Ultrasonic backscatter difference measurements of cancellous bone from the human femur: Relation to bone mineral density and microstructure

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Ultrasonic backscatter techniques are being developed to detect changes in cancellous bone caused by osteoporosis. One technique, called the backscatter difference technique, measures the power difference between two portions of a backscatter signal. The goal of the present study is to investigate how bone mineral density (BMD) and the microstructure of human cancellous bone influence four backscatter difference parameters: the normalized mean of the backscatter difference (nMBD) spectrum, the normalized slope of the backscatter difference spectrum, the normalized intercept of the backscatter difference spectrum, and the normalized backscatter amplitude ratio (nBAR). Ultrasonic measurements were performed with a 3.5 MHz broadband transducer on 54 specimens of human cancellous bone from the proximal femur. Volumetric BMD and the microstructural characteristics of the specimens were measured using x-ray micro-computed tomography. Of the four ultrasonic parameters studied, nMBD and nBAR demonstrated the strongest univariate correlations with density and microstructure. Multivariate analyses indicated that nMBD and nBAR depended on trabecular separation and possibly other microstructural characteristics of the specimens independently of BMD. These findings suggest that nMBD and nBAR may be sensitive to changes in the density and microstructure of bone caused by osteoporosis.

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I. INTRODUCTION

Ultrasonic devices, such as heel bone sonometers, are used to screen patients for osteoporosis, a degenerative bone disease that causes microstructural changes to bone resulting in increased bone fragility. Approximately 150×10^6 people worldwide are at risk of osteoporotic fracture, and the number is expected to double by 2040.¹ The clinical diagnosis of osteoporosis is based on dual energy x-ray absorptiometry (DXA) measurements of bone mineral density (BMD). However, heel bone sonometers have become popular for screening purposes because the devices are inexpensive, portable, and do not use ionizing radiation.

Heel bone sonometers operate by measuring the velocity and/or attenuation of ultrasonic pulses propagated through the calcaneus. Separate transducers are positioned on opposite sides of the bone to transmit and receive the ultrasonic pulses. The calcaneus is chosen as the measurement location because the bone is easily accessible from both sides and because the faces of the bone are relatively flat and perpendicular to the direction of wave propagation. In addition, the

calcaneus is a weight bearing bone that has a high volume fraction of cancellous bone tissue.

The calcaneus, like many other bones in the body such as the hip (proximal femur) and vertebrae, consists of an outer layer of cortical bone that surrounds an inner region of cancellous bone. Cancellous bone tissue is much more porous than cortical bone tissue. The microstructure of cancellous bone consists of a branched network of rod- and plate-like trabeculae that have a typical thickness and separation of $150 \mu\text{m}$ and $750 \mu\text{m}$, respectively, depending on several factors such as bone health and anatomic location.² As osteoporosis progresses, the trabeculae become thinner, sparser, and less well interconnected.³ These microstructural changes cause the bone to weaken, leading to increased risk of fracture. These changes are especially problematic because they may be irreversible, even with pharmacologic treatment.^{4,5}

There is interest in developing diagnostic techniques that are sensitive to changes in the microstructure of cancellous bone caused by osteoporosis. DXA systems are sensitive mainly to the concentration of bone mineral (hydroxyapatite). They do not have the resolution to directly image the trabecular microstructure of cancellous bone. However, changes in microstructure may influence how

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ultrasonic waves propagate through bone. Indeed, laboratory studies have found that the normalized broadband ultrasonic attenuation (nBUA) and speed of sound (SOS) depend on microstructural parameters such as trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp).^{6–9} Other studies have found that nBUA and SOS depend on measurement direction, indicating that ultrasonic waves are sensitive to the alignment and possibly the geometry of the trabeculae.^{10–14}

Heel bone sonometry was introduced more than 30 years ago. Since then, many new ultrasonic bone assessment techniques have been developed, including through-transmission measurements of attenuation and SOS at other skeletal locations, axial transmission measurements of guided waves along long bones, and pulse-echo measurements of cortical and cancellous bone. As new techniques emerge, it is both interesting and important to determine how microstructure influences measurements performed with these techniques.

Several new techniques are based on backscatter measurements of cancellous bone. Backscatter measurements are performed by propagating ultrasonic pulses into regions of cancellous bone and receiving the returned signal. Scattering occurs as the ultrasonic wave interacts with the trabeculae. Theoretical and experimental studies suggest that the backscattered wave is sensitive to the size, shape, orientation, and distribution of the trabeculae, as well as their composition.^{6–9,15–23} Backscatter measurements are convenient to perform because a single transducer can be used. This may facilitate easier access to central skeletal sites such as the hip and spine where approximately two-thirds of osteoporotic fractures occur.²⁴

Recently, a new backscatter technique was introduced that analyzes the power difference between two portions of a backscatter signal. Parameters based on the backscatter difference technique have demonstrated good correlations with bone density, indicating that they may be sensitive to changes in cancellous bone caused by osteoporosis.^{25–27} The backscatter difference technique may offer certain advantages over other backscatter techniques that have been proposed for bone assessment purposes. For example, backscatter difference measurements may be inherently less sensitive to errors caused by intervening tissues that lie between the transducer and ultrasonically interrogated region of bone.²⁸ In addition, backscatter difference parameters may not depend strongly on the portion of the backscatter signal that is analyzed.²⁷

The goal of the present study is to determine how backscatter difference measurements depend on BMD and the microstructural characteristics of human cancellous bone *in vitro*. Specifically, four backscatter difference parameters were measured: the normalized mean of the backscatter difference (nMBD) spectrum, the normalized slope of the backscatter difference (nSBD) spectrum, the normalized intercept of the backscatter difference (nIBD) spectrum, and the normalized backscatter amplitude ratio (nBAR). These represent all of the backscatter difference parameters that have been reported in the literature so far. BMD and the microstructural characteristics of bone were determined using x-ray micro-computed tomography (microCT).

II. METHODS

A. Specimen preparation

A total of 54 specimens of cancellous bone were prepared from the proximal ends of 14 left human femurs (8 females, 6 males, age = 49 ± 10 yr). A band saw was used to cut the specimens into 10 mm cubes. As shown in Fig. 1, specimens were oriented so that the axes of the cube were aligned with the anatomic axes of the proximal femur: anterior-posterior, medial-lateral, and proximal-distal. A water jet was used to remove marrow from the intertrabecular spaces. The specimens were placed in phosphate buffered saline solution and stored in refrigeration until the time of testing.

B. Micro-CT measurements

High-resolution microCT (μ CT40, Scanco Medical, Basserdorf, Switzerland) was used to scan and measure morphometric parameters of the bone specimens. The specimens were scanned at $10 \mu\text{m}$ resolution (isotropic voxel size) using an energy level of 55 kVp, an integration time of 200 ms, and an intensity of $145 \mu\text{A}$. Morphometric parameters were evaluated using a segmentation threshold of 220. The threshold was determined from the midpoint between the “bone” and “non-bone” peaks of the voxel brightness histogram.²⁹ The segmented images were compared to the original grayscale images to ensure that physiologically accurate representations were obtained. Images were generated from a $7 \text{ mm} \times 7 \text{ mm} \times 7 \text{ mm}$ region of interest (ROI) centered on the cube shaped specimen. Scanco’s built-in image analysis software was used to measure nine characteristics of the specimen: BMD, bone volume ratio (BV/TV), bone surface to volume ratio (BS/BV), connectivity (Conn-Dens.), structural model index (SMI), trabecular number (Tb.N), Tb.Th, Tb.Sp, and degree of anisotropy (DA). Table I provides a brief description of each parameter. The parameters can be categorized as either representing bone quantity or bone microstructure. BMD and BV/TV represent measures of bone quantity because they depend on the amount of

FIG. 1. Measurements were performed on cube shaped specimens of cancellous bone prepared from the proximal femur.

TABLE I. Description of microCT parameters measured in this study.

MicroCT parameter	Units	Description
BMD	mg/cm ³	Bone mineral density. Concentration of bone mineral (hydroxyapatite).
BV/TV		Bone volume ratio. Volume of bone relative to total scanned volume.
BS/BV	mm ⁻¹	Bone surface to volume ratio. Surface area of bone relative to bone volume.
Tb.Th	mm	Trabecular thickness.
Tb.N	mm ⁻¹	Trabecular number. Number of trabeculae per unit length.
Tb.Sp	mm	Trabecular separation.
SMI		Structural model index. 0 = ideal plate structure; 3 = ideal rod structure.
Conn-Dens.	mm ⁻³	Connectivity density. Number of connections between trabeculae normalized by total scanned volume.
DA		Degree of anisotropy. 1 = isotropic.

mineralized bone tissue, and not on how the tissue is organized throughout the volume. In contrast, the other seven microCT parameters (BS/BV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.Dens, and DA) represent measures of bone microstructure because they depend on how the mineralized tissue is organized and distributed throughout the volume.

C. Ultrasonic measurements

Bone specimens were placed on the bottom of a water filled tank after being vacuum degassed for 10 min. Ultrasonic backscatter measurements were performed using a broadband 3.5 MHz transducer (Panametrics V382, Waltham, MA) with a 12.7 mm diameter aperture and a 1.8 mm beam diameter at the focal distance of 50.8 mm. The transducer is similar to those used in several other backscatter studies of cancellous bone.^{7,21,23,27,30–34} The transducer was mounted to the end of a vertical arm connected to a custom three axis mechanical scanner that was used to scan the transducer over the specimen. The transducer was connected to an ultrasonic pulser/receiver (Panametrics 5077PR, Waltham, MA), and the received signals were digitized by a 12-bit digitizer (PicoScope 5244A, Pico Technology, Wales, England) operating at 500 MSa/s. Ultrasonic signals were analyzed from an 8 × 8 mm, 40 × 40 point ROI centered on the specimen. Measurements were performed along all six directions (anterior, posterior, medial, lateral, proximal, distal). Backscatter signals were digitized from each site of the scan and saved for analysis. Example signals are shown in Fig. 2.

D. Signal analysis

Signals were analyzed using software developed in LabVIEW (National Instruments, Austin, TX). As seen in Fig. 2(b), analysis gates labeled 1 and 2 were used to select two different portions of the signal for analysis from which the power spectra $P_1(f)$ and $P_2(f)$ were determined. The first analysis gate was delayed by an amount τ_d from the start of the backscatter signal. The two gates had the same duration (width) τ_w and were separated by an amount τ_s as shown. A rectangular windowing function was used for all gates. The spectra $P_1(f)$ and $P_2(f)$ were converted to dB and subtracted to obtain the backscatter difference spectrum $D(f)$,

$$D(f) = 10 \log_{10} P_1(f) - 10 \log_{10} P_2(f). \quad (1)$$

The difference spectrum was divided by the time between the centers of the two gates, $\tau_w + \tau_s$, to obtain a normalized difference spectrum $nD(f)$,

$$nD(f) = D(f)/(\tau_w + \tau_s). \quad (2)$$

Three backscatter difference parameters were determined from $nD(f)$. The nMBD was determined by frequency averaging $nD(f)$ from 2 to 5 MHz, a frequency range based on the -6 dB bandwidth of the transducer. The nSBD and the nIBD were determined from the slope and intercept, respectively, of a line fitted to the normalized difference spectrum $nD(f)$ over the same frequency range.

The backscatter signals also were analyzed in the time domain to determine a parameter called the nBAR. nBAR depends on the ratio of the root mean square voltage (V_{rms}) of the first and second gated portions of the backscatter signal

$$nBAR = \frac{\log_{10}(V_{rms1}/V_{rms2})}{(\tau_w + \tau_s)}. \quad (3)$$

Like the other three ultrasound parameters (nMBD, nSBD, and nIBD), nBAR is sensitive to the power difference between the two gated portions of the backscatter signal.

The four backscatter difference parameters nMBD, nSBD, nIBD, and nBAR were analyzed for 34 different combinations of gate delay (τ_d), gate width (τ_w), and gate separation (τ_s). The same combinations were used in a recent study that examined the effects of gate choice on backscatter difference measurements of bone.²⁷ Briefly, values for τ_d , τ_w , and τ_s ranged from 2 to 7 μ s, 1 to 3 μ s, and 0 to 5 μ s, respectively, in 1 μ s increments. These values were chosen so that the first gate avoided the echo from the front surface of the specimen, and both gated portions of the signal had a signal-to-noise ratio greater than 10 dB. Table II lists the 34 combinations of τ_d , τ_w , and τ_s that were analyzed.

E. Statistical analysis

A Shapiro-Wilk test was used to assess the normality of the distributions. Spearman's correlation coefficients were computed to examine univariate relationships between the ultrasonic parameters and the microCT parameters. Partial correlation analysis was used to examine relationships between the ultrasonic parameters and the microCT

FIG. 2. Backscatter signals and microCT images from a low (a), medium (b), and high (c) density specimen of bone. Backscatter measurements were performed by analyzing the power difference between two different gated portions of the backscatter signal labeled “1” and “2” (b). The gates were delayed by an amount τ_d from the echo from the front surface of the specimen. The gates had the same duration (width) τ_w and were separated by an amount τ_s .

parameters controlled for BMD. Stepwise multiple linear regression was used to determine optimal combinations of microCT parameters as predictors of the ultrasound parameters. All statistical computations were performed using SPSS statistics software (IBM Corporation, Armonk, NY). Statistical results were considered significant for $p < 0.05$.

III. RESULTS

Table III reports the mean, standard deviation, minimum, and maximum values of the microCT parameters. The

TABLE II. Combinations of gate delay (τ_d), gate width (τ_w), and gate separation (τ_s) analyzed in this study. A total of 34 combinations were analyzed.

τ_d (μ s)	τ_w (μ s)	τ_s (μ s)	τ_d (μ s)	τ_w (μ s)	τ_s (μ s)	τ_d (μ s)	τ_w (μ s)	τ_s (μ s)
2	1	0	3	1	0	4	1	3
2	1	1	3	1	1	4	2	0
2	1	2	3	1	2	4	2	1
2	1	3	3	1	3	5	1	0
2	1	4	3	1	4	5	1	1
2	1	5	3	2	0	5	1	2
2	2	0	3	2	1	5	2	0
2	2	1	3	2	2	6	1	0
2	2	2	3	3	0	6	1	1
2	2	3	4	1	0	7	1	0
2	3	0	4	1	1			
2	3	1	4	1	2			

Shapiro-Wilk test found that four of the microCT parameters (BMD, BV/TV, Tb.Th, and SMI) were not normally distributed, and two of the backscatter difference parameters (nSBD and nIBD) were not normally distributed for some gate combinations. Thus, Spearman’s rank correlation coefficients (denoted by ρ) were used to measure the strength of associations between two variables rather than Pearson’s correlation coefficients. Table IV reports Spearman’s rank correlation coefficients between the microCT parameters. Tables V–VIII report Spearman’s rank correlation coefficients between the ultrasound parameters and the microCT parameters for a subset of gate choices (even valued gate delays and gate separations).

TABLE III. Mean, standard deviation, minimum, and maximum values of microCT parameters from all specimens ($n = 54$).

MicroCT parameter	Mean	Standard Deviation	Minimum	Maximum
BMD (mg/cm^3)	208	91.1	85.1	512
BV/TV	0.177	0.090	0.059	0.477
BS/BV (mm^{-1})	17.4	4.69	7.43	26.4
Tb.Th (mm)	0.156	0.044	0.101	0.309
Tb.N (mm^{-1})	1.23	0.217	0.086	1.92
Tb.Sp (mm)	0.782	0.137	0.513	1.12
SMI	0.639	0.845	−2.06	2.45
Conn-Dens. (mm^{-3})	3.80	1.28	1.59	6.56
DA	1.97	0.331	1.33	2.69

TABLE IV. Spearman's rank correlation coefficients between microCT parameters. n.s. = not significant.

	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
BMD	1	0.99 ^a	-0.92 ^a	0.87 ^a	0.67 ^a	-0.66 ^a	-0.92 ^a	n.s.	0.35 ^b
BV/TV		1	-0.93 ^a	0.88 ^a	0.65 ^a	-0.65 ^a	-0.93 ^a	n.s.	0.36 ^c
BS/BV			1	-0.98 ^a	-0.42 ^c	0.42 ^c	0.87 ^a	n.s.	n.s.
Tb.Th				1	0.39 ^c	-0.40 ^c	-0.79 ^a	n.s.	n.s.
Tb.N					1	-0.99 ^a	-0.55 ^a	0.76 ^a	0.27 ^b
Tb.Sp						1	0.53 ^a	-0.75 ^a	-0.29 ^b
SMI							1	n.s.	-0.42 ^c
Conn-Dens.								1	n.s.
DA									1

^a $p < 0.0001$.^b $p < 0.05$.^c $p < 0.01$.TABLE V. Spearman's rank correlation coefficients between nMBD and the microCT parameters. Results are presented for a subset of the 34 gate combinations investigated (even values of τ_d and τ_s). n.s. = not significant.

$[\tau_d, \tau_w, \tau_s] (\mu s)$	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
[2,1,0]	0.80 ^a	0.80 ^a	-0.72 ^a	0.72 ^a	0.64 ^a	-0.64 ^a	-0.68 ^{a,d}	0.28 ^b	n.s.
[2,1,2]	0.87 ^a	0.88 ^a	-0.79 ^a	0.77 ^a	0.70 ^a	-0.70 ^a	-0.76 ^a	0.32 ^b	n.s.
[2,1,4]	0.90 ^a	0.91 ^a	-0.80 ^a	0.76 ^a	0.76 ^a	-0.76 ^{a,d}	-0.80 ^a	0.36 ^c	0.28 ^b
[2,2,0]	0.88 ^a	0.88 ^a	-0.79 ^a	0.80 ^a	0.76 ^a	-0.70 ^a	-0.77 ^a	0.29 ^b	n.s.
[2,2,2]	0.90 ^a	0.91 ^a	-0.80 ^a	0.77 ^a	0.75 ^a	-0.74 ^{a,d}	-0.79 ^a	0.36 ^c	n.s.
[2,3,0]	0.89 ^a	0.90 ^a	-0.80 ^a	0.76 ^a	0.72 ^a	-0.72 ^{a,d}	-0.79 ^a	0.33 ^b	n.s.
[4,1,0]	0.74 ^a	0.76 ^a	-0.68 ^a	0.66 ^a	0.54 ^a	-0.56 ^a	-0.63 ^a	n.s.	n.s.
[4,1,2]	0.91 ^a	0.91 ^a	-0.79 ^a	0.75 ^a	0.75 ^a	-0.76 ^{a,d}	-0.80 ^a	0.36 ^c	0.29 ^b
[4,2,0]	0.90 ^a	0.90 ^a	-0.78 ^a	0.74 ^a	0.74 ^a	-0.75 ^{a,d}	-0.80 ^a	0.36 ^{c,d}	n.s.
[6,1,0]	0.79 ^a	0.78 ^a	-0.67 ^a	0.62 ^a	0.70 ^a	-0.72 ^a	-0.73 ^a	0.33 ^b	0.30 ^b

^a $p < 0.0001$.^b $p < 0.05$.^c $p < 0.01$.^dSignificant partial correlation controlled for BMD ($p < 0.05$).TABLE VI. Spearman's rank correlation coefficients between nSBD and the microCT parameters. Results are presented for a subset of the 34 gate combinations investigated (even values of τ_d and τ_s). n.s. = not significant.

$[\tau_d, \tau_w, \tau_s] (\mu s)$	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
[2,1,0]	0.39 ^a	0.39 ^a	-0.43 ^a	0.45 ^b	0.36 ^a	-0.37 ^a	-0.32 ^c	n.s.	n.s.
[2,1,2]	0.57 ^d	0.55 ^d	-0.52 ^d	0.50 ^b	0.52 ^d	-0.52 ^d	-0.52 ^d	0.27 ^c	n.s.
[2,1,4]	0.66 ^d	0.66 ^d	-0.62 ^d	0.59 ^d	0.55 ^d	-0.56 ^d	-0.56 ^d	0.28 ^c	n.s.
[2,2,0]	0.68 ^d	0.69 ^d	-0.57 ^d	0.59 ^d	0.67 ^d	-0.62 ^{d,e}	-0.61 ^d	n.s.	0.28 ^c
[2,2,2]	0.64 ^d	0.64 ^d	-0.53 ^d	0.49 ^{b,c}	0.63 ^d	-0.63 ^{d,e}	-0.55 ^d	0.39 ^a	0.30 ^c
[2,3,0]	0.65 ^d	0.64 ^d	-0.54 ^d	0.51 ^{d,e}	0.67 ^{d,e}	-0.68 ^{d,e}	-0.56 ^d	0.41 ^{a,c}	0.27 ^c
[4,1,0]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
[4,1,2]	0.50 ^b	0.49 ^b	-0.45 ^b	0.41 ^a	0.42 ^a	-0.42 ^a	-0.46 ^b	0.29 ^c	n.s.
[4,2,0]	0.29 ^c	0.28 ^c	n.s.	n.s. ^c	n.s.	n.s.	n.s.	n.s.	n.s.
[6,1,0]	0.32 ^c	0.33 ^c	-0.34 ^c	0.32 ^c	n.s.	n.s.	-0.32 ^c	n.s.	n.s.

^a $p < 0.01$.^b $p < 0.001$.^c $p < 0.05$.^d $p < 0.0001$.^eSignificant partial correlation controlled for BMD ($p < 0.05$).

TABLE VII. Spearman's rank correlation coefficients between nIBD and the microCT parameters. Results are presented for a subset of the 34 gate choices investigated (even values of τ_d and τ_s). n.s. = not significant.

$[\tau_d, \tau_w, \tau_s] (\mu s)$	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
[2,1,0]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
[2,1,2]	0.63 ^a	0.66 ^a	-0.58 ^a	0.57 ^a	0.48 ^b	-0.49 ^b	-0.55 ^a	n.s.	n.s.
[2,1,4]	0.70 ^a	0.71 ^a	-0.58 ^a	0.56 ^a	0.58 ^a	-0.57 ^a	-0.63 ^a	n.s.	n.s.
[2,2,0]	0.50 ^b	0.49 ^b	-0.49 ^b	0.47 ^b	0.36 ^c	-0.32 ^d	-0.43 ^c	n.s.	n.s.
[2,2,2]	0.68 ^a	0.69 ^a	-0.62 ^a	0.60 ^a	0.51 ^a	-0.49 ^b	-0.60 ^a	n.s.	n.s.
[2,3,0]	0.61 ^a	0.63 ^a	-0.58 ^a	0.56 ^a	0.45 ^b	-0.43 ^c	-0.54 ^a	n.s.	n.s.
[4,1,0]	0.38 ^c	0.41 ^c	-0.37 ^c	0.37 ^c	0.28 ^d	-0.31 ^d	-0.29 ^d	n.s.	n.s.
[4,1,2]	0.62 ^a	0.63 ^a	-0.52 ^a	0.51 ^a	0.54 ^a	-0.55 ^a	-0.54 ^a	n.s.	n.s.
[4,2,0]	0.50 ^b	0.50 ^b	-0.44 ^b	0.45 ^b	0.41 ^c	-0.40 ^c	-0.44 ^b	n.s.	n.s.
[6,1,0]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

^a $p < 0.0001$.

^b $p < 0.001$.

^c $p < 0.01$.

^d $p < 0.05$.

Stronger correlations between the ultrasound and microCT parameters were generally observed for gate choices in which the center to center separation between the gates, $\tau_w + \tau_s$, was large and the delay, τ_d , was small. The gate choice that maximized $\tau_w + \tau_s$ and minimized τ_d was $\tau_d = 2 \mu s$, $\tau_w = 1 \mu s$, and $\tau_s = 5 \mu s$ (denoted [2,1,5]). Figure 3 shows the four ultrasound parameters (nMBD, nSBD, nIBD, and nBAR) plotted as functions of BMD for the [2,1,5] gate choice. Figure 4 shows nMBD plotted as a function of microCT parameters for the [2,1,5] gate choice.

On average, the strongest univariate predictors for the four backscatter difference parameters were those that represented bone quantity: BMD and BV/TV. However, moderate to strong univariate correlations were observed between the backscatter difference parameters and microCT parameters that represent microstructure: BS/BV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn-Dens., and DA. Statistically significant correlations were found between all of the backscatter difference parameters and all of the microstructural parameters for at

least one of the 34 gate choices analyzed. The lone exception was nIBD and DA for which no significant correlation was found for any gate choice. Among the four backscatter difference parameters, nMBD demonstrated the strongest correlations with microstructure for the most gate choices. nMBD demonstrated highly significant ($p < 0.0001$) correlations with Tb.Th, Tb.N, Tb.Sp, and SMI for every gate choice except [7,1,0]. Correlations with Conn-Dens. were weaker, but statistically significant ($p < 0.05$) for all gate choices. Weakest correlations were observed with DA.

As seen in Table IV, many of the microCT parameters exhibited a strong covariance with BMD. Partial correlation analysis was performed to determine if any of the backscatter difference parameters correlated with the microCT parameters independently of BMD. Significant partial correlations ($p < 0.05$) are indicated in Tables V–VIII with a dagger superscript. Counted over all 34 gate choices, nMBD and nBAR exhibited significant partial correlations with Tb.Sp for most gate choices.

TABLE VIII. Spearman's rank correlation coefficients between nBAR and the microCT parameters. Results are presented for a subset of the 34 gate choices investigated (even values of τ_d and τ_s). n.s. = not significant.

$[\tau_d, \tau_w, \tau_s] (\mu s)$	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
[2,1,0]	0.80 ^a	0.81 ^a	-0.72 ^a	0.71 ^a	0.66 ^a	-0.66 ^a	-0.70 ^a	0.30 ^b	n.s.
[2,1,2]	0.85 ^a	0.86 ^a	-0.76 ^a	0.74 ^a	0.70 ^a	-0.70 ^a	-0.74 ^a	0.34 ^b	n.s.
[2,1,4]	0.90 ^a	0.91 ^a	-0.78 ^a	0.75 ^a	0.77 ^a	-0.77 ^{a,c}	-0.80 ^a	0.36 ^c	0.28 ^b
[2,2,0]	0.86 ^a	0.86 ^a	-0.77 ^a	0.77 ^a	0.75 ^a	-0.68 ^a	-0.76 ^a	0.30 ^b	n.s.
[2,2,2]	0.90 ^a	0.91 ^a	-0.79 ^a	0.75 ^a	0.75 ^a	-0.74 ^{a,c}	-0.80 ^a	0.35 ^b	n.s.
[2,3,0]	0.88 ^a	0.89 ^a	-0.79 ^a	0.75 ^a	0.71 ^a	-0.71 ^a	-0.79 ^a	0.33 ^b	n.s.
[4,1,0]	0.65 ^a	0.67 ^a	-0.61 ^a	0.59 ^a	0.45 ^d	-0.47 ^d	-0.57 ^a	n.s.	n.s.
[4,1,2]	0.87 ^a	0.87 ^a	-0.73 ^a	0.69 ^a	0.76 ^a	-0.77 ^{a,c}	-0.77 ^a	0.38 ^{c,c}	0.30 ^b
[4,2,0]	0.89 ^a	0.89 ^a	-0.75 ^a	0.71 ^a	0.73 ^a	-0.73 ^{a,c}	-0.80 ^a	0.35 ^{c,c}	0.28 ^b
[6,1,0]	0.72 ^a	0.71 ^a	-0.57 ^a	0.52 ^{a,c}	0.72 ^a	-0.73 ^{a,c}	-0.67 ^a	0.36 ^c	0.34 ^b

^a $p < 0.0001$.

^b $p < 0.05$.

^c $p < 0.01$.

^d $p < 0.001$.

^eSignificant partial correlation controlled for BMD ($p < 0.05$).

FIG. 3. Backscatter difference parameters plotted as a function of BMD for a specific gate choice: $\tau_d = 2 \mu\text{s}$, $\tau_w = 1 \mu\text{s}$, and $\tau_s = 5 \mu\text{s}$. Each point represents the mean value of the indicated backscatter difference parameter averaged over all measurements on a specimen (9600 measurements total per specimen).

Some investigators have used multiple regression analysis techniques to investigate the relative roles of density and microstructure on ultrasonic measurements of cancellous bone.^{6–9} Table IX shows multivariate models that were generated for nMBD, nSBD, nIBD, and nBAR for the [2,1,5] gate choice using stepwise linear regression. Table IX reports the coefficient of determination for each model adjusted for the number of independent variables (R_{adj}^2) and the improvement (ΔR_{adj}^2) provided by the multivariate models. BMD and BV/TV were identified in the analysis as the sole predictors of nSBD and nIBD for the [2,1,5] gate choice. BMD was identified as the main predictor of nMBD and nBAR, but some microstructural parameters also were identified as predictors: Tb.Sp, BS/BV, and DA. Models that included microstructural parameters as predictors yielded better correlations ($\Delta R_{\text{adj}}^2 < 0.10$) than models based on bone quantity parameters alone. Interestingly, some of the microCT parameters that demonstrated strong univariate correlations with the backscatter difference parameters such as BV/TV, Tb.Th, and SMI did not frequently appear as predictors in the multivariate models. This may be due to their strong covariance with BMD (see Table IV).

IV. DISCUSSION

A. Influence of microstructure on the ultrasonic parameters

Ideally, methods used to assess fracture risk associated with osteoporosis should measure bone strength. However, the standard method for diagnosing osteoporosis, DXA, measures BMD which may only account for 60%–70% of the variation in bone strength.³⁵ The remaining variance is attributed to other factors such as microstructure. One of the driving forces behind ultrasonic bone assessment is the

desire to find techniques that are sensitive to factors other than density that influence bone strength. The idea that ultrasound may be able to detect osteoporotic changes in bone that are undetectable by DXA seems plausible given the fundamental differences between how acoustic and electromagnetic waves interact with matter.

The results of the present study can be used to determine if the measured ultrasonic parameters depend on microstructure independently of BMD. The present study uses microCT, not DXA, to measure BMD. However, the two techniques are similar in that they both use x rays to estimate the concentration of bone mineral (hydroxyapatite) in the tissue. Partial correlation analysis found that nMBD and nBAR demonstrated significant partial correlations with Tb.Sp controlled for BMD for 23 and 24 of the 34 gate choices analyzed, respectively. As seen in Table X, significant partial correlations were found between nMBD and Conn-Dens., nBAR and Conn-Dens., and nSBD and Tb.Sp for several (≥ 13) gate choices. All four backscatter difference parameters demonstrated a significant partial correlation with at least one microstructural parameter. Thus, it seems that microstructure may influence the backscatter difference parameters independently of BMD.

Similar observations may be drawn from the stepwise linear regression analysis. Tb.Sp, BS/BV, and DA were identified as predictors for nMBD and nBAR for many (8–23) of the 34 gate choices. Table XI summarizes the results of the stepwise linear regression analysis by counting the number of gate choices for which a given microCT parameter was identified in a multivariate model as a predictor for a backscatter difference parameter. For most (≥ 30) of the 34 gate choices, BMD was identified as a predictor for nMBD and nBAR. Tb.Sp was identified as a predictor of nMBD and nBAR for 23 gate choices. Thus, while nMBD and nBAR

FIG. 4. The backscatter difference parameter nMBD plotted as a function of microCT parameters for a specific gate choice: $\tau_d = 2 \mu\text{s}$, $\tau_w = 1 \mu\text{s}$, and $\tau_s = 5 \mu\text{s}$. Each point represents the mean value of nMBD averaged over all measurements on a specimen (9600 measurements total per specimen).

are strongly influenced by BMD, microstructure appears to play a significant role as well.

B. Comparison to previous studies

A small number of studies has examined the relationship between backscatter measurements of cancellous bone and microstructure. Most of these studies analyzed a parameter called broadband ultrasonic backscatter (BUB), which is determined from the frequency averaged backscatter coefficient. Chaffai *et al.* and Padilla *et al.* found highly significant ($p < 0.0001$) univariate correlations between BUB and

several microstructural characteristics of cancellous bone.^{6,8} However, they did not find a strong association between BUB and microstructure independent of density. Hakulinen *et al.* measured BUB over a wide range of frequencies, and found strong univariate correlations between BUB and microstructure.⁷ For measurements with a 1 MHz transducer, they found that multiple regression models based on DA and BV/TV accounted for an additional 10% of the variance in BUB compared to models based on BV/TV alone. Wear *et al.* measured the mid-band value of the backscatter coefficient (similar to BUB) and reported that BMD and BV/TV were the best univariate predictors of the backscatter

TABLE IX. Multivariate models for the four backscatter difference parameters for a specific gate choice: $\tau_d = 2 \mu\text{s}$, $\tau_w = 1 \mu\text{s}$, and $\tau_s = 5 \mu\text{s}$.

Dependent variable	Independent variables	R^2_{adi}	ΔR^2_{adi}
nMBD	BMD	0.83	
	BMD, Tb.Sp	0.85	0.02
	BMD, Tb.Sp, BS/BV	0.86	0.03
	BMD, Tb.Sp, BS/BV, DA	0.88	0.05
	Tb.Sp, BS/BV, DA	0.88	0.05
nSBD	BMD	0.54	
nIBD	BV/TV	0.52	
nBAR	BMD	0.77	
	BMD, Tb.Sp	0.80	0.03
	BMD, Tb.Sp, BS/BV	0.83	0.06
	Tb.Sp, BS/BV	0.84	0.07
	Tb.Sp, BS/BV, DA	0.86	0.09

measurements.⁹ Wear *et al.* found that a multiple regression model based on BV/TV and Tb.Th explained 5% more of the variance in BUB than BV/TV alone and 19% more of the variance than BMD alone.

The same four studies also measured the nBUA.⁶⁻⁹ The attenuating characteristics of cancellous bone are relevant to the present study because theoretical considerations indicate that the backscatter difference parameters are closely related to the attenuation coefficient.²⁵ Attenuation causes the amplitude of the backscatter signal to decrease with time to produce the power difference between the two gated portions of the signal that is analyzed to determine the backscatter difference parameters. The findings for nBUA in these previous studies were similar to the findings for BUB. nBUA generally demonstrated moderate to strong univariate correlations with density and several microstructural parameters. Bone quantity (BMD or BV/TV) typically explained most of the variance in nBUA, while microstructure accounted for a modest amount of additional variance (<10%).

So far, only one other study has directly investigated the effects of microstructure on backscatter difference measurements of cancellous bone. Malo *et al.* measured MBD (nMBD not normalized for gate choice) at the neck of intact femurs, and found significant univariate correlations with Tb.Sp, Tb.N, and SMI, but not Tb.Th.²² However, the correlations were not significant when adjusted for BMD. Interestingly, MBD measured at the trochanter did not demonstrate significant univariate correlations with microstructure.

The findings of the present study seem consistent with the findings of most of these previous studies. The backscatter difference parameters (nMBD, nSBD, nIBD, and nBAR) demonstrate strong ($p < 0.0001$) univariate

TABLE X. Number of gate choices (out of 34 total) that yielded significant ($p < 0.05$) partial correlations between the indicated backscatter difference parameters and microCT parameters controlled for BMD.

Backscatter difference parameter	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
nMBD	0	0	3	3	23	2	14	0
nSBD	0	1	8	8	13	0	6	0
nIBD	0	1	0	0	0	0	0	0
nBAR	0	0	7	0	24	1	15	0

correlations with density and several microstructural parameters (depending on ultrasonic parameter, microstructural parameter, and gate choice). The backscatter difference parameters also demonstrate significant partial correlations with several microstructural parameters when adjusted for BMD. In addition, multivariate models that incorporate microstructural parameters Tb.Sp, BS/BV, and DA along with BMD yield stronger adjusted correlations than univariate models in many cases.

C. Spatial averaging

The backscatter measurements reported in the present study were highly spatially averaged. The signals were acquired along six measurement directions (anterior, posterior, medial, lateral, proximal, distal) using a 40 point $\times$ 40 point ROI. Thus, each backscatter difference parameter (nMBD, nSBD, nIBD, and nBAR) was computed from 9600 measurement locations on each specimen that were averaged to yield a single spatially averaged value for that specimen.

Experimentally, it is common practice to spatially average backscatter measurements of cancellous bone because the backscatter signals are highly variable. The variability may be attributed to two main factors. First, the non-uniform nature of cancellous bone may cause the ultrasonic properties of the tissue to vary from one measurement location to another. Second, the phase-sensitive nature of the measurements may cause the backscatter signals to be sensitive to small displacements of the transducer. A backscatter signal is produced by the superposition of waves scattered from multiple trabeculae that interfere constructively and destructively with each other. When the transducer is moved to a slightly different measurement location, the phase relationship between the waves scattered by individual trabeculae may change appreciably, even if the physical characteristics of the trabeculae in the insonified volume do not. Transducer displacements as small as one half of the diameter of the

TABLE XI. Results of the stepwise linear regression analysis. Values in the table indicate the number of gate choices (out of 34 total) for which the indicated microCT parameter was identified as a significant predictor of the indicated backscatter difference parameter.

Backscatter difference parameter	BMD	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	SMI	Conn-Dens.	DA
nMBD	33	1	17	0	5	23	6	0	8
nSBD	15	5	2	3	6	8	1	0	0
nIBD	11	9	3	4	0	1	2	0	0
nBAR	30	2	16	3	7	23	3	0	16

ultrasonic beam are known to produce large changes in the spectral characteristics of backscatter signals from cancellous bone.³⁶

Clinical backscatter measurements of cancellous bone are expected to benefit from spatial averaging as well. However, the same kind of spatial averaging used in the present study cannot be used *in vivo* because most skeletal locations cannot be measured along three orthogonal axes. At the hip, the most accessible direction for backscatter measurements may be the anterior direction.³⁷ To simulate clinical measurements along a single direction, the statistical analyses of the present study were repeated for data acquired along the anterior direction only. The resulting correlations with density and microstructure were found to decrease in most cases. Using the [2,1,5] gate choice as an example, the Spearman's rank correlation coefficient between nMBD and BMD decreased by 22%. The correlation between nMBD and the other microCT parameters decreased by 12%–33% except for the correlation between nMBD and Conn-Dens., which increased by 7%. Similar results were obtained for nBAR. The multivariate analyses were affected as well. In most cases, the partial correlation coefficients were reduced, however, nMBD and nBAR continued to demonstrate significant ($p < 0.05$) partial correlations with several microstructural parameters, especially Tb.Sp.

When ultrasonic measurements are averaged along a single direction instead of all six available directions, the ultrasonically interrogated volume is reduced. This may partially explain why the correlations between the ultrasonic and microCT parameters are reduced in many cases. Another consideration involves the location of the ultrasonically interrogated region. For any single measurement direction, a gate choice $[\tau_d, \tau_w, \tau_s]$ interrogates a rectangular volume of bone that has dimensions $8\text{ mm} \times 8\text{ mm} \times L$, where $L = c(2\tau_w + \tau_s)/2$ and $c = 1.5 \times 10^3\text{ m/s}$ is the approximate SOS in cancellous bone. The interrogated volume lies a distance $c\tau_d/2$ below the incident surface of the cube shaped specimen. Using the [2,1,5] gate choice as a specific example, the ultrasonically interrogated region for a single measurement direction is an $8\text{ mm} \times 8\text{ mm} \times 5.3\text{ mm}$ volume that lies 1.5 mm below the incident surface. Recall that the microCT parameters are determined from a $7\text{ mm} \times 7\text{ mm} \times 7\text{ mm}$ ROI centered on the specimen. Thus, the ultrasonic and microCT measurements do not interrogate exactly the same regions of bone, which may negatively affect the correlations between the ultrasonic and microCT measurements. The impact depends on the amount of overlap between the two regions (which depends on gate choice) and the uniformity of each specimen. To address this issue, ultrasonic measurements were averaged along all six available directions to maximize the ultrasonically interrogated volume and to improve the overlap between the two regions.

D. Limitations

The main limitation of the present study involves the *in vitro* nature of the measurements. Measurements were performed on excised cubes of cancellous bone in a water tank at room temperature. *In vivo*, the measurement

conditions are different and susceptible to other sources of error. For example, reflection and refraction effects associated with the curved outer cortical layer of bone may redirect acoustic energy away from the desired ROI. In addition, the cortical layer may produce reverberations that diminish the signal-to-noise ratio of the measurements. The specimens measured in the present study were cubes of purely cancellous bone with the cortical bone removed. Also, the cube shaped geometry of the specimens allowed measurements to be performed along three orthogonal axes. Similar access to multiple measurement directions will not be possible *in vivo* at most skeletal locations, including the hip and spine.

Statistical limitations should be considered as well. Five of the seven microstructural parameters (BS/BV, Tb.Th, Tb.N, Tb.Sp, and SMI) were strongly covariant with both BMD and BV/TV ($p < 0.0001$). Such covariance limits the ability of the study to determine the independent contributions of bone microstructure to the variances in the reported ultrasonic parameters. Other investigators have made similar observations and suggested that microstructural parameters derived from microCT measurements may not be robust enough to highlight structural characteristics that impact ultrasonic properties independently of density.^{6,8,38}

V. SUMMARY AND CONCLUSIONS

Several backscatter techniques have been developed and studied for the purpose of ultrasonic bone assessment. The backscatter difference technique is a relatively new technique that may offer some advantages compared to other approaches. For example, the backscatter difference technique may be relatively insensitive to errors caused by intervening tissues that lie between the transducer and interrogated region of bone.²⁸ Also (when appropriately normalized), parameters based on the backscatter difference technique may not depend strongly on which portions of the backscatter signal are chosen for analysis.²⁷

Previous studies have shown that parameters derived from the backscatter difference technique demonstrate strong correlations with density, suggesting that these parameters may be able to detect changes in bone caused by osteoporosis.^{25–27} The present study examines the relationship between backscatter difference parameters and the microstructural characteristics of bone, which also are affected by osteoporosis. So far, a total of four backscatter difference parameters have been introduced in the literature for the purpose of ultrasonic bone assessment: nMBD, nSBD, nIBD, and nBAR.^{22,25–28} The present study examined all four parameters. In addition, the parameters were analyzed for several different gated portions of the backscatter signal.

The main findings of the present study are the following.

(1) Among the four backscatter difference parameters tested, nMBD and nBAR exhibit the strongest univariate correlations with bone quantity and microstructure. (2) While bone quantity (BMD and BV/TV) is a strong predictor of nMBD and nBAR, microstructure accounts for additional variance in these ultrasonic parameters. (3) Of the seven microstructural characteristics measured, Tb.Sp appears to have the strongest influence on nMBD and nBAR independent of

BMD. These findings suggest that parameters based on the backscatter difference technique are sensitive to changes in both the density and microstructure of cancellous bone caused by osteoporosis. Of the four parameters tested, nMBD and nBAR may be better suited for ultrasonic bone assessment based on their strong univariate correlations with density and microstructure as well as evidence that the parameters are sensitive to changes in microstructure, independent of density.

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