than conventional ultrasound parameters.

Quantitative ultrasound, based on transmission measurements, has shown potential to measure bone mineral density for the assessment of osteoporosis and fracture risk [47], however, further standardization efforts are necessary [48]. Soft tissue layers are typically considered as confounding variables to bone densitometry and their diagnostic potential remains largely unexplored.

Ultrasound elastography of muscles is a rapidly emerging field with many applications for systemic muscle diseases [49]. However, elastography ultrasound measures can be significantly influenced by position, pressure, inclination of the probe, and muscle activation state [49–52]. Phantom and breast studies showed independence of these factors with SoS-US. Temperature introduces small variation in SoS of the order of 2 m $s^{-1}$/°C at 37° C, which are here insignificant given the small range of body temperature. Available studies do not show a significant variation with probe direction or contractile activity in SoS-US [21, 53, 54].

SoS-US has a high potential for the differentiation of soft tissue types likewise muscle, glandular, and adipose tissue. A

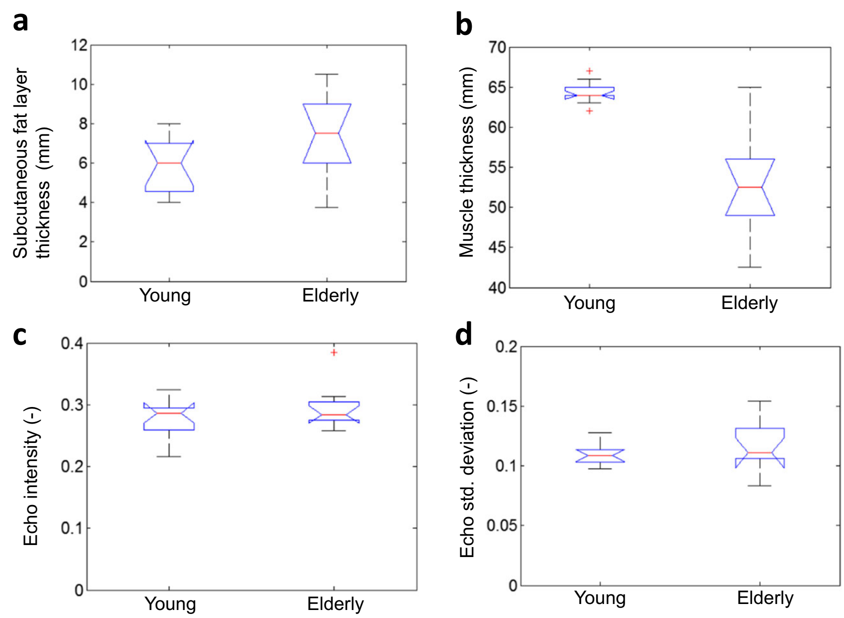



**Fig. 7** Classification results based on ultrasound thickness and echogenicity parameters. Boxplots are shown for subcutaneous fat layer thickness (**a**), muscle thickness (**b**), echo intensity (**c**) and echo standard deviation (**d**) for all test subjects ($N = 21$). Significant differences between elderly and young patients were only found for muscle thickness ($p < 0.001$)

recent study has applied SoS-US to the quantification of the breast density, that is, the amount of glandular and fatty tissue [23]. Studies in animal tissue have found that SoS in muscle tissue strongly correlates with adipose tissue content [55].

State-of-the-art SoS measurement systems are 3D US systems, which require the immersion of the tissue in a water bath, with transmit and receive units located in opposite sides of the tissue. These devices are bulky and the procedure is time costly, their application being also mostly limited to breast imaging. A recent work has explored the application of such systems to limb muscle speed of sound estimation [56]. The setup shows practical difficulties of immersing the limbs in water. Moreover, the presence of bones introduces large variations which distort the measurement.

Hand-held 2D SoS-US overcomes these limitations by using a simple reflector add-on to conventional 2D ultrasound. This eliminates the need of a water bath. Moreover, the measurements can be performed fast and comfortably similar to an ultrasound examination. The transmission is performed through a portion of the superficial posterior compartment of the calf muscle. Thus, bones do not influence the measurement. With the increasing portability of ultrasound systems, hand-held 2D SoS-US could be potentially used in in primary and hospital care.

We found an overall high acceptance rate for SoS-US for all women with higher rates among the elderly women. Hand-held SoS-US was a fast procedure, and provided a pleasant experience to the elderly participants, including the application of body gel, caring hands, as well as personal communication during the examination. The reason to choose the lower limb for our pilot study was that the lower limb is expected to have a greater impact on mobility. The posture of patients have been discussed for B-mode ultrasound. Most studies were performed with the patient supine for the anterior thigh and prone for the posterior thigh [36, 38–42]. Abe et al [37] generally performed their examination in standing position. Because of possible mobility-disability and cognitive impairment, we found a comfortable sitting position without the need to undress especially for the elderly women suitable and timesaving (Fig. 3).

In our proof-of-principle study the elderly females showed a significant lower SoS compared to the young adults, indicating a lower muscle to fat tissue ratio of the elderly women within the examined calf area. The ΔSoS of the elderly females was significantly higher than the young females indicating a higher heterogeneity of muscle, possibly "myosteatosis".

No significant SoS and ΔSoS differences between dominant and non-dominant legs of individuals were found, while SoS and ΔSoS were significantly different among age groups, indicating reproducibility of the measurements and age-related differences. A significant correlation was observed between SoS-US, hand grip strength ($r = 0.644$) and Tegner activity score ($rs = 0.709$). In comparison, the correlation between handgrip strength and Tegner activity score was slightly higher ($r = 0.794$) than the correlations with SoS. A possible reason for the differences between hand-grip strength and SoS calf measurements may be that age-related muscle loss does not advance in all anatomic regions at the same rate, as confirmed by a study with MRI [57]. Sarcopenia of the lower limb is greater than for the upper limb muscles since the effects of sarcopenia are generally more evident in antigravity muscle groups [33, 58].

There are some limitations to our study, such as the limited number of study participants. We compared the lower extremity (SoS-US) with the upper extremity (handgrip strength), as well as a standard non-imaging test (handgrip strength and Tegner activity score) with a novel imaging technique (SoS-US). Possible influence of osteoarthritis, diabetes, and other diseases for the different extremities and examination techniques was not considered. Further studies are needed to improve application of this novel technique, to compare it to other imaging modalities, and to examine the exact value for age-, cancer-, chronic-, traumatic disease, as well as life style-related (e.g., sports and nutrition) muscle changes.

In conclusion, there were significant differences of SoS and ΔSoS between young and elderly females, indicating a decrease in muscle mass, and an increase in subcutaneous and intermuscular adipose tissue in elderly females. Measurements were well tolerated and fast. The novel technique shows potential for sarcopenia quantification in primary and hospital care using a standard ultrasound machine.

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## Compliance with ethical standards

**Guarantor** The scientific guarantor of this publication is Marga Rominger.

**Conflict of interest** The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

**Statistics and biometry** No complex statistical methods were necessary for this paper.

**Informed consent** Written informed consent was obtained from all subjects (patients) in this study.

**Ethical approval** Institutional Review Board approval was obtained.

**Methodology**
• Prospective
• Case-control study
• Performed at one institution

## References

1. Buford TW, Lott DJ, Marzetti E et al (2012) Age-related differences in lower extremity tissue compartments and associations with physical function in older adults. Exp Gerontol 47:38–44
2. Shen Y, Hao Q, Zhou J, Dong B (2017) The impact of frailty and sarcopenia on postoperative outcomes in older patients undergoing gastrectomy surgery: a systematic review and meta-analysis. BMC Geriatr 17:188
3. van Vugt JLA, Alferink LJM, Buettner S et al (2018) A model including sarcopenia surpasses the MELD score in predicting waiting list mortality in cirrhotic liver transplant candidates. J Hepatol 68:707–714
4. van Vugt JLA, Buettner S, Levolger S et al (2017) Low skeletal muscle mass is associated with increased hospital expenditure in patients undergoing cancer surgery of the alimentary tract. PLoS One 12:e0186547
5. Boer BC, de Graaff F, Brusse-Keizer M et al (2016) Skeletal muscle mass and quality as risk factors for postoperative outcome after open colon resection for cancer. Int J Colorectal Dis 31:1117–1124
6. Cruz-Jentoft AJ, Baeyens JP, Bauer JM et al (2010) Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. Age Ageing 39:412–423
7. Fielding RA, Vellas B, Evans WJ et al (2011) Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. J Am Med Dir Assoc 12:249–256
8. Dawson-Hughes B, Bischoff-Ferrari H (2016) Considerations concerning the definition of sarcopenia. Osteoporos Int 27:3139–3144
9. Bischoff-Ferrari HA, Orav JE, Kanis JA et al (2015) Comparative performance of current definitions of sarcopenia against the prospective incidence of falls among community-dwelling seniors age 65 and older. Osteoporos Int 26:2793–2802
10. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R (2004) The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc 52:80–85
11. Boutin RD, Yao L, Canter RJ, Lenchik L (2015) Sarcopenia: current concepts and imaging implications. AJR Am J Roentgenol 205:W255–W266
12. Duric N, Boyd N, Littrup P et al (2013) Breast density measurements with ultrasound tomography: a comparison with film and digital mammography. Med Phys 40:013501
13. Duric N, Littrup P, Poulo L et al (2007) Detection of breast cancer with ultrasound tomography: first results with the computed ultrasound risk evaluation (CURE) prototype. Med Phys 34:773–785
14. Glide C, Duric N, Littrup P (2007) Novel approach to evaluating breast density utilizing ultrasound tomography. Med Phys 34:744–753
15. Glide-Hurst CK, Duric N, Littrup P (2008) Volumetric breast density evaluation from ultrasound tomography images. Med Phys 35:3988–3997
16. Khodr ZG, Sak MA, Pfeiffer RM et al (2015) Determinants of the reliability of ultrasound tomography sound speed estimates as a surrogate for volumetric breast density. Med Phys 42:5671–5678
17. O'Flynn EA, Fromageau J, Ledger AE et al (2017) Ultrasound tomography evaluation of breast density: a comparison with noncontrast magnetic resonance imaging. Invest Radiol 52:343–348
18. Sak M, Duric N, Littrup P et al (2017) Using speed of sound imaging to characterize breast density. Ultrasound Med Biol 43:91–103
19. Sak M, Duric N, Littrup P et al (2014) Comparison of sound speed measurements on two different ultrasound tomography devices. Proc SPIE Int Soc Opt Eng 9040:90400s
20. Sak M, Duric N, Littrup P et al (2013) Breast density measurements using ultrasound tomography for patients undergoing tamoxifen treatment. Proc SPIE Int Soc Opt Eng 8675:86751e
21. Sanabria SJ, Goksel O (2016) Hand-held sound-speed mammography based on ultrasound reflector tracking. In: Ourselin S et al (eds) Medical Image Computing and Computer-Assisted Intervention - MICCAI 2016, Part I, LNCS, 9900, 567–576
22. Sanabria SJ, Goksel O (2016) Hand-held medical ultrasound apparatus and system for determining a tomographic image. PCT/EP2016/070321 (patent pending)
23. Sanabria SJ, Goksel O, Martini K et al (2018) Breast-density assessment with hand-held ultrasound: a novel biomarker to assess breast cancer risk and to tailor screening? Eur Radiol. https://doi.org/10.1007/s00330-017-5287-9
24. Szabo T (2004) Diagnostic ultrasound imaging: inside out, 1st edn. Academic Press, Burlington
25. Thorngren KG, Werner CO (1979) Normal grip strength. Acta Orthop Scand 50:255–259

26. Desrosiers J, Bravo G, Hébert R, Dutil E (1995) Normative data for grip strength of elderly men and women. Am J Occup Ther 49:637–644
27. Fortbildungen für orthopädische Medizin und manuelle Therapie (FOMT) Report (2013) TAS − Tegner activity scale. https://www.fomt.info/Frageboegen/TAS-Tegner-acitivity-scale-deutsche-Version.pdf
28. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307–310
29. Stewart A (2010) Basic statistics and epidemiology: a practical guide, 3rd edn. Radcliffe Pub, Abingdon
30. Ticinesi A, Meschi T, Narici MV, Lauretani F, Maggio M (2017) Muscle ultrasound and sarcopenia in older individuals: a clinical perspective. J Am Med Dir Assoc 18:290–300
31. Muscaritoli M, Anker SD, Argilés J et al (2010) Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by special interest groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr 29:154–159
32. Sergi G, Trevisan C, Veronese N, Lucato P, Manzato E (2016) Imaging of sarcopenia. Eur J Radiol 85:1519–1524
33. Harris-Love MO, Monfaredi R, Ismail C, Blackman MR, Cleary K (2014) Quantitative ultrasound: measurement considerations for the assessment of muscular dystrophy and sarcopenia. Front Aging Neurosci 6:172
34. Narici MV, Maganaris CN, Reeves ND, Capodaglio P (2003) Effect of aging on human muscle architecture. J Appl Physiol (1985) 95: 2229–2234
35. Morse CI, Thom JM, Reeves ND, Birch KM, Narici MV (2005) In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. J Appl Physiol (1985) 99:1050–1055
36. Takai Y, Ohta M, Akagi R et al (2014) Applicability of ultrasound muscle thickness measurements for predicting fat-free mass in elderly population. J Nutr Health Aging 18:579–585
37. Abe T, Patterson KM, Stover CD et al (2014) Site-specific thigh muscle loss as an independent phenomenon for age-related muscle loss in middle-aged and older men and women. Age (Dordr) 36:9634
38. Berger J, Bunout D, Barrera G et al (2015) Rectus femoris (RF) ultrasound for the assessment of muscle mass in older people. Arch Gerontol Geriatr 61:33–38
39. Ismail C, Zabal J, Hernandez HJ et al (2015) Diagnostic ultrasound estimates of muscle mass and muscle quality discriminate between women with and without sarcopenia. Front Physiol 6:302
40. Minetto MA, Caresio C, Menapace T et al (2016) Ultrasound-based detection of low muscle mass for diagnosis of sarcopenia in older adults. PM R 8:453–462
41. Kuyumcu ME, Halil M, Kara Ö et al (2016) Ultrasonographic evaluation of the calf muscle mass and architecture in elderly patients with and without sarcopenia. Arch Gerontol Geriatr 65:218–224
42. Seymour JM, Ward K, Sidhu PS et al (2009) Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. Thorax 64:418–423
43. Can B, Kara M, Kara Ö, Ülger Z, Frontera WR, Özçakar L (2017) The value of musculoskeletal ultrasound in geriatric care and rehabilitation. Int J Rehabil Res 40:285–296
44. Reeves N, Maganaris C, Narici M (2004) Ultrasonographic assessment of human skeletal muscle size. Eur J Appl Physiol 91:116–118
45. Strasser E, Draskovits T, Praschak M, Quittan M, Graf A (2013) Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. Age (Dodr) 35:2377–1388
46. da Silva Pereira Júnior N, da Matta TT, Alvarenga AV, de Albuquerque Pereira WC, de Oliveira LF (2017) Reliability of ultrasound texture measures of biceps brachialis and gastrocnemius Lateralis muscles' images. Clin Physiol Funct Imaging 37:84–88
47. Hans D, Baim S (2017) Quantitative ultrasound (QUS) in the Management of Osteoporosis and assessment of fracture risk. J Clin Densitom 20:323–333
48. Krieg MA, Barkmann R, Gonnelli S et al (2008) Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD official positions. J Clin Densitom 11:163–187
49. Brandenburg JE, Eby SF, Song P et al (2014) Ultrasound elastography: the new frontier in direct measurement of muscle stiffness. Arch Phys Med Rehabil 95:2207–2219
50. Gennisson JL, Deffieux T, Macé E, Montaldo G, Fink M, Tanter M (2010) Viscoelastic and anisotropic mechanical properties of in vivo muscle tissue assessed by supersonic shear imaging. Ultrasound Med Biol 36:789–801
51. Kot BC, Zhang ZJ, Lee AW, Leung VY, Fu SN (2012) Elastic modulus of muscle and tendon with shear wave ultrasound elastography: variations with different technical settings. PLoS One 7:e44348
52. Nordez A, Hug F (2010) Muscle shear elastic modulus measured using supersonic shear imaging is highly related to muscle activity level. J Appl Physiol (1985) 108:1389–1394
53. Marsh RL (2016) Speed of sound in muscle for use in sonomicrometry. J Biomech 49:4138.4141
54. Topp KA, O'Brien WD (1998) Anisotropy of ultrasonic parameters in fresh rat skeletal muscle in vitro. In: Schneider SC, Levy M, McAvoy BR (eds) IEEE Ultrasonics Symposium, Sendai, Japan, pp 1369–1372
55. Park B, Whittaker AD, Miller RK, Hale DS (1994) Predicting intramuscular fat in beef longissimus muscle from speed of sound. J Anim Sci 72:109–116
56. Qu X, Azuma T, Lin H et al (2017) Limb muscle sound speed estimation by ultrasound computed tomography excluding receivers in bone shadow. In: N Duric, B Heyde (eds) Proceedings of the SPIE, Volume 10139, id. 101391B 8 pp
57. Janssen I, Heymsfield SB, Wang ZM, Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol (1985) 89:81–88
58. Narici MV, Maffulli N (2010) Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull 95:139–159