Calcif Tissue Int (2002) 71:133–140
DOI: 10.1007/s00223-001-1107-x

Calcified Tissue International

Assessment of the Bone Status of Nigerian Children and Adolescents with Sickle Cell Disease Using Calcaneal Ultrasound and Serum Markers of Bone Metabolism

D. J. VanderJagt,[1] C. Bonnett,[1] S. N. Okolo,[2] R. H. Glew[1]

[1]Department of Biochemistry and Molecular Biology, University of New Mexico, School of Medicine, Albuquerque, NM 87131, USA
[2]Department of Paediatrics, Jos University Teaching Hospital, Jos, Nigeria

Received: 18 September 2001 / Accepted: 14 March 2002 / Online publication: 24 July 2002

Abstract. Growth and skeletal maturation are impaired in sickle cell disease (SCD). SCD is also associated with decreased bone mineral density (BMD) as determined by dual X-ray and photon absorptiometry. Quantitative ultrasound (US), which is as good a predictor of fracture as absorptiometry, provides additional information about bone architecture and elasticity. It is not known if the quantitative US parameters, broadband ultrasound attenuation (BUA) and speed of sound (SOS), are affected in children and adolescents with SCD. We therefore compared the bones of 80 children with SCD in Nigeria to those of age- and gender-matched controls using calcaneal ultrasound and the serum bone markers N-telopeptide of type1 collagen (NTx) and bone-specific alkaline phosphatase (BSAP), which are indicators of bone resorption and formation, respectively. BUA, which is reflective of BMD, was significantly lower for both the male and female SCD subjects compared with controls (86 vs 113 dB/MHz, $P < 0.001$ and 87 vs 100 dB/MHz, $P < 0.001$, respectively). However, SOS, which is more indicative of bone elasticity, was significantly different only for the male SCD subjects. Both NTx and BSAP were significantly reduced in the serum of the male and female SCD subjects. Correlations between BUA and serum NTx were found for both female controls and SCD subjects ($r = 0.58$, $P < 0.001$ and $r = 0.32$, $P = 0.05$, respectively), but not for the male subjects or controls. Significant correlations between BUA and BSAP were observed only for the female controls. In summary, we have shown that US analysis, in combination with serum markers of bone metabolism, can be used to distinguish bone development in children with SCD from that of nonaffected controls.

Key words: Calcaneal ultrasound — Sickle cell disease — Serum NTx — Bone-specific alkaline phosphatase — Bioelectrical impedance analysis — Nigeria

This work was supported by a Minority International Research Training (MIRT) grant from the Fogarty International Center of the National Institutes of Health.
Correspondence to: R. H. Glew

Sickle cell disease (SCD) in children is associated with impaired growth and skeletal maturation [1–5]. The delayed growth in SCD patients has been attributed to a hypermetabolic state resulting from increases in bone marrow activity and cardiac output secondary to chronic anemia [6–8] and to acquired deficiencies of specific micronutrients and trace minerals that are required for growth [9–12].

In addition to perturbations in body composition, children with SCD also exhibit a wide spectrum of bone abnormalities [13–15] The increased need for red blood cell production leads to bone marrow hyperplasia and ultimately to a decrease in the trabecular network of bone [16]. Frequent infarctions compromise the supply of blood and nutrients to bones and can result in decreased formation of new bone, and the frequent occurrence of osteomyelitis can cause destruction of existing bone. In combination, these processes lead to increased risk for osteopenia and fractures in patients with SCD [17–19].

Studies using dual-photon absorptiometry (DPA) have documented significantly lower bone mineral densities in the lumbar spine of both boys and girls with SCD compared with normal subjects [18, 19]. It is widely recognized that although bone densitometry and dual X-ray absorptiometry (DXA) provide the most accurate measurements of BMD, these methods necessitate exposure of the patient to small amounts of ionizing radiation. Ultrasound, on the other hand, does not use ionizing radiation and provides additional information regarding bone elasticity and microarchitecture [20], thereby making it a suitable method for monitoring the efficacy of therapeutic interventions. Quantitative ultrasound has been shown to be as good at predicting osteoporotic fractures as BMD and can predict fracture risk independent of BMD [21, 22].

Since US parameters are not affected by the size of bone, the method is useful in examining bones of growing children [23]. Jaworski et al. [24] demonstrated that US analysis can distinguish healthy controls from children with chronic disorders that are associated with osteopenia such as osteogenesis imperfecta, hypercalciuria, and steroid-induced osteoporosis. Whether the quantitative ultrasound parameters, BUA and SOS, are affected in children with SCD has yet to be determined.

Information regarding the metabolic state of bone can be obtained by measuring the serum levels of specific markers of bone turnover. For example, the N-terminal telopeptide of type 1 collagen (NTx), which is produced during normal degradation of bone collagen, is a highly specific indicator of bone resorption [25–27]. Bone-specific alkaline phosphatase (BSAP) reflects osteoblast activity and is used to monitor patients with osteoporosis or other metabolic bone diseases [28, 29].

In this study, we examined the bone status of children with SCD in Nigeria using quantitative ultrasound analysis of the calcaneus to determine if US can distinguish the bone quality of children with SCD from their healthy counterparts. Serum markers of bone turnover were also determined and correlations between the US parameters and serum markers of bone metabolism were also explored.

Experimental Subjects

Sickle cell subjects (39 males and 41 females) were recruited from among the patients presenting at the Sickle Cell Clinic at the Jos University Teaching Hospital (JUTH) in Jos, Nigeria. The SS genotype of each subject was confirmed by cellulose acetate electrophoresis of red blood cell lysates. Controls (41 males and 38 females) of the same age range were recruited from among the patients presenting at the JUTH Paediatrics Clinic for routine immunizations or checkups and from among the children of the staff of JUTH. Blood samples were obtained for the determination of bone turnover markers at the time that the ultrasound measurements were made. An additional 51 healthy male controls and 71 female controls were recruited at the National Hospital Abuja, Abuja, Nigeria for the measurement of ultrasound parameters only. This study was approved by the Ethics Review Committee of JUTH and by the Human Research Review Committee of the University of New Mexico School Health Sciences Center, Albuquerque, NM.

Methods

Anthropometric Measurements

Weights and heights of subjects and controls were measured while they were wearing light clothing and no footwear. The height of each subject, measured to the nearest 0.1 cm, was obtained using a wall-mounted stadiometer. Weight was recorded to the nearest 0.25 kg. Z-scores for weight and height were calculated for both the subjects with SCD and the controls using the reference data from the National Center for Health Statistics [30]. These data are considered suitable for international use by the World Health Organization [31]. Body mass index (BMI) was expressed as kg/m^2. Mid-arm circumference and triceps skinfold measurements were also obtained. Information regarding Tanner staging or history of fractures was not available for the subjects in this study.

Bioelectrical Impedance Measurements

Body composition measurements were made using bioelectrical impedance analysis (BIA). Resistance (R) and reactance (X_c) were determined using a portable bioelectrical impedance analyzer (BIA-Quantum, RJL, Inc., Clinton Township, MI). These parameters were used to calculate fat-free mass (FFM) employing age- and gender-appropriate equations, as described elsewhere [32]. Body fat (BF) was calculated as the difference between total weight and FFM.

Quantitative Ultrasound Measurements

Ultrasound measurements were made using the Achilles$^+$ ultrasonometer (Lunar Corporation, Madison, WI, USA) according to the manufacturer's instructions. The Achilles$^+$ is an immersion-type instrument which uses a temperature-controlled water bath. Each subject was seated with his or her right foot placed in the heel bath of the instrument. For the few subjects with a foot length less than 22 cm, the position of the foot was adjusted to align the transducer with the optimal site of the calcaneus [33] by using foot shims provided with the instrument, as described by Jaworski et al. [23]. After the introduction of water containing surfactant into the heel bath, BUA (dB/MHz) and speed of sound transmission (SOS, m/sec) measurements were made. The stiffness index (SI) was calculated using the instrument software according to the following equation:

$$SI = (0.67 \times BUA) + (0.28 \times SOS) - 420.$$

The calculated SI parameter normalizes the BUA and SOS measurements and corrects for any temperature variations [34].

Biochemical Markers of Bone Turnover

N-telopeptide of Type 1 Collagen (NTx) in Serum. The concentration of NTx in serum was measured by a competitive enzyme-labeled immunoassay (Osteomark NTx Assay, Ostex International, Inc., Seattle, WA, USA). Absorbance at 605 nm was measured using an automated plate reader, and the concentration of NTx in the sample was calculated by a calibration curve constructed with NTx standards. NTx concentrations are reported as nanomoles of bone collagen equivalents (nmole BCE) per liter of serum.

Bone-Specific Alkaline Phosphatase (BSAP). The concentration of BSAP in serum was determined using an enzyme immunoassay (Alkaphase B®, Metra Biosystems, Mountain View, CA, USA). This assay is highly specific for bone alkaline phosphatase (AP) and cross-reacts less than 8% with liver AP and not to any significant extent with other AP isoenzymes. The color developed during the reaction of the BSAP and the substrate p-nitrophenylphosphate (pNPP) was measured at 405 nm using an automated plate reader. BSAP concentrations in the unknowns were calculated by a calibration curve fitted with a quadratic equation and are expressed in units per liter (U/l). Each unit represents one µmole of pNPP hydrolyzed per min at 25°C.

Table 1. Summary of the anthropometric characteristics of the sickle cell subjects and controls

	Male SCD subjects (n = 39)	Controls (n = 41)	*P*-value	Female SCD subjects (n = 41)	Controls (n = 38)	*P*-value
Age (yrs)	14 (9–19)[a]	14 (8.9–19)	NS	13 (8.6–22)	13 (8.5–20)	NS
Weight (kg)	30.0 (13.0–60.0)	42.5 (25.0–75.0)	<0.001	29.0 (14.0–69.0)	39.5 (25.0–63.0)	0.003
Weight z-score	−2.41 (1.02)[b]	−0.69 (1.02)	<0.001	−1.78 (1.02)	−0.48 (0.80)	<0.001
Height (cm)	138 (108–121)	152 (121–188)	<0.001	137 (105–164)	151 (122–170)	0.001
Height z-score	−2.67 (1.29)[b]	−0.71 (1.32)	<0.001	−1.97 (1.30)	−0.39 (0.91)	< 0.001
BMI (kg/m^2)	15.6 (11.1–22.6)	18.1 (13.5–22.9)	<0.001	15.7 (6.8–25.7)	17.2 (14.6–22.6)	0.045
MAC (cm)	16.8 (12.7–27.4)	20.5 (6.5–28.3)	<0.001	16.9 (13.9–27.2)	20.0 (12.5–26.0)	<0.001
TSF (mm)	6.00 (2.75–23.5)	6.75 (3.00–23.7)	NS	8.80 (4.0–28.0)	10.6 (5.0–26.0)	NS
FFM (kg)	21.6 (9.84–44.2)	31.5 (17.7–58.6)	<0.001	20.0 (9.70–43.5)	29.5 (16.2–43.7)	<0.001
FFM (% BW)	75.3 (69.9–81.9)	79.4 (69.0–87.5)	<0.001	70.2 (56.5–79.0)	74.3 (65.0–83.4)	<0.001
BF (kg)	7.16 (3.15–16.8)	8.3 (4.4–19.4)	0.01	8.43 (5.83–25.5)	9.58 (4.81–25.5)	NS
BF (% BW)	24.6 (18.1–30.0)	20.6 (12.4–30.9)	<0.001	29.7 (20.9–43.5)	25.7 (16.6–34.9)	NS

[a] Median (minimum − maximum);
[b] mean (SD)
NS, not significant, $P > 0.05$; BMI, body mass index; MAC, mid-arm circumference; TSF, triceps skinfold; FFM, fat-free mass; BF, body fat; BW, body weight

Statistical Analyses

Statistical analyses were performed with the Number Cruncher Statistical Software program (NCSS 2000, Kaysville, UT). Results are expressed as the median (minimum-maximum). Comparisons between sickle cell subjects and controls were made using the two-sample *t*-test. For those variables that were not normally distributed, the Mann-Whitney rank-sum test was used. Pearson rank correlation coefficients were calculated to determine the relationships among anthropometric characteristics, serum concentrations of bone markers and ultrasound parameters.

The lines of best fit for the relationships between the US parameters (BUA, SOS, and SI) and age for the healthy controls were obtained using the SigmaPlot 5 program (SPSS Inc., Chicago, IL). The SigmaPlot curve fitter function utilizes the Marquardt-Levenberg algorithm to determine the coefficients of the independent variables that give the best fit between the equation and the data. The 5% and 95% confidence intervals for the population data were also computed.

Results

Anthropometric Characteristics

The male and female subjects with SCD were closely matched by age to their respective controls (Table 1). The male SCD subjects and controls ranged from 8.9 to 19 years with a median of 14 years. The female SCD subjects spanned a slightly wider age range than the males (8.6–22 years), with a median of 13 years. The median age of the female control subjects was also 13 years.

The weight, height, BMI, mid-arm circumference, %FFM, FFM, and BF of the male subjects with SCD were all significantly lower than the corresponding control values ($P < 0.001$, Table 1). The mean z-scores for height and weight for the male subjects were significantly different from those of the control subjects (Table 1). Whereas 40% of the male controls had weight z-scores less than −1, 76% of the male subjects with SCD had weight z-scores lower than −1. Although the body fat content of the male SCD subjects was significantly lower than that of the controls, their body fat when expressed as a percent of total weight was greater. This anomaly was probably due to the greater deficit in FFM than BF in the subjects with SCD.

Similar deficits in weight, height, BMI, MAC, FFM, and %FFM were observed for the female subjects with SCD (Table 1). Only 20% of the female control subjects had weight and height z-scores less than −1 compared with the female SCD subjects in whom more than 75% had weight and height z-scores less than −1. However, there was no difference in BF or %BF between the female subjects with SCD and their controls. Collectively, these anthropometric data indicated that the male and female SCD patients were stunted and moderately malnourished relative to the subjects who served as controls.

Ultrasound Measurements of the Calcaneus

The ultrasound parameters for the SCD subjects and controls are summarized in Table 2. The broadband ultrasound attenuation (BUA), which is a function mainly of bone density, was significantly lower in both the SCD males and SCD females relative to their respective controls ($P < 0.001$). The relationship between BUA and age for female SCD subjects is shown in Figure 1A: the solid line represents the predicted value of BUA for age based on data for the healthy controls and the dashed lines represent the 5th and 95th percentiles for the predicted values for healthy Nigerian females in the age range of 8–20 years (n = 109). The

Table 2. Ultrasound parameters and serum markers of bone turnover in Nigerian children with SCD and controls

	Male SCD subjects (n = 39)	Male controls (n = 41)	*P*-value	Female SCD subjects (n = 41)	Female controls (n = 38)	*P*-value
BUA (dB/MHz)	86 (61–118)[a]	113 (82–157)	< 0.001	87 (69–136)	101 (62–138)	< 0.001
SOS (m/sec)	1586 (1493–1641)	1555 (1507–1614)	0.005	1555 (1486–1681)	1541 (1505–1597)	NS
SI	78 (49–108)	88 (67–129)	< 0.001	73 (48–113)	77 (55–117)	NS
NTx (BCE/l)	69.9 (28.9–144)	86.1 (10.1–195)	0.01	57.6 (16–155)	81.5 (5.6–143)	NS
BSAP (U/l)	71.9 (41.4–165)	106 (28.4–288)	0.005	71.8 (14.6–146)	101 (9.7–240)	0.002
BSAP/NTx	1.06 (0.51–2.53)	1.17 (0.55–3.41)	NS	1.23 (0.17–14.7)	1.56 (0.69–4.49)	NS

[a] Median (minimum – maximum); BUA, broadband ultrasound attenuation; SOS, speed of sound; SI, stiffness index; NTx, N-terminal telopeptide of collagen; BCE/l, bone collagen equivalents/l; BSAP, bone-specific alkaline phosphatase

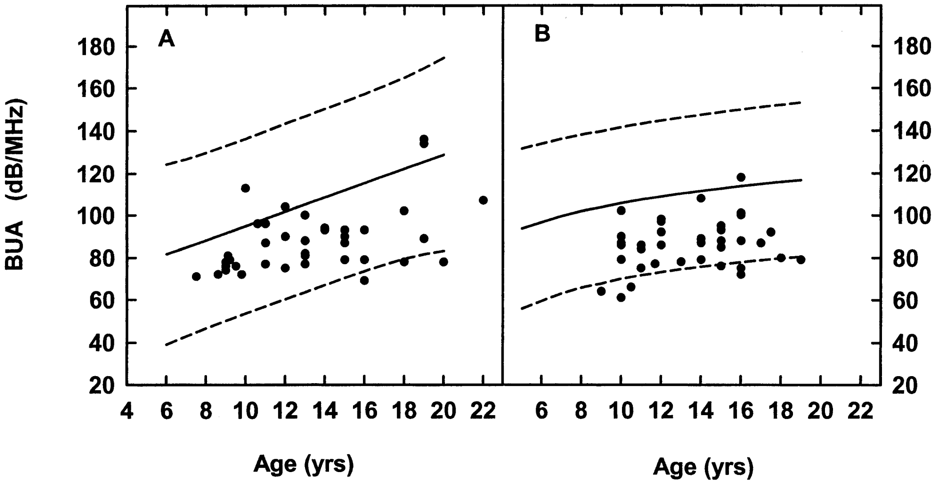

Fig. 1. (**A**) The change in BUA with age for female SCD subjects, $n = 41$ (●). The solid line represents the predicted value for BUA with age based on the data for 109 Nigerian female controls. The dashed lines represent the 5th and 95th confidence limits for the female controls. (**B**) The change in BUA with age for the male SCD subjects, n = 39 (●). The solid line represents the predicted value for BUA with age based on the data for 92 Nigerian male control subjects. The dashed lines represent the 5th and 95th confidence limits for male controls.

BUA values of the majority of the female SCD subjects fell between the 50th and 5th percentiles defined by the control population. In a multiple regression model with BUA serving as the independent variable and age, weight and FFM as the independent variables, only age was found to be a significant determinant of BUA for the female controls. In contrast, using the same model, weight was found to be the only significant determinant of BUA for the female SCD subjects.

The relationships between BUA and age for the male SCD subjects is shown in Figure 1B. All male SCD subjects, with the exception of one, had a BUA value below the predicted value, and the BUA value of several of them fell below the 5th percentile for the healthy controls.

The relationships between SOS and age for the female and male SCD subjects are shown in Figures 2A and B, respectively. Whereas the majority of the male SCD subjects had SOS values above the predicted values for healthy controls, the SOS values for all of the female SCD subjects, except for one, fell within the 5th and 95th percentiles of the predicted values for age.

The relationships between SI and age for the female and male SCD subjects are shown in Figures 3A and B, respectively. A significant difference in the SI was obtained for male SCD subjects and male controls, but no difference was evident between the SI values for the female SCD subjects and controls.

Biochemical Markers of Bone Turnover

The serum biochemical markers of the SCD subjects and controls are summarized in Table 2. The serum levels of NTx for the male SCD subjects were significantly lower than for their corresponding controls (69.9 vs. 86.1 BCE/l, respectively; $P = 0.01$). Although the median serum NTx concentration for the female SCD subjects was lower than that for the controls, the difference was insignificant. In contrast, both the male and female SCD subjects had significantly lower serum levels of BSAP compared with their respective controls. Because serum NTx and BSAP levels are indicative of bone resorption and synthesis, respectively, we calculated the ratio of BSAP to NTx. We found no difference in the BSAP/NTx ratio between the subjects with SCD and controls, most likely because both serum markers were similarly reduced in the subjects with SCD.

Significant correlations between both serum NTx and BSAP and age were obtained for the female SCD subjects and female controls (Table 3). On the other hand,

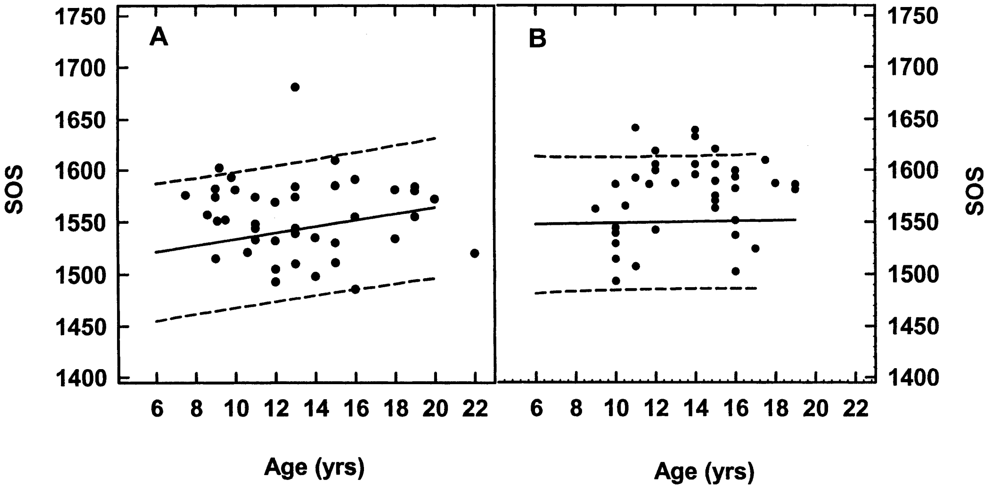

Fig. 2. (**A**) The change in SOS with age for the female SCD subjects, n = 41 (●). The solid line represents the predicted value for BUA with age based on the data for 109 Nigerian female controls. The dashed lines represent the 5th and 95th confidence limits for the female controls. (**B**) The change in SOS with age for the male SCD subjects, n = 39 (●). The solid line represents the predicted value for BUA with age based on the data for 92 Nigerian male controls. The dashed lines represent the 5th and 95th confidence limits for male controls.

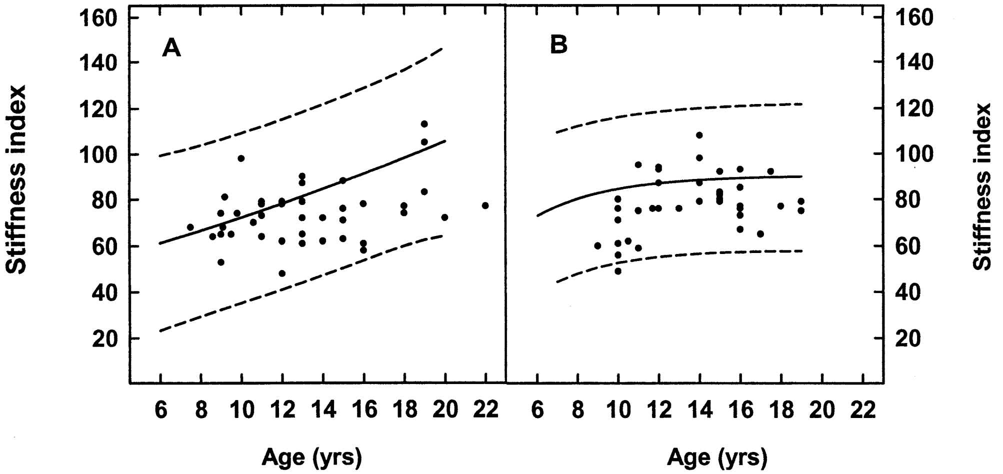

Fig. 3. (**A**) The change in SI with age for the female SCD subjects, n = 41 (●). The solid line represents the predicted value for BUA with age based on the data for 109 Nigerian female controls. The dashed lines represent the 5th and 95th confidence limits for the female controls. (**B**) The change in SI with age for the male SCD subjects, n = 39 (●). The solid line represents the predicted value for BUA with age based on the data for 92 Nigerian male controls. The dashed lines represent the 5th and 95th confidence limits for male controls.

neither the male SCD subjects nor the male controls showed a significant relationship between their serum NTx or BSAP values and age (Table 3).

Significant negative correlations between BUA and NTx were obtained for both the female SCD subjects and controls; however, this relationship was stronger for the female controls ($r = -0.58$, $P < 0.001$ vs. $r = -0.32$, $P = 0.05$) (Fig. 4A). The serum NTx concentration was not significantly correlated with SOS for either the female SCD subjects or the female controls (Table 3). Serum BSAP was inversely correlated with BUA for both the female controls and female SCD subjects (Fig. 5A). As was true for the NTx levels, a stronger relationship between BUA and BSAP was observed for the female controls compared with the SCD subjects ($r = -0.63$, $P < 0.001$ vs. $r = -0.30$, $P = 0.05$, Fig. 5 A,B). The only significant correlation of SOS with a serum marker was obtained for BSAP in the female controls (Table 3). No significant correlations were observed between either bone marker and any of the ultrasound parameters for the male SCD subjects or controls.

Discussion

In this study we have demonstrated that quantitative ultrasound and serum markers of bone metabolism can be used to provide insights into the quality and metabolic status of bones of children with SCD. Overall, on the basis of both physical and biochemical measurements, the bone status of Nigerian boys and girls with SCD appears to be inferior to that of their age- and gender-matched controls.

Of the two US parameters, BUA and SOS, the former showed the greater discrimination between the SCD subjects and healthy controls. Although both BUA and SOS are thought to reflect the number, thickness, and orientation of trabeculae, the BUA value is regarded as more reflective of bone mineral density [35]. In contrast, SOS is considered to be more related to the elasticity of bone [35]. The lack of a significant change in SOS with increasing age for the SCD and control subjects in our study corroborates the results reported by Schonau et al. [36]. In their study of the bones of healthy German children using US ve-

Table 3. Correlation of serum bone markers with anthopometric characteristics and ultrasound parameters

	Males				Females			
	NTx	r	BSAP		NTx	r	BSAP	
	SCD	Controls	SCD	Controls	SCD	Controls	SCD	Controls
Age	0.17	0.07	0.06	0.19	−0.50[a]	−0.82[a]	0.57 [a]	0.70[a]
BMI	0.09	−0.06	0.08	0.05	−0.28	−0.49[d]	−0.53 [a]	−0.53[a]
FFM	0.31	−0.16	0.26	−0.07	−0.45[b]	−0.54[a]	−0.48[b]	−0.55[a]
BF	0.02	−0.11	−0.01	−0.09	−0.39[c]	−0.69[a]	−0.55[a]	−0.59[a]
BUA	0.21	−0.07	0.21	−0.08	−0.32[e]	−0.58[a]	−0.30	−0.63[a]
SOS	−0.15	−0.02	−0.40	−0.11	−0.13	−0.26	−0.11	−0.44[b]

[a] $P = 0.001$
[b] $P = 0.002$
[c] $P = 0.01$
[d] $P = 0.02$
[e] $P = 0.05$

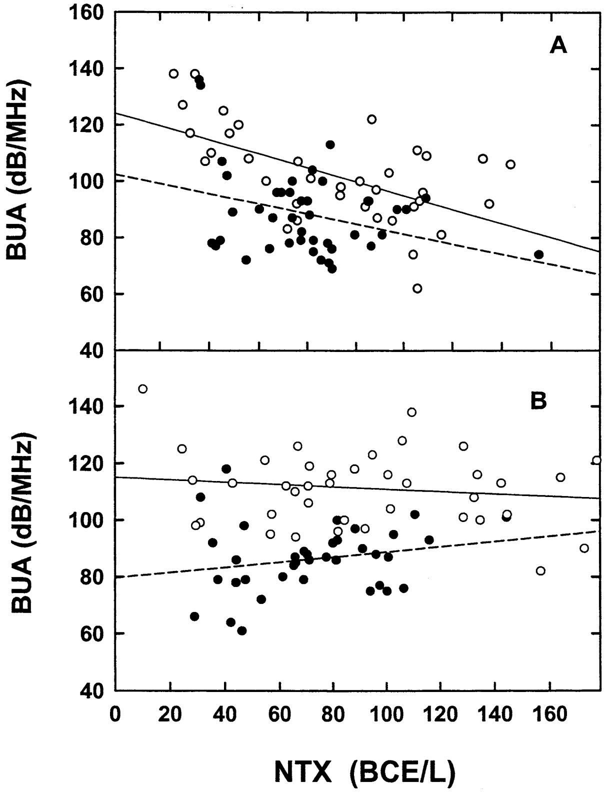

Fig. 4. (**A**) The relationship between BUA and serum NTx for female SCD subjects (●, r = −0.32, $P = 0.05$) and female controls (○, r = −0.58, $P = 0.001$); (**B**), the relationship between BUA serum NTx for male subjects with SCD (●, r = 0.21, NS) and male controls (○, −0.07, NS).

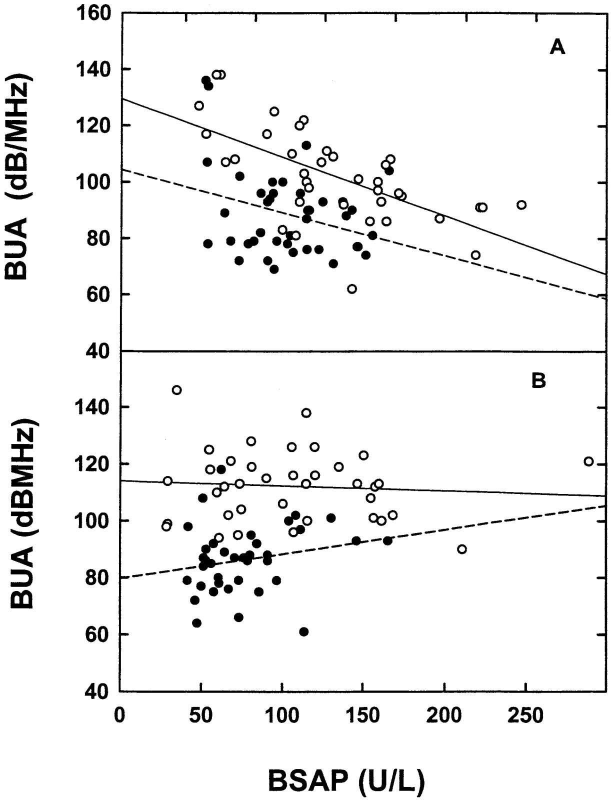

Fig. 5. (**A**) The relationship between BUA and serum BSAP for female subjects with SCD (●, r = −0.30, NS) and female controls (○, r = −0.63, $P = 0.001$). (**B**) The relationship between BUA and serum BSAP for male subjects with SCD (●, r = 0.21, NS) and male controls (○, r = −0.08, NS).

locity of the calcaneus, thumb, and patella, they found that whereas the US velocity through the thumb and patella increased significantly with age, the velocity through the calcaneus was age-independent.

The impairment in bone growth associated with SCD may be partly due to nutritional factors. It is widely recognized that children with SCD are often malnourished as the result of their increased rate of metabolism or because of deficiencies of specific nutrients required for growth. In addition, body weight is a significant determinant of BMD. We [32] and others [5–7, 12, 37] have shown that children with SCD have decreased total body weight and fat-free mass compared with children without the disease.

IGF1 is a growth hormone (GH)-dependent peptide that circulates in the plasma bound primarily to IGFBP-3. IGF-1 is a potent stimulator of bone formation, and administration of IGF-1 has been shown to promote bone formation in animals and humans [38, 39]. IGFBP-3 prolongs the half-life of IGF-1 and the bioactivity of IGF1 is dependent on the presence of IGFBP-3. Furthermore, the levels of circulating IGF1 and IGFBP3 are related to nutritional status [40, 41]. Soliman et al. [19], in their study of the relationship of bone density and growth factors, reported that children with SCD have lower circulating levels of IGF-1 and IGFBP-3 compared with controls of the same age and gender. In addition, 40% of their SCD subjects showed a defective GH response on provocation.

Bone density is dependent not only on chronological age but on bone age and pubertal stage as well. Barden et al. [5] reported a greater than 1 year delay in the bone age of African-American children with SCD. Children with SCD also experience delayed sexual development [12, 13], and delayed puberty is known to have an effect on the accretion of peak BMD. Finkelstein et al. [42, 43] demonstrated that otherwise healthy adult men with a history of constitutionally delayed puberty have a decreased radial and spinal bone mineral density. Since the timing of puberty can be a critical determinant of peak bone mass, the bone density acquired during adolescence may therefore influence the risk for fracture later in life. Although pubertal stage is a determinant of bone density in growing children, van den Bergh et al. [44], using multiple step-wise regression analysis of the calcaneal ultrasound of healthy male and female children and adolescents, reported that after correction for age and weight, Tanner stage was not an independent determinant of BUA. Our finding that age was the main determinant of BUA for the female controls, whereas weight was the main factor determining BUA in female SCD subjects, indicates that in the malnourished state, weight may be more important than age in influencing the acquisition of bone mineral density.

Nutritional supplementation has been suggested as a means for overcoming the growth deficit in children with SCD. However, only limited data are available regarding the efficacy of caloric supplementation in SCD [45]. If nutritional supplementation were shown to be an effective way to increase the overall growth and development of children with SCD, a method for determining the effect of dietary intervention on the bones of children with this disease would be important. Because US uses no ionizing radiation, frequent repetitive measurements would not expose the subject to any hazard, thereby providing a convenient and safe means of following bone development in children.

The significantly lower levels of serum bone markers that we observed for the subjects with SCD in this study may be due to the malnourished state of the subjects. Body composition parameters in this study, and a previous study in which we determined the body composition and serum prealbumin concentrations in controls and SCD subjects in Nigeria [46], indicate that the latter are less well nourished than children who do not have this disease. Although there are few data regarding bone marker levels in children, it is known that the levels of certain bone markers change during growth, particularly during puberty [47]. Our observation of significantly different levels of serum bone markers in SCD subjects and controls in the present study may be due in part to the fact that the SCD patients and controls were not matched for Tanner staging. In contrast to our results, Soliman et al. [19] found normal levels of bone ALP in 28 prepubertal children with SCD. In a study of young adults with SCD in Saudi Arabia, Mohammed et al. [48] reported increased serum levels of ALP and increased urinary excretion of hydroxyproline which they attributed either to delayed growth or to increased destruction of bone.

Although the two biochemical markers of bone turnover (NTx and BSAP) and the results of US analysis both distinguished the male and female SCD children from their respective controls, significant correlations between serum bone markers and ultrasound parameters were obtained only for the female subjects (Table 3). The different findings for male and female subjects may be related to their varying response of the bone markers to hormones related to sexual development. We did not measure the levels of sex hormones in the subjects who participated in this study, nor did we obtain information regarding Tanner staging.

In summary, we have determined that US analysis, in combination with serum markers of bone metabolism, can be used to distinguish the bone development of children with SCD from that of nonaffected controls. Because of the noninvasive nature and portability of ultrasound, it should be a useful method for monitoring interventions aimed at improving the overall nutritional status and bone quality in children with SCD.

References

1. Phebus CK, Gloninger MF, Maciak BJ (1984) Growth patterns by age and sex in children with sickle cell disease. J Pediatr 105:28–33
2. Singhal A, Thomas P, Cook R, Wierenga K, Serjeant GR (1994) Delayed adolescent growth in homozygous sickle cell disease. Arch Dis Child 71:404–408
3. Henderson RA, Saavedra JM, Dover GJ (1994) Prevalence of impaired growth in children with homozygous sickle cell anemia. Am J Med Sci 307:405–407
4. Luban BL, Leiken SL, August GA (1982) Growth and development in sickle cell anemia. Am J Pediatr Hematol Oncol 4:61–65
5. Barden EM, Zemel BS, Kawchak DA, Goran MI, Obene-Frempong K, Stallings VA (2000) Total and resting energy expenditure in children with sickle cell disease. J Pediatr 136:73–79
6. Badaloo A, Jackson AA, Jahoor F (1989) Whole body protein turnover and resting metabolic rate in homozygous sickle cell disease. Clin Sci 77:93–97
7. Salman EK, Haymond MW, Bayne E, Sager BK, Wiisanen A, Pitel P (1996) Protein and energy metabolism in prepubertal children with sickle cell anemia. Pediatr Res 40:34–40

8. Borel MJ, Buchowski MS, Turner EA, Peeler BB, Goldstein RE, Flakoll PJ (1998) Alterations in basal nutrient metabolism increase resting energy expenditure in sickle cell disease. Am J Physiol 274 (E 37):357–364
9. Finan AC, Elmer MA, Sasanow SR, McKinney S, Russell MO, Gill FM (1988) Nutritional factors and growth in children with sickle cell disease. Am J Dis Child 142:237–240
10. Phebus CK, Maciak BJ, Gloninger MF, Paul HS (1988) Zinc status of children with sickle cell disease. Am J Hematol 29:67–73
11. Prasad AS, Cossack ZT (1984) Zinc supplementation and growth in sickle cell disease. Ann Int Med 100:367–371
12. Leonard MB, Zemel BS, Kawchak DA, Obene-Frempong K, Stallings VA (1998) Plasma zinc status, growth, and maturation in children with sickle cell disease. J Pediatr 132:467–471
13. Serjeant GR (1992) Sickle cell disease. Oxford University Press, Oxford
14. Omojola MF, Annobil S, Adzaku F, Addae SK, Mohammed S (1993) Bone changes in sickle cell anaemia. East Afr Med J 70:154–158
15. Bennett OM, Namnyak SS (1990) Bone and joint manifestations of sickle cell anemia. J Bone Jt Surg 72B:494–499
16. Johanson NA (1990) Musculoskeletal problems in hemoglobinopathy. Orthop Clin North Am 21:191–198
17. Al-Awamy B, Sumer T, Naeem MA, Al-Mouzan M (1986) Pathological fracture of vertebral column in association with sickle cell anemia in Saudi Arabia. Trop Geogr Med 38:421–424
18. Brinker MR, Thomas KA, Meyers SJ, Texada T, Humbert JR, Cook SD, Gitter R (1998) Bone mineral density of the lumbar spine and proximal femur is decreased in children with sickle cell anemia. Am J Orthop 27:43–49
19. Soliman AT, Bererhi H, Darwish A, Alzalabani MM, Wall Y, Ansari B (1998) Decreased bone mineral density in prepubertal children with sickle cell disease: correlation with growth parameters, degree of siderosis and secretion of growth factors. J Trop Pediatr 44:194–198
20. Gluer CC, Wu CY, Jergas M, Goldstein SA, Genant HK (1994) Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 55:46–52
21. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, Delmas PD, Pouilles JM, Breart G, Meunier PJ (1996) Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. Lancet 348:511–514
22. Bauer DC, Gluer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM (1997) Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. Arch Intern Med 157:629–634
23. Jaworski M, Lebiedowski M, Lorenc RS, Trempe J (1995) Ultrasound bone measurements in pediatric subjects. Calcif Tissue Int 56:368–371
24. Jaworski M, Lebiedowski M, Lorenc RS (1996) Evaluation of ultrasound measurement in physiological and pathological conditions in children. In: Schonau E (ed) Pediatric osteology: new development and diagnostic therapy. Elsevier, Amsterdam, pp 169–174
25. Eyre DR (1997) Bone markers as tools in osteoporosis management. Spine 22:17S–24S
26. Clemens JD, Herrick MV, Singer FR, Eyre DR (1997) Evidence that serum NTx (collagen type-1-N-telopeptides) can act as immunochemical markers of bone resorption. Clin Chem 43:2058–2063
27. Cabrera CD, Henriquez MS, Traba ML, Villafane EA, Dela Piedra C (1998) Biochemical markers of bone formation in the study of postmenopausal osteoporosis. Osteoporos Int 8:147–151
28. Kyd PA, DeVooght K, Kerkoff F, Thomas E, Fairney A (1998) Clinical usefulness of bone alkaline phosphatase in osteoporosis. Ann Clin Biochem 35:717–725
29. Piovesan A, Berritu A, Torta M, Cannone R, Sperone P, Panero A, Gorzegno G, Termine A, Dogliotti L, Angeli A (1997) Comparison of assay of total and bone-specific alkaline phosphatase in the assessment of osteoblast activity in patients with metabolic bone disease. Calcif Tissue Int 61:362–369
30. United States Public Health Service, Health Resources Administration, NCHS growth charts, Rockville, MD (1976) (HRA 76-1120,5,3)
31. World Health Organization (1983) Measuring change in nutritional status: guidelines for assessing the nutritional impact of supplementary feeding programmes for vulnerable groups. WHO, Geneva
32. VanderJagt DJ, Okolo SN, Rabasa AI, Glew RH (2000) Bioelectrical impedance analysis of the body composition of Nigerian children with sickle cell disease. J Trop Pediatr 46:67–72
33. Zulf Mughal M, Lorenc R (1999) Assessment of bone status in children using quantitative ultrasound techniques. In: Njeh CF, Hans D, Fuerst T, Gluer C, Genant H (eds) Quantitative ultrasound: assessment of osteoporosis and bone status. Martin Dunitz Ltd, London, pp 315
34. Hans D, Arlot ME, Schott AM, Roux JP, Kotzki PO, Meunier PJ (1995) Do ultrasound measurements of the os calcis reflect the bone microarchitecture more than the bone mass? A two-dimensional histomorphometric study. Bone 16:295–300
35. Morita R, Yamamoto I, Yuu I, Hamanaka Y, Ohta T, Takada M, Matsushita R, Masuda K (1997) Quantitative ultrasound for the assessment of bone status. Osteoporos Int 7:S128–S134
36. Schonau E, Radermacher A, Wentzlik U, Klein K, Michalk D (1994) The determination of ultrasound velocity in the os calcis, thumb and patella during childhood. Eur J Pediatr 153:252–256
37. Singhal A, Davies P, Sahota A, Thomas PW, Serjeant GR (1993) Resting metabolic rate in homozygous sickle cell disease. Am J Clin Nutr 57:32–34
38. Mohan S, Bayiing DJ (1991) Bone growth factors. Clin Orthop 263:30–48
39. Ebeling PR, Jones JD, O'Fallon WM, Janes CH, Riggs BL (1993) Short-term effects of recombinant human insulin-like growth factor 1 on bone turnover in normal women. J Clin Endocrinol Metab 77:1384–1387
40. Clemmons D, Synder DK, Busby WH (1991) Variables controlling the secretion of insulin-like growth factor binding protein-2 in normal human subjects. J Clin Endocrinol Metab 73:727–733
41. Isley WL, Underweed LE, Clemmons DR (1983) Dietary components that regulate serum somatomedin C in humans. J Clin Invest 71:175–182
42. Finkelstein JS, Neer RM, Biller BMK, Cawford JD, Klibanski A (1992) Osteopenia in men with a history of delayed puberty. N Engl J Med 326:600–604
43. Finkelstein JS, Klibanski A, Neer RM (1996) A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. J Clin Endocrinol Metab 81:1152–1155
44. van den Bergh JPW, Noordam C, Ozyilmaz A, Hermus ARMM, Smals AAGH, Otten BJ (2000) Calcaneal ultrasound imaging in healthy children and adolescents: relation of the ultrasound parameters BUA and SOS to age, body weight, height, foot dimensions and pubertal stage. Osteoporos Int 11:967–976
45. Heyman MB, Vichinsky E, Katz R, Gaffield B, Hurst D, Castillo R (1985) Growth retardation in sickle cell disease treated by nutritional support. Lancet 1:903–906
46. VanderJagt DJ, Kanellis GJ, Isichei C, Pastuszyn A, Glew RH (1997) Serum and urinary amino acids levels in sickle cell disease. J Trop Pediatr 43:220–225
47. Szulc P, Seeman E, Delmas PD (2000) Biochemical measurements of bone turnover in children and adolescents. Osteoporosis Int 11:281–294
48. Mohammed SM, Suleiman SA, Addae SK, Annobil SH, Adzaku FK, Kadoummi OF, Richards JT (1991) Urinary hydroxyproline and serum alkaline phosphatase in sickle cell disease. Clin Chim Acta 203:285–294