Ultrasound in Med. & Biol., Vol. 00, No. 00, pp. 1–9, 2022
Copyright © 2022 World Federation for Ultrasound in Medicine & Biology. All rights reserved.
Printed in the USA. All rights reserved.
0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2022.09.007

● *Original Contribution*

ULTRASONIC THROUGH-TRANSMISSION MEASUREMENTS OF HUMAN MUSCULOSKELETAL AND FAT PROPERTIES

Dongsheng Bi,*,# Lingwei Shi,*,# Chengcheng Liu,† Boyi Li,† Ying Li,* Lawrence H. Le,‡ Jingchun Luo,§ Sijia Wang,¶ and Dean Ta*,†,§

* Center for Biomedical Engineering, School of Information Science and Technology, Fudan University, Shanghai, China; † Academy for Engineering and Technology, Fudan University, Shanghai, China; ‡ Department of Radiology and Diagnostic Imaging, University of Alberta, Edmonton, Alberta, Canada; § Human Phenome Institute, Fudan University, Shanghai, China; and ¶ Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai, China

(*Received* 16 *June* 2022; *revised* 7 *September* 2022; *in final form* 9 *September* 2022)

Abstract—The study described here was aimed at investigating the feasibility of using the ultrasonic through-transmission technique to estimate human musculoskeletal and fat properties. Five hundred eighty-two volunteers were assessed by dual-energy X-ray absorptiometry (DXA) and ultrasonic transmission techniques. Bone mineral density (BMD), muscle and fat mass were measured for both legs and the whole body. Hip BMD and spine BMD were also measured. Ultrasonic transmission measurements were performed on the heel, and the measured parameters were broadband ultrasound attenuation (BUA), speed of sound (SOS), ultrasonic stiffness index (SI), *T*-score and *Z*-score, which were significantly correlated with all measured BMDs. The optimal correlation was observed between SI and left-leg BMD ($p < 0.001$) before and after adjustment for age, sex and body mass index (BMI). The linear and partial correlation analyses revealed that BUA and SOS were closely associated with muscle and fat mass, respectively. Multiple regressions revealed that muscle and fat mass significantly contributed to the prediction of transmission parameters, explaining up to 17.83% ($p < 0.001$) variance independently of BMD. The results suggest that the ultrasonic through-transmission technique could help in the clinical diagnosis of skeletal and muscular system diseases. (E-mail: tda@fudan.edu.cn) **© 2022 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Musculoskeletal diseases, Bone mineral density, Muscle, Fat, Ultrasonic transmission technique.**

INTRODUCTION

Musculoskeletal disorders (MSDs) are symptoms in multiple body areas and systems, such as osteoporosis and fractures occurring in bones, sarcopenia occurring in muscles, osteoarthritis occurring in joints and low-back-pain symptoms according to World Health Organization. MSDs severely threaten the health of human beings, causing high morbidity and mortality to the aging population (Walsh et al. 2006; Edwards et al. 2015) and the working population (Valachi and Valachi 2003; Chiasson et al. 2012). Work-related MSDs lead to severe threats to the labor force, economics and workers' life (Bhattacharya 2014; Bevan 2015). Getting older is also one of the predominant factors increasing the risk of developing serious MSDs, accompanied by the deterioration of the musculoskeletal system. MSDs have already been a worldwide major health care concern, suggesting the urgent need for the early diagnosis and prevention of MSDs.

Decreasing bone mineral density (BMD) and decreasing muscle mass are closely related and are known indicators of osteoporosis and sarcopenia (*i.e.,* two typical MSDs) (Edwards et al. 2015; Qi et al. 2019; Sutter et al. 2019). Osteoporosis characterized by bone loss is closely associated with sarcopenia characterized by muscle loss (Walsh et al. 2006; Lima et al. 2019). Moreover, the screening for sarcopenia simultaneously with osteoporosis has been suggested because of the close association between muscle loss and bone loss (Walsh et al. 2006). Almost 50% of osteoporotic postmenopausal women were diagnosed with sarcopenia (Walsh et al. 2006), emphasizing the importance of assessing bone and muscle properties simultaneously.

Address correspondence to: Dean Ta, Center for Biomedical Engineering, Fudan University, 2005 Songhu Road, Yangpu District, Shanghai 200438, China. E-mail: tda@fudan.edu.cn

# These authors have contributed equally to this work and share the first authorship.

Current modalities assessing bone and skeletal muscle properties include magnetic resonance imaging (MRI), computed tomography (CT), dual-energy X-ray absorptiometry (DXA) and ultrasound (Guerri et al. 2018). MRI has high resolution but at a high cost. Both MRI and CT possess the disadvantages of time-consuming imaging analysis and technical difficulty in performing measurements (Pahor et al. 2009). CT exposes patients to greater amounts of radiation during consecutive scans compared with the DXA technique. DXA has the ability to assess BMD and muscle properties and is regarded as the clinical gold standard for osteoporosis screening and the most commonly used technique for sarcopenia assessment. However, the X-ray ionizing radiation of DXA is harmful to human health, limiting the real-time measurements of musculoskeletal properties. In addition, DXA measurements require patients to keep still during the scan lasting around 10 min. It also takes time for professional technologists to analyze BMD and body composition. Recently, based on the imaging modality, musculoskeletal ultrasound has been used to assess skeletal muscle size (Rahmani et al. 2015), Achilles tendon (Lalumiere et al. 2020) and diagnose shoulder lesions such as tendinitis (Chen et al. 2011; Chang et al. 2016), low back pain (Cheung et al. 2020) and spinal cord injury (Dudley-Javoroski et al. 2010).

As a non−ionizing-radiation and more convenient quantitative ultrasound (QUS) technique, the ultrasonic through-transmission technique has been adopted in characterization of heel BMD since 1984, shedding light on the ultrasonic diagnosis of osteoporosis (Langton et al. 1984). Ultrasonic parameters can be obtained by placing the heel between two fixed unfocused transducers responsible for transmitting and receiving ultrasonic signals (Wear 2020). It takes less than 1 min to perform one transmission measurement and obtain ultrasonic parameters (*e.g.,* broadband ultrasound attenuation [BUA] and speed of sound [SOS]). BUA and SOS can characterize the attenuation and velocity properties of the measured biological tissues, respectively (Langton and Njeh 2008; Daugschies et al. 2015). The ultrasonic transmission technique has been applied in measurement of BMD (Chaffaí et al. 2002; Nicholson and Bouxsein 2002; Wear et al. 2000) and mechanical properties (Langton et al. 1996; Wear et al. 2017), monitoring of bone loss during bed rest (Laugier et al. 2000; Qin et al. 2019) and discrimination of fracture and osteoporosis (Schott et al. 1995; Turner et al. 1995; Hans et al. 1996; Bauer et al. 1997; Thompson et al. 1998; Roux et al. 2001). The interaction between ultrasound and musculoskeletal tissues suggested the potential feasibility of ultrasonic assessment of musculoskeletal properties. However, whether the ultrasonic transmission technique can assess BMD and muscle and fat properties remains speculative and requires investigation.

The study described here investigated the feasibility of ultrasonic through-transmission measurements of human musculoskeletal and fat properties with 582 recruited volunteers. Linear and partial correlation analyses were conducted among ultrasonic transmission and musculoskeletal parameters. The study also investigated the contribution of muscle and fat mass to ultrasonic parameters independently of BMD by multiple regression analysis.

METHODS

Study volunteers

The present study recruited 582 volunteers without musculoskeletal diseases, and was approved by the ethics committee of the School of Life Sciences of Fudan University. Written informed consent was obtained from all volunteers. The volunteers ranged from 20 to 60 y with a mean age of 33 ± 11 y. There were 252 male volunteers with a mean age of 34 ± 10 y (range: 20−60) and 330 female volunteers with a mean age of 32 ± 11 y (range: 21−60). Body mass index (BMI) ranged from 15.9 to 35.5 kg/m^2 with a mean of 22.8±3.2. The volunteers were all measured by DXA and ultrasonic through-transmission techniques. Figure 1 is a schematic of (a) musculoskeletal and fat property measurements and (b) ultrasonic transmission measurements, with *green* indicating the bone and *red* indicating the muscle.

Measurements of musculoskeletal and fat properties

The Lunar iDXA (GE Healthcare, General Electric Co., Boston, MA, USA) was adopted to measure BMD and muscle and fat mass. Quality assurance was performed by a technician with the equipped quality assurance block and aluminum spine phantom. Then the standard test program was implemented to measure musculoskeletal properties. For BMD measurements, the spine, femur and legs were tested because they are significant weight-bearing bones. We measured the BMD of the L1−L4 lumbar vertebrae (spine BMD), dual femur (hip BMD), whole body (BMD-W), left leg (BMD-leg-L) and right leg (BMD-leg-R). Body composition was analyzed to measure muscle and fat mass from the whole body (Muscle-W and Fat-W) and left and right legs (Muscle-leg-L, Muscle-leg-R, Fat-leg-L, Fat-leg-R), which were rich in skeletal muscle. The measurement of musculoskeletal properties is illustrated in Figure 1a. Table 1 lists the statistics of the aforementioned musculoskeletal and fat parameters.

Ultrasonic through-transmission measurements

As illustrated in Figure 1b, ultrasonic through-transmission measurements of the left heel were performed with a GE Achilles EXPII (GE Healthcare). A daily

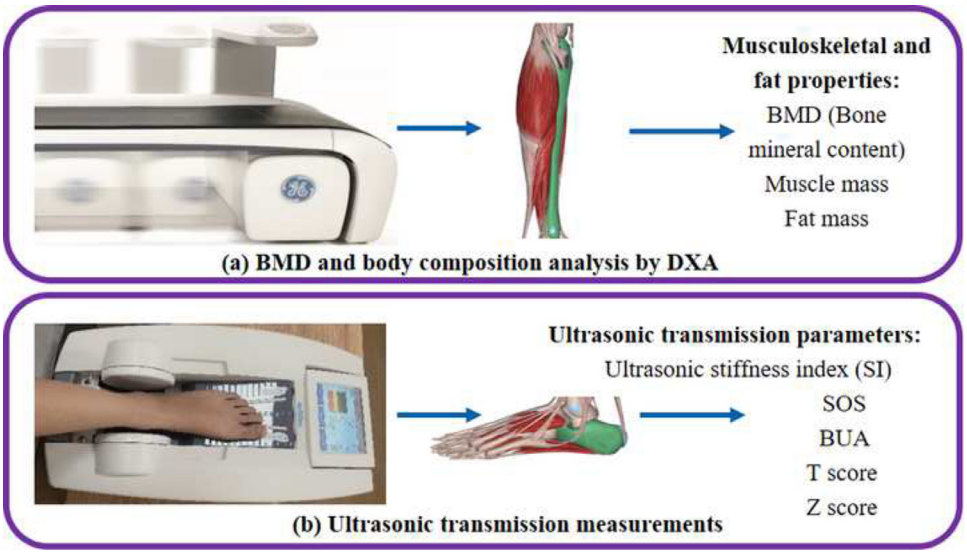

Fig. 1. Schematic of (a) musculoskeletal and fat property measurements and (b) ultrasonic transmission measurements. The *green* represents bone and the *red* represents muscle. BMD = bone mineral density; BUA =broadband ultrasound attenuation; DXA = dual-energy X-ray absorptiometry.

quality assurance test, taking around 30 s, was performed with a standard test cylinder. Then the scan of the heel takes 10 s with water inside the inflated membranes surrounding the calcaneus. Five measurements were performed on each volunteer, and the statistics of ultrasonic transmission parameters are summarized in Table 1. The measured transmission parameters are broadband ultrasound attenuation (BUA), speed of sound (SOS), ultrasonic stiffness index (SI), *T*-score and *Z*-score. BUA and SOS are commonly measured parameters, and SI is a linear combination of BUA and SOS derived from the equation SI = (0.67 * BUA + 0.28 * SOS) − 420 (Greenspan et al. 1997; Wear and Armstrong 2001; Economos et al. 2014). Therefore, SI reflects the comprehensive information of ultrasonic attenuation and velocity properties of the calcaneal system. SI also has better

Table 1. Statistics of anthropometric characteristics, bone mineral density, muscle mass, fat mass and ultrasonic transmission parameters

Parameter (582 volunteers)	Units	Mean	Standard deviation	Minimum	Maximum
Anthropometric characteristics					
Age	y	33	11	20	60
Body mass index	kg/m^2	22.8	3.2	15.9	35.5
Body weight	kg	62.5	11.6	41.9	103.0
Height	m	1.66	8.38	1.47	1.93
Bone mineral density					
Whole body	g/cm^2	1.17	0.10	0.92	1.46
Left leg	g/cm^2	1.17	0.13	0.85	1.63
Right leg	g/cm^2	1.18	0.13	0.88	1.61
Hip	g/cm^2	1.00	0.12	0.64	1.39
Spine	g/cm^2	1.17	0.13	0.84	1.54
Muscle mass					
Whole body	kg	41.84	8.33	25.46	64.98
Left leg	kg	7.08	1.63	3.90	11.36
Right leg	kg	7.18	1.66	4.08	11.78
Fat mass					
Whole body	kg	18.71	6.05	5.51	44.53
Left leg	kg	2.96	0.94	0.94	8.10
Right leg	kg	2.98	0.94	0.87	8.09
Ultrasonic transmission parameters					
Stiffness index	1	98	16	55	159
T-score	1	−0.1	1.1	−3.4	3.7
Z-score	1	0.2	1.1	−2.6	4.6
Speed of sound	m/s	1587	39	1401	1714
Broadband ultrasound attenuation	dB/MHz	111	12	75	149

repeatability compared with SOS or BUA alone (Economos et al. 2014). *T*- and *Z*-scores were derived for the ultrasonic diagnosis of osteoporosis.

Statistical analysis

The Kolmogorov–Smirnov test revealed that all measured BMDs and ultrasonic parameters followed the normal distribution, while an abnormal distribution was observed for muscle and fat properties. Pearson's linear correlation analysis was performed for two parameters obeying a normal distribution. Spearman's correlation coefficient was calculated if there was one parameter with an abnormal distribution. The correlations were regarded as significant at a p value <0.05. We analyzed the partial correlations among ultrasonic transmission and musculoskeletal parameters after adjustment for potential confounders including age, sex and BMI. Multiple regressions were implemented to investigate the contribution of muscle and fat to transmission parameters beyond that provided in BMD. The dependent variables were transmission parameters, and the two independent variables were BMD and another musculoskeletal feature (muscle or fat mass). The calculated increase in the adjusted squared correlation coefficient (ΔR^2) represented the additional variance explained by muscle or fat mass independently of BMD. All statistical analysis was implemented in MATLAB 2020b (The MathWorks, Natick, MA, USA).

RESULTS

Associations among ultrasonic transmission and musculoskeletal parameters

Table 2 lists correlations among ultrasonic transmission, musculoskeletal and fat parameters. Ultrasonic transmission parameters were positively correlated with all bone densities at the significance level of 0.001 ($0.35 < R < 0.46$ for BMD-W, $0.27 < R < 0.47$ for leg BMD, $0.35 < R < 0.47$ for hip BMD, and $0.24 < R < 0.36$ for spine BMD). Figure 2 illustrates the associations between SI, *T*-score and left-leg BMD, respectively. SI was significantly correlated with all musculoskeletal parameters ($0.11 < |R| < 0.47$, $p < 0.05$). For the assessment of muscle and fat mass, only BUA was positively correlated with muscle mass ($0.29 < |R| < 0.31$, $p < 0.001$), while SOS only negatively correlated with fat mass ($0.14 < |R| < 0.25$, $p < 0.001$). Table 3 lists the correlations among musculoskeletal and fat parameters. Close associations were observed between BMD and muscle mass ($0.71 < R < 0.72$, $p < 0.001$, for legs, and $R = 0.50$, $p < 0.001$, for the whole body). BMD and fat mass also had significant correlations ($0.14 < R < 0.15$, $p < 0.001$, for legs, $R = 0.20$, $p < 0.001$, for the whole body).

Partial correlation analysis

By partial correlation analysis, Table 4 lists the partial correlations among ultrasonic transmission and musculoskeletal and fat parameters after adjustment for age, sex and BMI. The optimal correlation was still observed between SI and left-leg BMD ($R = 0.51$, $p < 0.001$). All ultrasonic transmission parameters remained positively associated with BMD at all measured sites ($0.31 < R < 0.51$, $p < 0.001$), which was consistent with previous results, and turned out to be significantly correlated with muscle and fat mass of the whole body ($0.10 < |R| < 0.28$, $p < 0.05$). After adjustment, the correlations among transmission parameters and fat mass were more pronounced, with the significance level changing from $p < 0.05$ and $p < 0.01$ to $p < 0.001$. Similar to the results of linear correlations, BUA and SOS were still closely associated with muscle and fat mass, respectively.

Multiple regression analysis predicting ultrasonic properties

Figure 2 also illustrates the multiple regressions for predicting ultrasonic SI and *T*-score, respectively, and the additional variance (ΔR^2) explained by left-leg muscle mass independently of left-leg BMD. By adjusting left-leg BMD as a covariate, left-leg muscle mass explained an additional 6.56% ($p < 0.001$) of the variance of SI (Fig. 2b) and up to 17.83% ($p < 0.001$) of the

Table 2. Correlations among ultrasonic transmission, musculoskeletal and fat parameters

	Bone mineral density					Muscle			Fat		
	Whole body	Left leg	Right leg	Hip	Spine	Whole body	Left leg	Right leg	Whole body	Left leg	Right leg
Stiffness index	0.46*	0.47*	0.46*	0.47*	0.34*	0.12†	0.15*	0.13†	−0.17*	−0.11†	−0.11‡
T-Score	0.35*	0.28*	0.27*	0.42*	0.36*	−0.13†	−0.10‡	−0.12†	−0.18*	n.s.	n.s.
Z-Score	0.43*	0.40*	0.39*	0.43*	0.34*	n.s.	n.s.	n.s.	−0.13†	−0.10‡	−0.10‡
Speed of sound	0.41*	0.40*	0.40*	0.44*	0.33*	n.s.	n.s.	n.s.	−0.25*	−0.14*	−0.15*
BUA	0.37*	0.41*	0.39*	0.35*	0.24*	0.29*	0.31*	0.29*	n.s.	n.s.	n.s.

BUA = broadband ultrasound attenuation; n.s. = not significant.
* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

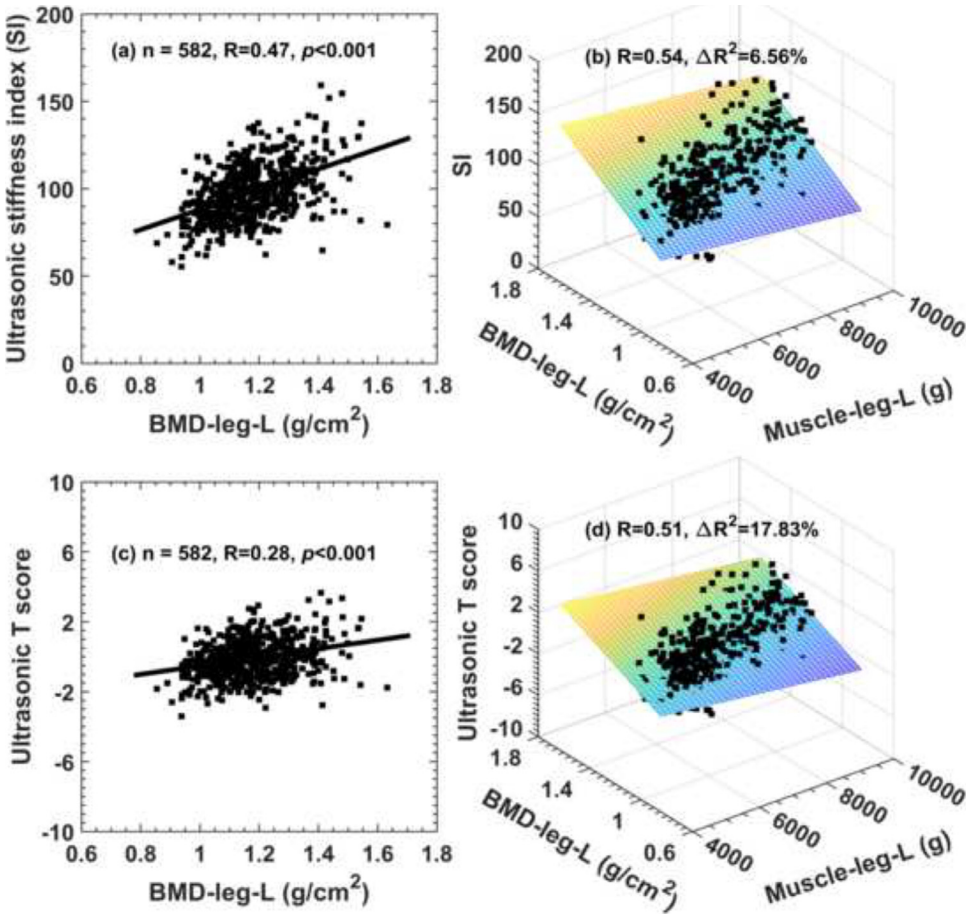

Fig. 2. Associations between (a) SI, (c) *T*-score and left-leg BMD and the multiple regressions for predicting (b) SI and (d) *T*-score with the additional variance explained by left-leg muscle mass independently of left-leg BMD. BMD = bone mineral density; leg-L = left leg; leg-R = right leg; SI = ultrasonic stiffness index.

variance of the ultrasonic *T*-score (Fig. 2d). Similarly, independently of left-leg BMD, left-leg fat mass also significantly contributed to predicting transmission parameters with an additional small 1.01% variance for SI ($p < 0.01$) and 1.55% variance for SOS ($p < 0.001$).

DISCUSSION

Foot MSDs and the feasibility of using the ultrasonic technique to estimate muscle and fat tissues

Foot musculoskeletal disorders were accompanied by reduced heel muscle function, and smaller volume and strength of foot muscles were observed in individuals with foot MSDs (*i.e.,* plantar heel pain) (Osborne et al. 2019). The thickness and stiffness of the heel fat pad in obese individuals were observed to be significantly higher than those in normal individuals, suggesting a higher incidence of foot MSDs in overweight and obese individuals (Taş et al. 2017). The assessment of heel muscle and fat mass is of great importance to the early diagnosis of foot MSDs. A significant indicator of foot MSDs, BMI, was reported to be positively correlated with the thickness of the heel fat pad (Taş et al. 2017). Similarly, our study observed the close and positive correlation between BMI and fat mass ($0.40 < R < 0.60$, $p < 0.001$), as well as the correlation between BMI and muscle mass ($0.40 < R < 0.59$, $p < 0.001$).

After exclusion of BMI (Table 4), the differences in ultrasonic attenuation and velocity properties in muscle and fat tissues may still account for the significant associations between BUA and muscle mass ($p < 0.001$) and between SOS and fat mass ($p < 0.001$). The independent contributions of muscle and fat mass to ultrasonic properties indicate that the ultrasonic through-transmission technique might provide insights into the assessment of musculoskeletal disorders. The aforementioned results and ultrasonic interrogation of biological tissues including calcaneal muscle and fat provide evidence of the feasibility of using the ultrasonic through-transmission technique to estimate skeletal muscle and fat mass.

Table 3. Correlations among bone mineral density, muscle and fat parameters

	Bone mineral density					Muscle			Fat		
	Whole body	Left leg	Right leg	Hip	Spine	Whole body	Left leg	Right leg	Whole body	Left leg	Right leg
Bone mineral density	1	—	—	—	—	—	—	—	—	—	—
Whole body											
Left leg	0.87*	1	—	—	—	—	—	—	—	—	—
Right leg	0.87*	0.98*	1	—	—	—	—	—	—	—	—
Hip	0.78*	0.70*	0.70*	1	—	—	—	—	—	—	—
Spine	0.70*	0.47*	0.47*	0.65*	1	—	—	—	—	—	—
Muscle	0.50*	0.71*	0.72*	0.34*	0.13†	1	—	—	—	—	—
Whole body											
Left leg	0.50*	0.72*	0.72*	0.33*	0.13†	0.97*	1	—	—	—	—
Right leg	0.49*	0.71*	0.72*	0.33*	0.13†	0.97*	0.98*	1	—	—	—
Fat	0.20*	0.14*	0.15*	0.15*	0.18*	0.23*	0.28*	0.28*	1	—	—
Whole body											
Left leg	n.s.	n.s.	n.s.	n.s.	0.18*	−0.12†	n.s.	n.s.	0.79*	1	—
Right leg	n.s.	n.s.	n.s.	n.s.	0.18*	−0.11†	n.s.	n.s.	0.79*	0.98*	1

n.s. = not significant.
* $p < 0.001$.
† $p < 0.01$.

BUA and SOS in assessment of musculoskeletal and fat properties

Ultrasonic attenuation and velocity properties were verified as effective in estimating muscle and fat mass. For linear and partial correlations, the present study revealed both the positive associations between BUA and muscle mass ($p < 0.001$) and the negative associations between SOS and fat mass ($p < 0.001$). After adjustment for potential confounders, the observed persistent associations indicated that the close relationship was independent of age, sex and BMI. The results suggested that the ultrasonic properties of attenuation (BUA) and velocity (SOS) were significant predictors of muscle and fat properties.

BUA and SOS were closely associated with bone characteristics such as bone density (Qin et al. 2019; Lee et al. 2022), mechanical parameters (Hakulinen et al. 2005; Wear et al. 2017) and microstructure parameters (Chaffaí et al. 2002; Wear 2020). BUA had a strong correlation ($R^2 = 0.69$) with DXA heel BMD, and was adopted to evaluate disuse-induced bone loss during 90-d bed rest (Qin et al. 2019). Significantly higher values of BUA (23.6%) and SOS (1.8%) were reported in a high-BMD group than in a low-BMD group (Lee et al. 2022), providing evidence of the positive correlations of BMD with SOS and BUA observed in Tables 2 and 4.

Ultrasonic property differences in bone, muscle and fat tissues may account for the aforementioned associations. The SOS of fat tissue (1450 m/s) is smaller than that of muscle tissue (1600 m/s) and also smaller than that of bone tissue (~4000 m/s) (Amin 1989; Bushberg et al. 2011). Therefore, the greater fat mass indicates the smaller SOS averaged in these biological tissues, explaining the negative correlation between SOS and fat mass (Tables 2 and 4). The attenuation coefficient of fat (0.61 dB/cm) is smaller than that of muscle (0.7−1.4 dB/cm) at the frequency of 1 MHz (Amin 1989). Muscle also has a larger density (1.07 mg/cm^3) and acoustic impedance (1.71×10^6 kg/m^{2}s) compared with the density (0.92 mg/cm^3) and acoustic impedance (1.34×10^6 kg/m^2·s) of fat (Bushberg et al. 2011), accompanied by

Table 4. Correlations among ultrasonic transmission, musculoskeletal and fat parameters after adjustment for age, sex and body mass index

	Bone mineral density					Muscle			Fat		
	Whole body	Left leg	Right leg	Hip	Spine	Whole body	Left leg	Right leg	Whole body	Left leg	Right leg
Stiffness index	0.47*	0.51*	0.49*	0.45*	0.35*	0.16*	0.15*	0.11†	−0.25*	−0.16*	−0.16*
T-score	0.47*	0.50*	0.49*	0.45*	0.35*	0.15*	0.14*	0.11‡	−0.25*	−0.17*	−0.16*
Z-score	0.46*	0.50*	0.48*	0.44*	0.34*	0.15*	0.14*	0.10‡	−0.23*	−0.17*	−0.17*
Speed of sound	0.48*	0.51*	0.51*	0.46*	0.36*	0.10‡	n.s.	n.s.	−0.28*	−0.21*	−0.22*
BUA	0.34*	0.38*	0.35*	0.31*	0.26*	0.23*	0.24*	0.20*	−0.14†	n.s.	n.s.

BUA = broadband ultrasound attenuation; n.s. = not significant.
* $p < 0.001$.
† $p < 0.01$.
‡ $p < 0.05$.

more attenuation in muscle tissues when ultrasound reflects and scatters in muscle tissue fibers. Therefore, more muscle mass will cause more attenuation, leading to a positive correlation between BUA and muscle mass. Stronger ultrasound attenuation in bone tissues also results in a positive correlation between BUA and BMD at multiple sites (Tables 2 and 4).

Contribution of muscle and fat mass in predicting ultrasonic properties

Ultrasonic transmission measurements provided muscle and fat information beyond that contained in BMD. Considering the close associations between BMD and muscle, the contribution of muscle to ultrasonic parameters should be investigated independently of BMD. Because the left calcaneus was not measured by DXA and was physically adjacent to the left leg, the left-leg BMD was taken as the covariate to investigate the contributions of left-leg muscle and fat in predicting ultrasonic properties. The maximum additional 17.83% of the variance explained by muscle revealed that ultrasonic transmission measurements may provide additional muscular properties independently of BMD measurements. A small variance of ultrasonic properties was attributable to fat mass. Another variance may be induced by the mismatch between the ultrasonically interrogated heel tissues and the tissues measured by DXA.

The remaining variability in predicting ultrasonic transmission properties may lie in other properties such as structural (Nicholson et al. 1998; Chaffaí et al. 2002; Wear et al. 2012), mechanical (Hans et al. 1999; Wear et al. 2017) and composition (Hoffmeister et al. 2002) properties. Some *in vitro* studies have reported the additional variance provided by microstructure parameters independently of BMD (Wear et al. 2012), bone volume fraction (Chaffaí et al. 2002) and bone apparent density (Nicholson et al. 1998). Together with BMD, elasticity and anisotropy information introduced 96%−98% variance in SOS (Hans et al. 1999). SOS and BUA yielded significant changes during the demineralization and decollagenization of bone specimens, indicating the potential contribution of collagen and mineral content in explaining ultrasonic transmission properties (Hoffmeister et al. 2002).

The influencing factors of ultrasonic properties

The present study adopted the GE Achilles instrument to measure ultrasonic properties because of its stability (Economos et al. 2014) and ability to measure bone properties (*i.e.,* discriminating osteoporosis [Greenspan et al. 1997; Jin et al. 2010] and hip fracture [Schott et al. 1995]). The precision errors of four calcaneal instruments based on the QUS technique were compared, and the ultrasonic SI of the GE Achilles possessed the smallest inter-observer, positioning and short-term precision errors, which were statistically similar to those of calcaneal DXA (Greenspan et al. 1997). A previous study also validated the internal stability of GE Achilles machines and the good repeatability of ultrasonic SI based on consistent measurements (Economos et al. 2014). The measured ultrasonic properties may slightly change with different ultrasonic devices.

Body weight was positively correlated with BUA ($R = 0.17$, $p < 0.001$) and negatively correlated with SOS ($R = -0.11$, $p < 0.05$), which was consistent with previous studies (Correa-Rodríguez et al. 2016; Forero-Bogotá et al. 2017). Significantly higher BUA values were reported in overweight and obese adolescents as compared with normal adolescents (Rodríguez et al. 2014), also indicating the effect of body weight on ultrasonic properties.

Ultrasonic measurements along different directions will determine ultrasonic interrogated bone tissue with different shapes, which may produce different values of SOS and BUA and further influence the correlations between ultrasonic parameters and bone properties. The curved shape of cortical bone, acting as an acoustic lens, may distort the wavefronts and induce phase cancellation effects (Hoffmeister et al. 2011). The curved calcaneal surfaces have induced the artifacts of normalized BUA caused by phase cancellation (Langton and Subhan 2001). To investigate the effects of measurement directions on ultrasonic parameters, *in vitro* ultrasonic measurements were performed along the proximal−distal (PD), medial−lateral (ML) and anterior−posterior (AP) orientations (Liu et al. 2018). Ultrasonic parameters averaged over these anatomical orientations exhibited higher correlations with bone features than those obtained with only one orientation (Liu et al. 2018). However, only medial−lateral orientation is accessible for *in vivo* ultrasonic measurements of the calcaneal system. The effects of bone shape on ultrasonic parameters are worthy of further investigation.

Limitations

One limitation of the study lies in the tissue mismatch between ultrasound and DXA measurements. Ultrasonic measurements were performed on the heel, whereas heel musculoskeletal properties were not derived by DXA measurements. Ultrasonic measurements could be performed on more body sites (*e.g.,* legs) for the assessment of site-matched musculoskeletal properties. The present study analyzed mainly 2-D BMD data, and 3-D structural information of the bone should be analyzed to investigate ultrasonic anisotropic scattering in cancellous bone in the future. Another limitation of the study is that the recruited volunteers were all from

China, and a study with volunteers from different countries and races is needed.

CONCLUSIONS

Ultrasonic through-transmission measurements could estimate not only skeletal characteristics but also muscle and fat content. Ultrasonic properties of attenuation (BUA) and velocity (SOS) were verified as significant predictors for characterizing muscle and fat mass. Independently of BMD, muscle properties contributed significantly in predicting ultrasonic properties, with an additional explained 17.83% of the variance at best. The results suggested that the ultrasonic transmission technique has potential in the assessment of musculoskeletal diseases.

Acknowledgments—We are grateful to all volunteers for their cooperation and to Chen Ding (professor at the Human Phenome Institute, Fudan University) for organizing the experiment. This work was supported by the Shanghai Municipal Science and Technology Major Project (Grant No. 2017SHZDZX01), the National Natural Science Foundation of China (11874289, 11804056, 12122403 and 12034005), the Shanghai Science and Technology Innovation Plan (20S31901300, 19441903400), the China Postdoctoral Science Foundation (2021M690709), the Shanghai Rising-Star Program (21QC1400100) and the China Scholarship Council (202106100122).

Conflict of interest disclosure—The authors declare no conflicts of interest.

Data availability statement—The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Amin VR. Ultrasonic attenuation estimation for tissue characterization. MS thesis. : Iowa State University; 1989.

Bauer DC, Glüer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM. Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women: A prospective study. Arch Intern Med 1997;157:629–634.

Bevan S. Economic impact of musculoskeletal disorders (MSDs) on work in Europe. Best Pract Res Clin Rheumatol 2015;29:356–373.

Bhattacharya A. Costs of occupational musculoskeletal disorders (MSDs) in the United States. Int J Ind Ergon 2014;44:448–454.

Bushberg JT, Seibert JA, Leidholdt EM, Boone JM. The essential physics of medical imaging. 3rd edition Philadelphia: Lippincott Williams & Wilkins; 2011.

Chaffaí S, Peyrin F, Nuzzo S, Porcher R, Berger G, Laugier P. Ultrasonic characterization of human cancellous bone using transmission and backscatter measurements: Relationships to density and microstructure. Bone 2002;30:229–237.

Chang RF, Lee CC, Lo CM. Computer-aided diagnosis of different rotator cuff lesions using shoulder musculoskeletal ultrasound. Ultrasound Med Biol 2016;42:2315–2322.

Chen HS, Lin SH, Hsu YH, Chen SC, Kang JH. A comparison of physical examinations with musculoskeletal ultrasound in the diagnosis of biceps long head tendinitis. Ultrasound Med Biol 2011;37:1392–1398.

Cheung WK, Cheung JPY, Lee WN. Role of ultrasound in low back pain: A review. Ultrasound Med Biol 2020;46:1344–1358.

Chiasson MÈ, Imbeau D, Aubry K, Delisle A. Comparing the results of eight methods used to evaluate risk factors associated with musculoskeletal disorders. Int J Ind Ergon 2012;42:478–488.

Correa-Rodríguez M, Schmidt-RioValle J, González-Jiménez E, Rueda-Medina B. The effects of body composition, dietary intake, and physical activity on calcaneus quantitative ultrasound in Spanish young adults. Biol Res Nurs 2016;18:439–444.

Daugschies M, Brixen K, Hermann P, Rohde K, Glüer CC, Barkmann R. Quantitative ultrasound measurements at the heel: Improvement of short- and mid-term speed of sound precision. Ultrasound Med Biol 2015;41:858–870.

Dudley-Javoroski S, McMullen T, Borgwardt MR, Peranich LM, Shields RK. Reliability and responsiveness of musculoskeletal ultrasound in subjects with and without spinal cord injury. Ultrasound Med Biol 2010;36:1594–1607.

Economos CD, Sacheck JM, Wacker W, Shea K, Naumova EN. Precision of Lunar Achilles+ bone quality measurements: Time dependency and multiple machine use in field studies. Br J Radiol 2014;80:919–925.

Edwards MH, Dennison EM, Aihie Sayer A, Fielding R, Cooper C. Osteoporosis and sarcopenia in older age. Bone 2015;80:126–130.

Forero-Bogotá M, Ojeda-Pardo M, García-Hermoso A, Correa-Bautista J, González-Jiménez E, Schmidt-Ríovalle J, Navarro-Pérez C, Gracia-Marco L, Vlachopoulos D, Martínez-Torres J, Ramírez-Vélez R. Body composition, nutritional profile and muscular fitness affect bone health in a sample of schoolchildren from Colombia: The FUPRECOL study. Nutrients 2017;9:106.

Greenspan SL, Bouxsein ML, Melton ME, Kolodny AH, Orwoll ES. Precision and discriminatory ability of calcaneal bone assessment technologies. J Bone Miner Res 1997;12:1303–1313.

Guerri S, Mercatelli D, Aparisi Gómez MP, Napoli A, Battista G, Guglielmi G, Bazzocchi A. Quantitative imaging techniques for the assessment of osteoporosis and sarcopenia. Quant Imaging Med Surg 2018;8:60–85.

Hakulinen MA, Day JS, Töyräs J, Timonen M, Kröger H, Weinans H, Kiviranta I, Jurvelin JS. Prediction of density and mechanical properties of human trabecular bone in vitro by using ultrasound transmission and backscattering measurements at 0.2–6.7 MHz frequency range. Phys Med Biol 2005;50:1629–1642.

Hans D, Dargent-Molina P, Schott AM, Sebert JL, Meunier PJ. Ultrasonographic heel measurements to predict hip fracture in elderly women: The EPIDOS Prospective Study. Lancet 1996;348:511–514.

Hans D, Wu C, Njeh CF, Zhao S, Augat P, Newitt D, Link T, Lu Y, Majumdar S, Genant HK. Ultrasound velocity of trabecular cubes reflects mainly bone density and elasticity. Calcif Tissue Int 1999;64:18–23.

Hoffmeister BK, Whitten S, Kaste S, Rho J. Effect of collagen and mineral content on the high-frequency ultrasonic properties of human cancellous bone. Osteoporosis Int 2002;13:26–32.

Hoffmeister BK, Holt AP, Kaste SC. Effect of the cortex on ultrasonic backscatter measurements of cancellous bone. Phys Med Biol 2011;56:6243–6255.

Jin N, Lin S, Zhang Y, Chen F. Assess the discrimination of Achilles InSight calcaneus quantitative ultrasound device for osteoporosis in Chinese women: Compared with dual energy X-ray absorptiometry measurements. Eur J Radiol 2010;76:265–268.

Lalumiere M, Larivière C, Nadeau M-J, Paquette P, Lamontagne M, Desmeules F, Gagnon DH. Proposing a minimal data set of musculoskeletal ultrasound imaging biomarkers to inform clinical practice: An analysis founded on the Achilles tendon. Ultrasound Med Biol 2020;46:2222–2235.

Langton CM, Njeh CF. The measurement of broadband ultrasonic attenuation in cancellous bone—A review of the science and technology. IEEE Trans Ultrason Ferroelectr Freq Control 2008;55:1546–1554.

Langton CM, Subhan M. Computer and experimental simulation of a cortical end-plate phase cancellation artefact in the measurement of BUA at the calcaneus. J Physiol Meas 2001;22:1–8.

Langton C, Palmer S, Porter R. The measurement of broadband ultrasonic attenuation in cancellous bone. Eng Med 1984;13:89–91.

Langton CM, Njeh CF, Hodgskinson R, Currey JD. Prediction of mechanical properties of the human calcaneus by broadband ultrasonic attenuation. Bone 1996;18:495–503.

Laugier P, Novikov V, Elmann-Larsen B, Berger G. Quantitative ultrasound imaging of the calcaneus: Precision and variations during a 120-day bed rest. Calcif Tissue Int 2000;66:16–21.

Lee S, Fujita C, Satoh A. Baseline body composition and physical activity level recommended for optimal bone mineral density in young women. Women's Health Rep (New Rochelle, NY) 2022;3:351–358.

Lima RM, De Oliveira RJ, Raposo R, Neri SGR, Gadelha AB. Stages of sarcopenia, bone mineral density, and the prevalence of osteoporosis in older women. Archi Osteoporos 2019;14:38.

Liu C, Li B, Diwu Q, Li Y, Zhang R, Ta D, Wang W. Relationships of ultrasonic backscatter with bone densities and microstructure in bovine cancellous bone. IEEE Trans Ultrason Ferroelectr Freq Control 2018;65:2311–2321.

Nicholson PHF, Bouxsein ML. Bone marrow influences quantitative ultrasound measurements in human cancellous bone. Ultrasound Med Biol 2002;28:369–375.

Nicholson P, Muller R, Lowet G, Cheng X, Hildebrand T, Ruegsegger P, Perre G, Dequeker J, Boonen S. Do quantitative ultrasound measurements reflect structure independently of density in human vertebral cancellous bone?. Bone 1998;23:425.

Osborne JWA, Menz HB, Whittaker GA, Landorf KB. Muscle function and muscle size differences in people with and without plantar heel pain: A systematic review. J Orthop Sports Phys Ther 2019;49:925–933.

Pahor M, Manini T, Cesari M. Sarcopenia: Clinical evaluation, biological markers and other evaluation tools. J Nutr Health Aging 2009;13:724–728.

Qi H, Sheng Y, Chen S, Wang S, Zhang A, Cai J, Lai B, Ding G. Bone mineral density and trabecular bone score in Chinese subjects with sarcopenia. Aging Clin Exp Res 2019;31:1549–1556.

Qin YX, Xia Y, Muir J, Lin W, Rubin CT. Quantitative ultrasound imaging monitoring progressive disuse osteopenia and mechanical stimulation mitigation in calcaneus region through a 90-day bed rest human study. J Orthop Translat 2019;18:48–58.

Rahmani N, Mohseni-Bandpei MA, Vameghi R, Salavati M, Abdollahi I. Application of ultrasonography in the assessment of skeletal muscles in children with and without neuromuscular disorders: A systematic review. Ultrasound Med Biol 2015;41:2275–2283.

Rodríguez M, Medina BR, Jiménez E, Pérez C, Schmidt-Riovalle J. The levels of bone mineralization are influenced by body composition in children and adolescents. Nutr Hosp 2014;30:763–768.

Roux C, Roberjot V, Porcher R, Kolta S, Dougados M, Laugier P. Ultrasonic backscatter and transmission parameters at the os calcis in postmenopausal osteoporosis. J Bone Miner Res 2001;16:1353–1362.

Schott AM, Weill-Engerer S, Hans D, Duboeuf F, Delmas PD, Meunier PJ. Ultrasound discriminates patients with hip fracture equally well as dual energy X-ray absorptiometry and independently of bone mineral density. J Bone Miner Res 1995;10:243–249.

Sutter T, Toumi H, Valery A, El Hage R, Pinti A, Lespessailles E. Relationships between muscle mass, strength and regional bone mineral density in young men. PLoS One 2019;14 e0213681.

Taş S, Bek N, Ruhi Onur M, Korkusuz F. Effects of body mass index on mechanical properties of the plantar fascia and heel pad in asymptomatic participants. Foot Ankle Int 2017;38:779–784.

Thompson P, Taylor J, Fisher A, Oliver R. Quantitative heel ultrasound in 3180 women between 45 and 75 years of age: Compliance, normal ranges and relationship to fracture history. Osteoporos Int 1998;8:211–214.

Turner CH, Peacock M, Timmerman L, Neal JM, Johnston CC. Calcaneal ultrasonic measurements discriminate hip fracture independently of bone mass. Osteoporos Int 1995;5:130–135.

Valachi B, Valachi K. Mechanisms leading to musculoskeletal disorders in dentistry. J Am Dent Assoc 2003;134:1344–1350.

Walsh MC, Hunter GR, Livingstone MB. Sarcopenia in premenopausal and postmenopausal women with osteopenia, osteoporosis and normal bone mineral density. Osteoporos Int 2006;17:61–67.

Wear KA. Mechanisms of interaction of ultrasound with cancellous bone: A review. IEEE Trans Ultrason Ferroelectr Freq Control 2020;67:454–482.

Wear KA, Armstrong DW. Relationships among calcaneal backscatter, attenuation, sound speed, hip bone mineral density, and age in normal adult women. J Acoust Soc Am 2001;110:573–578.

Wear KA, Stuber AP, Reynolds JC. Relationships of ultrasonic backscatter with ultrasonic attenuation, sound speed and bone mineral density in human calcaneus. Ultrasound Med Biol 2000;26:1311–1316.

Wear KA, Nagaraja S, Dreher ML, Gibson SL. Relationships of quantitative ultrasound parameters with cancellous bone microstructure in human calcaneus in vitro. J Acoust Soc Am 2012;131:1605–1612.

Wear KA, Nagaraja S, Dreher ML, Sadoughi S, Zhu S, Keaveny TM. Relationships among ultrasonic and mechanical properties of cancellous bone in human calcaneus in vitro. Bone 2017;103:93–101.