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Ultrasonic Backscatter Technique for Assessing and Monitoring Neonatal Cancellous Bone Status in Vivo

WEIYING MAO[1], YANG DU[1], CHENGCHENG LIU[2], BOYI LI[3], DEAN TA[3,4], CHAO CHEN[1], AND RONG ZHANG[1]

[1]Department of Neonatology, Children's Hospital of Fudan University, Shanghai 201102, China
[2]School of Physics Science and Engineering, Institute of Acoustics, Tongji University, Shanghai 200092, China
[3]Department of Electronic Engineering, Fudan University, Shanghai 200433, China
[4]State Key Laboratory of ASIC and System, Fudan University, Shanghai 200433, China

Corresponding author: Rong Zhang (zr_kitty@163.com)

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ABSTRACT Metabolic bone disease (MBD) impacts the prognosis of premature infants. There is an urgent need for a portable, noninvasive, and radiation-free method for assessing neonatal bone status. The objective of this study is to evaluate the feasibility of the ultrasonic backscatter technique for assessing and monitoring cancellous bone status in neonates. Ultrasonic backscatter measurements were performed on 766 infants at birth and followed up weekly during hospitalization, utilizing transducers with central frequencies of 3.5 MHz and 5.0 MHz. Backscatter parameters, including apparent integrated backscatter (AIB), frequency intercept of apparent backscatter (FIAB), and frequency slope of apparent backscatter (FSAB) were calculated. Correlations were analyzed with both anthropometric and biochemical indices. We found that AIB ($|r| = 0.40-0.47, p < 0.001$), FIAB ($|r| = 0.36-0.45, p < 0.001$), and FSAB ($|r| = 0.10-0.25$, $p < 0.01$) were significantly correlated with gestational age, weight, length, and head circumference at birth at both the 3.5 MHz and 5.0 MHz frequencies. Backscatter parameters showed regular changes at different postnatal age and correlations with anthropometric indices persisted during the first month of life. Significant differences were also found in subgroup analyses based on gestational age, birth weight, gender, multiple births, and intrauterine growth. AIB and FIAB were shown to be more effective than FSAB. This study suggests the ultrasonic backscatter technique is feasible for neonatal cancellous bone status evaluation at birth and for dynamic monitoring.

INDEX TERMS Metabolic bone disease, neonate, ultrasonic backscatter technique.

I. INTRODUCTION

Incidences of metabolic bone disease (MBD) increase year by year, with improving survival rates for preterm infants, particularly those born at ≤ 28 weeks or ≤ 1000 g [1], [2]. Early diagnosis and treatment of MBD is crucial as it is one of the diseases that contributes to poor short-term and long-term outcomes of prematurity [3]. Assessment of bone status can result in beneficial information for diagnosing and treating MBD in its early stages.

Traditional evaluation methods include biochemical indices and radiology. However, there is as yet no satisfactory method for neonates in terms of feasibility, accuracy or precision. Biochemical indices, such as serum calcium, phosphorus, and alkaline phosphatase (AKP) only reflect bone transformation indirectly and do not significantly correlate with bone mineral density (BMD). In addition, repeated blood sampling is inconvenient for preterm infants.

X-ray plain films, one of the most frequently used radiological methods, is insensitive to bone loss $< 30\%$, meaning it cannot be used for early diagnosis. Dual energy X-ray absorptiometry (DEXA) is the gold standard for osteoporosis in adults but is not suitable for neonates due to radiation and bedside unavailability. Quantitative computed tomography (QCT) is limited for the same reasons as DEXA. Radiological methods are based on BMD measurement,

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which reflects bone quantity. Bone status mainly depends on bone quantity, but also correlates with microstructure characteristics and mechanical properties, typically known as bone quality. Both quantity and quality must be measured to facilitate a better understanding of bone nutritional status.

Quantitative ultrasound (QUS) is a low-cost, non-ionizing, and portable method which was developed in the early 1980s. Studies have shown that QUS not only correlates with BMD but also has the potential to evaluate bone structural and mechanical properties. The most commonly used parameters – broadband ultrasonic attenuation (BUA) and speed of sound (SOS) in transmission mode – are known to be good indicators of bone status and predict fracture risk [4]–[9]. However, ultrasonic transmission techniques are limited due to their physical properties. They require a pair of transducers to transmit and receive signals, meaning they are restricted to a few peripheral skeletal sites. Furthermore, scattering and dispersion are unavoidable through ultrasound propagation but are not considered during transmission measurements. In contrast, ultrasonic backscatter technique, which utilizes only one transducer to transmit and receive signals, has easier access to central body skeletons which have higher fracture risk, such as hips and spines [10], [11]. Ultrasonic backscatter parameters, based on the theory of scattering heterogeneous materials, have been proved useful for assessing microstructure of cancellous bone (such as the calcaneus) [12]–[16]. Meanwhile, the metabolic rate of cancellous bone is eight times higher than that of cortical bone, meaning changes to bone status can be identified earlier. Ultrasonic backscatter from calcaneus in adults has been revealed highly correlated with BMD calculated by DEXA and QCT, and thus considered a promising alternative to detect osteoporosis [17], [18]. However, there are few studies focused on neonates.

In 2013, Zhang *et al.* [19] applied the ultrasonic backscatter technique to neonatal cancellous bone assessment for the first time and found a significant correlation between apparent backscatter coefficient (BSC) and gestational age ($r = 0.47$, $p < 0.001$) as well as birth weight ($r = 0.47$, $p < 0.001$) and length at birth ($r = 0.43$, $p < 0.001$) at 5.0 MHz frequency. Guo *et al.* [20] also found that BSC was positively correlated with gestational age and birth weight of term infants at both 2.25 MHz and 3.5 MHz and were able to establish a preliminary BSC reference range. Recently, Liu *et al.* [21] tried several novel backscatter parameters to characterize neonatal cancellous bone status. They clarified the influence of signal selection at 3.5 MHz frequency, including apparent integrated backscatter (AIB), frequency intercept of apparent backscatter (FIAB), and frequency slope of apparent backscatter (FSAB). However, available data are still limited. To our best knowledge, there are no relevant reports exploring how these parameters keep track of bone status during the neonatal period.

In this study, we first apply AIB, FIAB, and FSAB to regular bone status evaluation for neonates. The objective of this study is to evaluate the feasibility of the ultrasonic backscatter technique for assessing and monitoring cancellous bone status in neonates by analyzing relationships between AIB, FIAB, and FSAB and anthropometric indices, as well as serum biochemical indices, using the central frequencies of 3.5 MHz and 5.0 MHz.

II. METHODS

A. PARTICIPANTS

A total of 766 neonates were enrolled within 48 hours of birth between March 1st, 2016 and September 15th, 2018, from the Department of Neonatology, Children's Hospital of Fudan University. This sample included 324 full-term infants and 442 preterm infants. Infants who had been born with congenital malformations, inherited metabolic diseases, abnormalities of digestive system or bone diseases were excluded. Enrolled infants were divided into subgroups according to gestational age, birth weight, gender, multiple births, and intrauterine growth.

The study was given approval by the Ethics Committee of Children's Hospital of Fudan University (No. 25/2016). Informed consent was obtained from parents of all participants.

B. CLINICAL DATA COLLECTION

Neonatal data including gestational age, birth weight, gender, number of births, length, and head circumference were collected from medical records. For each neonate, weight, length, and head circumference were remeasured at each follow-up time point. Biochemical indices, including serum calcium, phosphorus, and AKP, were routinely tested and collected every week or every other week according to clinical treatment.

C. ULTRASONIC BACKSCATTER MEASUREMENTS

Ultrasound measurements were taken within 48 hours of birth and repeated every seven days until discharge. For each measurement, a planar transducer was placed vertically on the medial side of the infant's heel, where soft tissue and cortical bone lying between the transducer and regions of interest was thin, and the surface plain enough for ultrasound detection. The central frequencies of the transducers (Panametrics, Waltham, MA, USA) were 3.5 MHz (V546; diameter, 0.25 in; -6dB bandwidth, 3.25 MHz [range, 1.60 to 4.91 MHz]) and 5.0 MHz (V543; diameter, 0.25 in; -6dB bandwidth, 5.15 MHz [range, 3.20 to 7.11 MHz]). Ultrasonic gel (Aloka Medical Equipment, Shanghai, China) was used to couple the transducer with soft tissue on the calcaneus.

In this study, we applied a novel ultrasonic backscatter bone diagnostic instrument (UBBD; Fudan University, Shanghai, China) to acquire and process backscatter signals. The UBBD instrument, with a sampling frequency of 50.0 MHz, emitted a bipolar short pulse with a voltage of approximately $\pm$ 50 V to excite the transducer. It conducted amplification and a 14-bit analog to digital conversion after receiving ultrasonic backscatter signals by the

TABLE 1. Anthropometric characteristics of the participants at birth.

Group	< 28 weeks	28-31^{+6} weeks	32-36^{+6} weeks	≥ 37 weeks	Total
Number	18	104	320	324	766
Gestational age (days, mean ± SD)	189 ± 7	211 ± 8	241 ± 9	275 ± 9	250 ± 26
Birth weight (g, mean ± SD)	967 ± 140	1455 ± 307	2156 ± 472	3289 ± 556	2512 ± 868
Length (cm, mean ± SD)	34.3 ± 2.3	38.9 ± 2.9	43.9 ± 3.3	49.5 ± 2.5	45.3 ± 5.0
Head circumference (cm, mean ± SD)	24.7 ± 1.2	27.6 ± 1.8	30.8 ± 2.3	33.8 ± 2.5	31.5 ± 3.3
Male/Female	8/10	52/52	185/135	176/148	421/345
Singleton/Twins	8/10	76/28	222/98	317/7	623/143
SGA/AGA/LGA	2/14/2	7/88/9	68/241/11	44/254/26	121/597/48

SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age

same transducer. The signals were denoised by averaging 128 waveforms in the time domain and then stored for further analysis.

The apparent backscatter transfer function (ABTF) was defined as:

$$ABTF = 10\,log_{10}\left(\frac{P_{specimen}(f)}{P_{reference}(f)}\right), \quad (1)$$

over the -6dB bandwidth [22]. $P_{specimen(f)}$ and $P_{reference(f)}$ referred to frequency-dependent power obtained through Fast Fourier Transform (FFT) in the selected region of the backscatter and reference signal, respectively. The reference signal was reflected by a polished steel plate immersed in pure water. The ABTF was not spatially averaged. AIB was determined by frequency-averaged ABTF over the analysis bandwidth:

$$AIB = \frac{\int_{f_{\min}}^{f_{\max}} ABTF\,\mathrm{d}f}{f_{\max} - f_{\min}}; \quad (2)$$

$f_{\max}$ and $f_{\min}$ corresponded to the -6dB frequency band of the transducer. FSAB was the slope of a linear fit of the ABTF over the same bandwidth, while FIAB was the zero-frequency intercept of the fitted line.

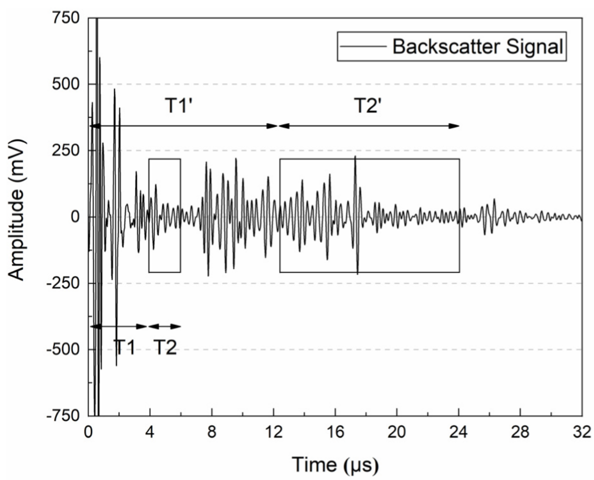

FIGURE 1. A backscatter signal of an infant born at 29 weeks' gestation at 3.5 MHz frequency.

Fig. 1 displays a typical backscatter signal obtained from the calcaneus of a female infant born at 29 weeks' gestation at 3.5 MHz frequency. A Hamming window was utilized to select signals of interest (SOI) for calculation of ultrasonic backscatter parameters. For AIB and FIAB, gate was 2 μs in width (T2) and started 4 μs (T1) after the start of the signal at both 3.5 MHz (left boxed region) and 5.0 MHz frequency. For FSAB, gate width (T2') was 12 μs and gate delay (T1') was 12.5 μs at 3.5 MHz frequency (right boxed region) and 11.5 μs at 5.0 MHz frequency. The SOIs were decided based on previous study by Liu *et al.* [21], as well as optimization of the correlations between backscatter parameters and anthropometric and biochemical indices.

D. STATISTICAL ANALYSIS

MATLAB R2018b (MathWorks, Natick, MA, USA) was utilized to analyze ultrasonic backscatter signals as well as to calculate the parameters. Statistical analyses were carried out with SPSS 22.0 (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant. The Kolmogorov–Smirnov test was used to check the normality of variables. Results showed all neonatal anthropometric and ultrasonic backscatter parameters were non-normally distributed. Therefore, differences in ultrasonic backscatter parameters between male and female infants were determined using the Mann-Whitney U test, as well as differences between singletons and twins. Differences among subgroups divided by gestational age, birth weight, or intrauterine growth were determined using the Kruskal-Wallis H test. Relationships between different parameters were measured using Spearman's correlation analysis.

III. RESULTS

A. BASELINE CHARACTERISTICS

All enrolled infants were divided into four groups according to their gestational age. Preterm infants born at < 28 weeks, 28-31^{+6} weeks and 32-36^{+6} weeks were defined as A-1, 2, 3, while term infants born ≥ 37 weeks' gestation were defined as A-4. Infants were also sorted into five groups (W1-5) by birth weight: < 1500 g, 1500-1999 g, 2000-2499 g, 2500-2999 g, ≥ 3000 g. Anthropological characteristics can be seen in Table 1.

B. GESTATIONAL AGE

As Fig. 2 demonstrates, schematic plots of distributions of AIB and FIAB relative to gestational age at 3.5 MHz and 5.0 MHz yielded a positive linear regression, indicating a

TABLE 2. Comparisons of AIB, FIAB, and FSAB among gestational age groups.

Group	Number	AIB (dB, mean ± SD)		FIAB (dB, mean ± SD)		FSAB (dB/MHz, mean ± SD)	
		3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz
A-1	18	-47.7 ± 8.9^{a§}	-53.4 ± 6.2^{a§}	-50.1 ± 20.4^{a§b*}	-54.3 ± 11.4^{a§}	-1.0 ± 4.3	-0.8 ± 1.4^{a**}
A-2	104	-47.1 ± 6.1^{a§b§}	-51.6 ± 6.7^{a§b*}	-48.9 ± 15.1^{a§b§}	-53.4 ± 14.7^{a§b§}	-1.3 ± 3.5^{a§b§}	-0.9 ± 2.0^{a§b§}
A-3	320	-43.4 ± 6.7^{a§}	-49.3 ± 6.5^{a§}	-38.3 ± 15.5^{a§}	-44.9 ± 14.5^{a§}	-2.9 ± 3.7	-1.9 ± 2.0^{a*}
A-4	324	-39.3 ± 7.5	-45.4 ± 6.8	-32.6 ± 16.1	-37.3 ± 13.9	-2.9 ± 4.2	-2.3 ± 2.4
p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[a]Significantly different from A-4; [b]Significantly different from A-3; $^{§}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$

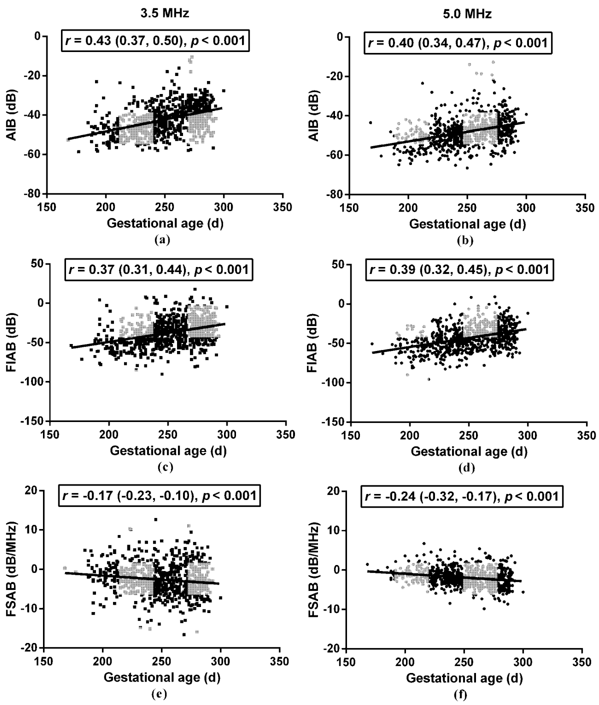

FIGURE 2. Gestational age positively correlated with AIB (a-b) and FIAB (c-d), and negatively correlated with FSAB (e-f) at 3.5 MHz and 5.0 MHz frequencies.

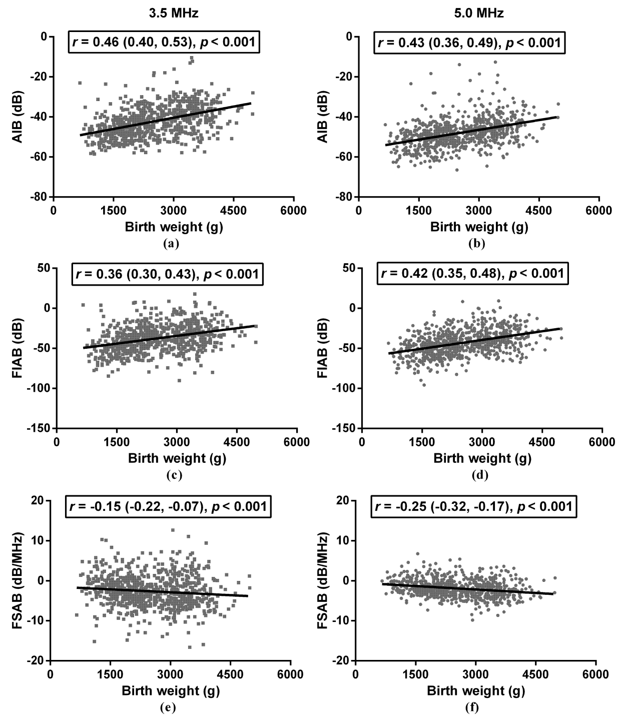

FIGURE 3. Birth weight positively correlated with AIB (a-b) and FIAB (c-d), and negatively correlated with FSAB (e-f) at 3.5 MHz and 5.0 MHz frequencies.

moderate correlation ($|r|$ for AIB = 0.40-0.43, $p < 0.001$; $|r|$ or FIAB = 0.37-0.39, $p < 0.001$). However, FSAB revealed a negative and relatively weak correlation with gestational age ($|r| = 0.17 - 0.24$, $p < 0.001$). Comparisons between different gestational age groups (see Table 2) indicated that AIB and FIAB were significantly higher in term infants than all groups of preterm infants at both frequencies.

Significantly lower FSAB for term infants was only observed at the 5.0 MHz frequency. All three backscatter parameters in group A-2 demonstrated significant differences from those in group A-3.

C. BIRTH WEIGHT

Fig. 3 shows that AIB and FIAB were positively correlated with birth weight ($|r|$ for AIB = 0.43-0.46, $p < 0.001$; $|r|$ for FIAB = 0.36-0.42, $p < 0.001$) while FSAB demonstrated a negative trend at both frequencies ($|r| = 0.15 - 0.25$, $p < 0.001$). The correlation of FSAB with birth weight was weaker than that of AIB and FIAB. For the subgroups divided by birth weight, significant differences were found in most pairs of comparisons for AIB and FIAB, but few were found for FSAB (see Table 3).

D. LENGTH AND HEAD CIRCUMFERENCE AT BIRTH

Length and head circumference were positively correlated with AIB and FIAB and negatively with FSAB at both frequencies. This was in line with gestational age and birth weight (see Table 4). FSAB also demonstrated a correlation coefficient lower than AIB and FIAB.

E. BIOCHEMICAL INDICES AT BIRTH

According to Table 5, all three backscatter parameters showed a weak but significant correlation with AKP at both the 3.5 MHz and 5.0 MHz frequencies ($|r| = 0.08 - 0.13$, $p < 0.05$). Significant positive correlations were found

TABLE 3. Comparisons of AIB, FIAB, and FSAB among birth weight groups.

Group	Number	AIB (dB, mean ± SD)		FIAB (dB, mean ± SD)		FSAB (dB/MHz, mean ± SD)	
		3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz
W-1	54	-49.1 ± 6.2[a§b§c**]	-54.9 ± 5.4[a§b§c§]	-52.9 ± 13.5[a§b§c§]	-60.2 ± 12.2[a§b§c§d*]	-1.5 ± 2.5[a**b*]	-1.0 ± 1.4[a§]
W-2	112	-46.3 ± 5.3[a§b§]	-52.0 ± 5.8[a§b§c*]	-45.4 ± 14.0[a§b§]	-51.6 ± 11.8[a§b§c§]	-2.4 ± 3.7	-1.7 ± 1.8
W-3	136	-44.7 ± 5.6[a§b§]	-49.3 ± 6.1[a§]	-41.0 ± 14.7[a§b§]	-46.5 ± 13.9[a§b**]	-2.6 ± 3.8	-1.6 ± 2.1[a**]
W-4	198	-41.3 ± 7.3[a**]	-47.6 ± 6.2[a§]	-33.8 ± 15.7	-40.2 ± 14.1	-2.9 ± 3.9	-1.7 ± 2.3[a**]
W-5	266	-38.6 ± 7.6	-44.8 ± 7.1	-32.2 ± 16.8	-36.3 ± 14.0	-2.8 ± 4.4	-2.4 ± 2.3
p		< 0.001	< 0.001	< 0.001	< 0.001	0.015	0.015

[a]Significantly different from W-5; [b]Significantly different from W-4; [c]Significantly different from W-3; [d]Significantly different from W-2;
[§]$p < 0.001$; [**]$p < 0.01$; [*]$p < 0.05$

TABLE 4. Correlations between ultrasonic backscatter parameters and length as well as head circumference at birth.

	AIB		FIAB		FSAB	
	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz
Length						
Spearman *r*	0.47	0.45	0.39	0.45	-0.10	-0.17
(95% CI)	(0.41, 0.54)	(0.39, 0.51)	(0.33, 0.46)	(0.38, 0.51)	(-0.18, -0.02)	(-0.25, -0.09)
p value	< 0.001	< 0.001	< 0.001	< 0.001	0.009	< 0.001
Head Circumference						
Spearman *r*	0.45	0.41	0.37	0.42	-0.14	-0.24
(95% CI)	(0.39, 0.51)	(0.35, 0.47)	(0.30, 0.43)	(0.36, 0.48)	(-0.21, -0.06)	(-0.31, -0.16)
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

TABLE 5. Correlations between ultrasonic backscatter parameters and serum biochemical indices at birth.

	AIB		FIAB		FSAB	
	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz	3.5 MHz	5.0 MHz
Alkaline phosphatase						
Spearman *r*	-0.10	-0.10	-0.13	-0.08	0.13	0.13
p value	0.008	0.007	0.001	0.045	0.001	< 0.001
Calcium						
Spearman *r*	0.26	0.26	0.24	0.25	-0.03	-0.04
p value	< 0.001	< 0.001	< 0.001	< 0.001	0.483	0.364
Phosphorus						
Spearman *r*	0.003	0.02	0.01	0.02	0.12	0.19
p value	0.934	0.552	0.732	0.545	0.002	< 0.001

between serum calcium and AIB and FIAB, as well as between serum phosphorus and FSAB at both frequencies.

F. SUBGROUP ANALYSIS AT BIRTH

Fig. 4 shows that FIAB was significantly higher in males than females as well as that large-for-gestational-age (LGA) infants had a higher AIB compared to small-for-gestational-age (SGA) infants at the 3.5 MHz frequency. Single birth infants had higher AIB and FIAB than twins at 3.5 MHz frequency. Significantly higher AIB and FIAB, but lower FSAB in male, single birth, and LGA infants were found at 5.0 MHz frequency when compared with females, twins, and SGA infants, respectively. No significant differences were found between other pairs of subgroups.

G. FOLLOW-UP MEASUREMENTS

As most infants were discharged before the fifth follow-up test, only the first five measurements were analyzed. As can be seen in Table 6, participants' mean gestational age decreased as follow-up tests continued. Fig. 5 shows a decline of AIB and FIAB at 3.5MHz and 5.0 MHz frequencies, while the FSAB trend was uncertain.

TABLE 6. Gestational age of the participants at different time points.

Time point (days after birth)	Number	Gestational age (days, mean ± SD)
At birth	766	250 ± 26
7	298	237 ± 23
14	158	224 ± 18
21	94	219 ± 16
28	78	214 ± 15

Supplemental Table S1-S6 illustrate that AIB and FIAB were both positively correlated with corrected gestational age, weight, length, and head circumference at both frequencies for all measurements, except for FIAB with length at the fifth time point at 3.5 MHz frequency. Significantly negative correlations between FSAB and these anthropometric indices persisted throughout the follow-up period.

For biochemical indices, all three backscatter parameters maintained significant correlations with AKP, while the

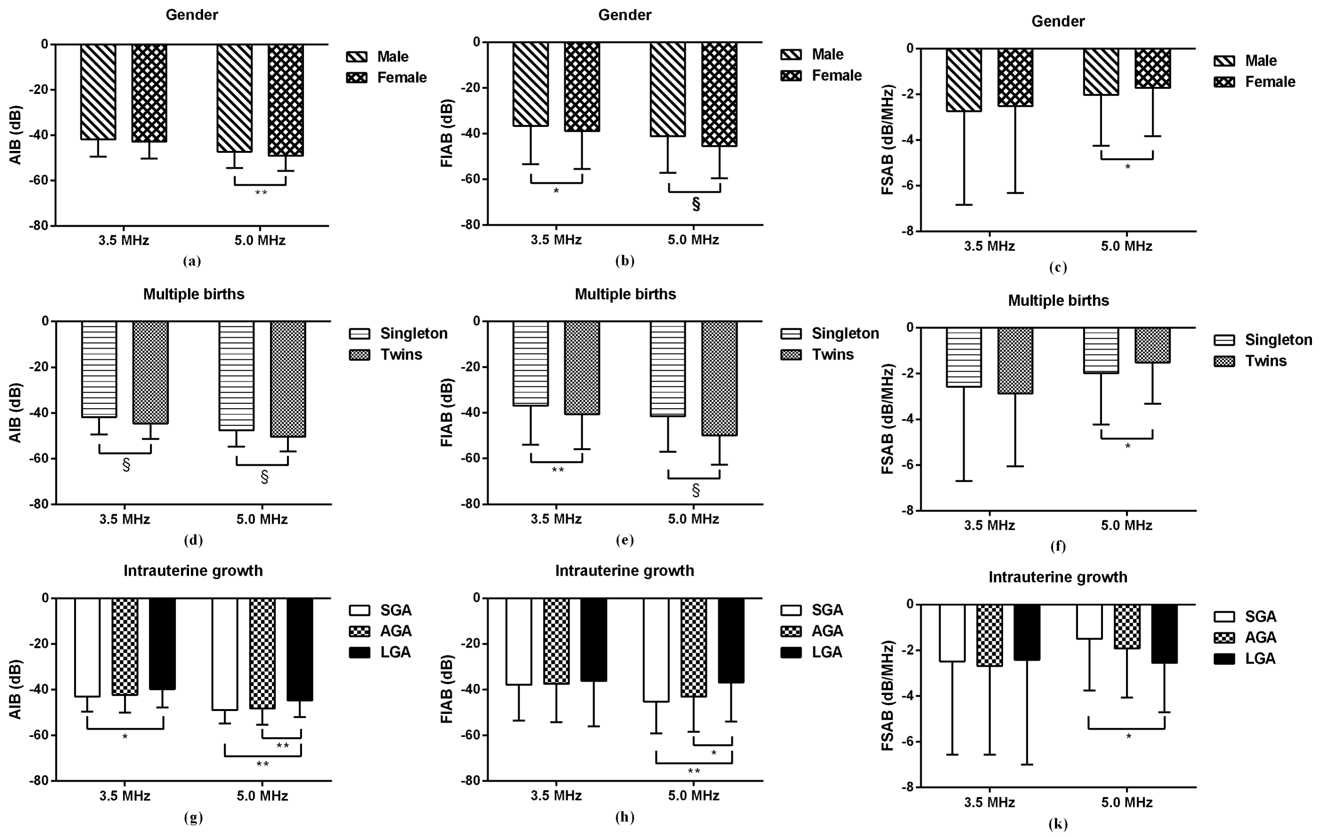

FIGURE 4. Backscatter parameters were significantly different between subgroups based on gender (a-c; 421 males and 345 females), multiple births (d-f; 623 single birth infants and 143 twins) and intrauterine growth (g-k; 121 SGA, 597 AGA and 48 LGA infants). Data were presented as mean ± SD. *$p < 0.05$; **$p < 0.01$; $^{\S}p < 0.001$.

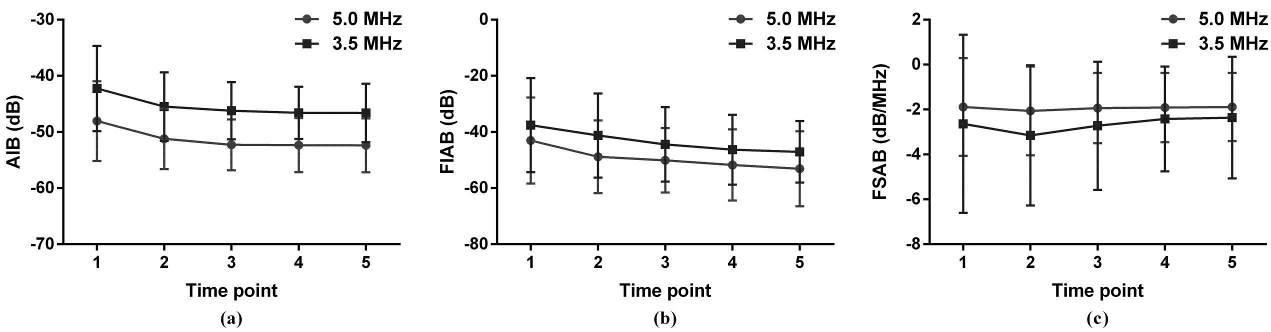

FIGURE 5. Backscatter parameters changed regularly during the follow-up period. (a) AIB. (b) FIAB. (c) FSAB. Time points 1-5 represented measurements at birth and days 7, 14, 21 and 28 after birth, respectively. Data were presented as mean ± SD ($n_1 = 766$, $n_2 = 298$, $n_3 = 158$, $n_4 = 94$, $n_5 = 78$).

duration varied from one week to a month. AIB was found to be negatively correlated with serum calcium and positively correlated with serum phosphorus at the second time point at both frequencies. Hardly any other significant correlations were observed during the follow-up tests.

IV. DISCUSSION

MBD is a common complication of prematurity that has a poor prognosis, accompanied by rachitic changes, fractures, and restricted growth as well as chronic diseases in adulthood [23]. Given that the incidence and survival rate of preterm infants who are at a high risk of MBD have increased in recent years, there is an urgent need for a portable, non-invasive, and radiation-free method to evaluate bone status. Many studies have focused on in vitro materials or adults and have suggested that the ultrasonic backscatter technique is probably an effective approach. However, few studies have been carried out with neonates.

AIB, FIAB, and FSAB are frequency-dependent ultrasonic backscatter parameters which are derived from ABTF, and which could be determined without knowledge of ultrasonic properties of the intervening tissue through the signal propagation path. This means that they are applicable to clinical practice. In addition to evaluation at birth, this is the first study to utilize AIB, FIAB, and FSAB for dynamic monitoring of bone status in neonates.

A. GESTATIONAL AGE AND BIRTH WEIGHT

All three backscatter parameters had significant correlations with gestational age and birth weight at both the 3.5 MHz and 5.0 MHz frequencies, reflecting fetal growth and maturation. It is known that most calcium and phosphorus deposition in fetuses occur during the final trimester of gestation, leading to improved bone density and strength in term infants compared to preterm infants [24]–[26]. The AIB and FSAB results were aligned with those of previous studies. Liu *et al.* [21] found a positive correlation between AIB and gestational age (maximum $r = 0.45$, $p < 0.001$), as well as birth weight (maximum $r = 0.50$, $p < 0.001$) for a short T1 (< 8 μs) at 3.5 MHz frequency. FIAB behaved similarly. In contrast, FSAB was found to be positively correlated with gestational age and birth weight when T1 was long (> 10 μs), which did not align with our results. AIB, FIAB, and FSAB are ultrasonic apparent backscatter parameters, and are not compensated for attenuation and diffraction along the ultrasound propagation path. Correlations depend on which is the leading effect; backscatter or attenuation. Hoffmeister *et al.* [27], [28] have inferred that FSAB was dominated by frequency-dependent attenuation and showed stronger correlations with a longer T1. This may explain why FSAB resulted in a negative correlation in our study. In addition, it has been noted that correlation coefficients of FSAB with anthropometric indices were remarkably lower than those of AIB and FIAB. Subgroup analyses also suggest that both AIB and FIAB are more sensitive than FSAB to distinctions among infants of different gestational age and birth weight, indicating that AIB and FIAB are more effective parameters for neonates.

B. LENGTH AND HEAD CIRCUMFERENCE AT BIRTH

AIB, FIAB, and FSAB were all significantly correlated with length and head circumference at birth at both frequencies. Birth length is known to be closely associated with fetal skeletal development. Rubinacci *et al.* [29] found a significant correlation between SOS, another QUS parameter, and birth length ($r = 0.64$, $p < 0.001$), in line with our results. Head circumference, in contrast, is far more influenced by neurodevelopment. Zhang *et al.* [19] found no significant correlation between calcaneal BSC and head circumference at any frequency, including 3.5 MHz and 5.0 MHz. However, Akcakus *et al.* [30] demonstrated that, consistent with our results, whole-body BMD and content correlated significantly with head circumference at birth.

C. BIOCHEMICAL INDICES

Correlations between each backscatter parameter and exclusive AKP were weak at birth and most disappeared within a month after birth. Relationships with serum phosphorus were also unclear, whereas serum phosphorus and AKP have been regarded as good predictors for MBD in previous studies. Peak AKP exceeding 650 IU/L was discovered to be closely related to decreased bone mineralization at discharge for extremely low birth weight infants with 80% sensitivity and 64% specificity (AUC 0.70, $p = 0.005$) [31]. Backstrom *et al.* [32] revealed that serum AKP $>$ 900 IU/L combined with phosphorus $<$ 1.8 mmol/L led to a sensitivity of 100% at a specificity of 70% for low BMD. Harrison and Gibson [33] also demonstrated a combination of serum phosphorus and AKP levels increased the sensitivity of MBD prediction among infants at high risk. A possible reason for the poor correlations in this study is that recorded serum phosphorus and AKP merely reflected levels of these biochemical indices at the time of blood sampling, and that they fluctuated prior to bone mass reduction and microstructure changes.

It is noteworthy that serum calcium at birth was positively correlated with AIB and FIAB at both frequencies, in line with gestational age, birth weight, length, and head circumference. The serum calcium level is not generally considered a useful indicator for bone mineralization, in agreement with our follow-up results, as it is strongly influenced by parathyroid hormone (PTH) and could maintain typical levels even in calcium deficiency cases. Hung *et al.* [34], for example, reported similar serum calcium levels before eleven weeks postnatal age in preterm infants with and without osteopenia according to radiographic evidence of diminished bone density. However, Liu *et al.* [35] revealed that some bone microstructural features, including mean trabecular separation (Tb.Sp), trabecular number density (Tb.N), connective density (Conn.D) as well as degree of anisotropy (DA), still contributed significantly to AIB and FIAB after adjustment for BMD ($\Delta r^2 = 1.1\%–7.5\%$, $p < 0.05$). Therefore, the association between serum calcium and ultrasonic parameters may be partially attributed to bone microstructure properties.

D. GENDER

The significantly higher AIB and FIAB as well as lower FSAB in male newborns at 5.0 MHz frequency suggest a better bone nutritional status compared to females. AIB and FSAB failed to show gender differences at 3.5 MHz frequency. Chen *et al.* [36] observed that male gender was correlated with increased tibial bone SOS at birth, although many studies have not found a difference in bone metabolism across genders [37]. Fetal bone growth is affected by several factors including heredity, diet, physical activity, PTH, PTH-related protein, and so on, but not sex steroids [38], [39]. This means that the reason for the differences observed between males and females here remains unknown.

E. MULTIPLE BIRTHS

AIB and FIAB were remarkably different between single births and twins at both 3.5 MHz and 5.0 MHz frequencies. FSAB demonstrated a difference only at 5.0 MHz frequency. This may indicate that the bone status is better in single birth infants, although results must be interpreted with caution as anthropometric indices were not balanced between subgroups. Gestational age, birth weight, length, and head circumference were all found to be significantly lower in twins than in single birth infants ($p < 0.001$), to some extent enlarging the ultrasonic backscatter difference. In contrast, studies [40], [41] have revealed no difference in body composition in clinically normally grown neonates, whether they were single births or not, even though multiple pregnancies are well recognized to be related to intrauterine growth restriction and preterm births.

F. INTRAUTERINE GROWTH

Significant differences were found in each backscatter parameter between LGA and SGA infants at 5.0 MHz frequency, but only AIB of LGA infants differed from that of SGA infants at 3.5 MHz frequency. Koklu *et al.* [42] observed that whole-body BMD measured by DEXA at birth was higher in LGA and lower in SGA when compared to appropriate-for-gestational-age (AGA) infants, which aligns with our findings. In contrast, Li *et al.* [43] found that BMD did not differ in very low birth weight infants between AGA and SGA babies. Whether or not SGA is associated with reduced BMD during the neonatal period remains controversial. Further research is needed in this area.

G. FOLLOW-UP MEASUREMENTS

Relationships between backscatter parameters with corrected gestational age, weight, length, and head circumference of the infants maintained their significance by the time of the fifth ultrasonic measurements. The regular decreases of AIB and FIAB suggest a deteriorating bone status over the first month of life. It has been confirmed that bone mineral contents reduce dramatically as early as the first week after birth [44], while MBD is typically found within the first 6-16 weeks of life [23]. However, the variation trend of FSAB at either of the frequencies fails to be determined.

H. CENTRAL FREQUENCIES OF THE TRANSDUCER

AIB, FIAB, and FSAB at 5.0 MHz frequency were found to be more sensitive to differences of cancellous bone status between subgroups based on gender, multiple births, and intrauterine growth, although there was not enough evidence for better correlation coefficients with anthropometric and biochemical indices compared to those at 3.5 MHz frequency. Researchers have suggested that higher frequencies with shorter wavelengths are more appropriate for bone status evaluation for preterm infants, given their trabecular thickness and number density as well as other microstructural information [19]. Transducers which have relatively higher central frequencies may be preferred in future research.

One limitation of this study is that follow-up measurements were discontinued after discharge. Consequently, most follow-up data were collected from preterm infants who had a similar physiological status and so may fail to demonstrate true correlations with ultrasonic backscatter parameters. In addition, neither ABTF nor the ultrasonic parameters were spatially averaged in this study, as they were limited by the in vivo research condition. This means that the anisotropy of cancellous bone has the potential to be a source of random error [45], [46].

V. CONCLUSION

In this study, we applied ultrasonic backscatter parameters AIB, FIAB, and FSAB to cancellous bone status evaluation in neonates. We found a significant correlation with corrected gestational age, weight, length, and head circumference in the neonatal period, which suggests that the ultrasonic backscatter technique is feasible for assessing and monitoring neonatal bone status. AIB and FIAB may be considered more effective parameters as they have tended to have higher correlation coefficients and are more sensitive to differences between subgroups compared to FSAB. Further studies that enroll lager populations are needed to improve the sensitivity and accuracy of ultrasonic backscatter technique for neonatal bone status evaluation and to establish a preliminary normal reference range for neonates, especially those who are at high risk of MBD.

APPENDIX

Follow-up results are shown in supplemental Table S1-S6.

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WEIYING MAO was born in Zhejiang, China, in 1994. She received the M.D. degree from the Shanghai Medical College, Fudan University, Shanghai, China, in 2017.

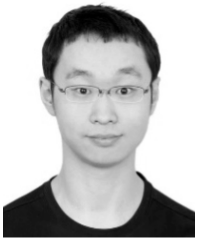
She is currently a Resident Doctor with the Children's Hospital of Fudan University. Her research interest includes the use of ultrasound in metabolic bone disease.

YANG DU was born in Heilongjiang, China, in 1995. He received the M.D. degree from the Shanghai Medical College, Fudan University, Shanghai, China, in 2018.

He is currently a Resident Doctor with the Children's Hospital of Fudan University. His research interests include newborn medicine and statistical analysis.

CHENGCHENG LIU was born in Shandong, China, in 1987. He received the B.S. and Ph.D. degrees from the Department of Electronic Engineering, Fudan University, China, in 2009 and 2014, respectively.

He was a Postdoctoral Researcher with the Department of Electronic Engineering, Fudan University, from 2014 to 2016. He is currently an Assistant Professor with the School of Physics Science and Engineering, Institute of Acoustics, Tongji University. His research interests include biomedical ultrasound, ultrasonic signal processing, and ultrasonic imaging.



BOYI LI was born in Hohhot, China, in 1990. He received the B.S. degree from the Department of Information and Engineering Technology, Sichuan Agricultural University, Ya'an, China, in 2012, and the M.S. degree from the Department of Electronic and Information Engineering, Inner Mongolia University, Hohhot, in 2015. He is currently pursuing the Ph.D. degree in biomedical engineering with the Department of Electronic Engineering, Fudan University, Shanghai, China.

His main research interests include biomedical ultrasound, signal processing, and statistical analysis.

DEAN TA was born in China, in 1972. He received the M.S. degree from the Institute of Acoustics, Shaanxi Normal University, Xi'an, China, and the Ph.D. degree from Tongji University, Shanghai, China, in 1999 and 2002, respectively.

From 2002 to 2004, he was a Postdoctoral Researcher with the Department of Electronic Engineering, Fudan University, Shanghai, China, where he is currently a Full Professor and the Vice Head. He was the principal investigator of more than 20 projects. In the last decade, he has contributed over 200 article and coauthored five books and 17 patents. His research interests include biomedical ultrasound and diagnosis system, medical signal processing, the generation, propagation and applications of ultrasonic guided waves in bone and NDT and E, and the applications of numerical techniques in ultrasound.

Dr. Ta is also the Vice President of the Acoustical Society of China (ASC) and the Chairman of Biomed Ultrasound Eng Speciality of ASC. He was selected as the first Wei Moan Acoustics Award (2013) and the Outstanding Young Scientist Grant of NSF China (2015).

CHAO CHEN was born in Zhejiang, China, in 1960. He received the M.D. degree from the Medical School, Wenzhou Medical College, Wenzhou, China, in 1982, and the Ph.D. degree from Shanghai Medical University, Shanghai, China, in 1992.

From 1995 to 1996, he was a Postdoctoral Researcher with the Laboratory of Neonatology, Department of Pediatrics, Medical School, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA. He is currently a Professor and the Director of the Department of Neonatology, Children's Hospital of Fudan University, Shanghai. He has contributed more than 250 articles. His research interests include neonatal intensive care and treatment and management of premature infants.

Dr. Chen is also the Vice-Chairman of the Society of Perinatology of Chinese Medical Association, the Vice-Chairman of the Society of Neonatology of Chinese Medical Association, and a member of Board of Chinese Journal of Pediatrics. He has chaired the six National Nature Science Foundation of China.

RONG ZHANG was born in Jiangxi, China, in 1976. She received the M.D. degree from Shanghai Medical University, Shanghai, China, in 1999, and the Ph.D. degree in pediatrics from Fudan University, Shanghai, in 2015.

From 2006 to 2007, she completed her Fellowship Training at Stollery Children's Hospital, Edmonton, AB, Canada. She is currently a Neonatologist with the Children's Hospital of Fudan University, Shanghai. She has been working in neonatology for nearly 20 years. Her research interests include the nutritional treatment of preterm and the use of ultrasound in metabolic bone disease.

Dr. Zhang chairs the National Nature Science Foundation of China.