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ORIGINAL ARTICLE

Bone mass of Spanish school children: impact of anthropometric, dietary and body composition factors

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Abstract The purpose of this study was to: (a) determine the relationship between quantitative ultrasound (QUS) results and anthropometric, dietary and body composition factors and establish reference ranges for amplitude-dependent speed of sound (Ad-SoS) in the phalanges and broadband ultrasound attenuation (BUA) in the calcaneus of children from Extremadura, Spain, and (b) to present reference curves for this population. Healthy children ($n = 245$), aged 4–16 years, were included (124 girls and 121 boys). Phalangeal and calcaneal QUS measurements were performed using DBM Sonic Bone Profiler and McCue CUBA Clinical ultrasound devices, respectively. Weight, height and body mass index (BMI) were evaluated by anthropometric methods. Fat percentage, fat mass, lean mass (FFM) and total body water (TBWater) were evaluated by bioelectrical impedance measurements using a Holtain body composition analyzer. Food intake was evaluated by a 7-day food record. A gender analysis revealed that Ad-SoS and BUA parameters increased significantly with age and that both positively correlated with age, weight, height, BMI, FFM and TBWater. For both genders, Ad-SoS showed significant and positive correlations with age, weight, height, BMI, FFM, BUA and TBWater.

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Keywords Bone mass · Amplitude-dependent speed of sound · Broadband ultrasound attenuation · Body composition

Abbreviations

Ad-SoS	Amplitude-dependent speed of sound
BUA	Broadband ultrasound attenuation
FP	Fat percentage
FM	Fat mass
FFM	Lean mass
TBWater	Total body water

Introduction

The measurement of bone mineral status is a useful tool for identifying children who may be at an increased risk of osteoporosis in adulthood. Dual energy X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography may be used for this purpose, but exposure to ionizing radiation is a limiting factor that prevents studies in large populations of children.

Quantitative ultrasound (QUS) methods have been developed to assess bone mineral status at some peripheral skeletal sites. Ultrasound has a special appeal for use in children because of its speed, low cost, lack of ionizing radiation and portability; thus, ultrasound is particularly indicated to assess bone mineral status in children. Different methods for the assessment of bone mineral density (BMD) are in use, and individual studies have been performed to evaluate the dependency of ultrasound velocity on BMD [1–3]. Changes in ultrasound measurements have been shown to correlate with BMD changes in children and adolescents and can discriminate between normal and low bone density [4]. Physical properties measured by ultrasound depend on ultrasonic waves properties. Ultrasonic waves are elastic waves that have the potential to probe multiple bone properties, such as bone density, material properties, microarchitecture and even macrostructure. The age-related loss of both cortical and cancellous bone substantially increases the fragility of bone. The assumption underlying QUS is that these alterations can be determined through non-invasive low-frequency ultrasound measurements [5]. Most of the clinical QUS devices measure amplitude-dependent speed of sound (Ad-SoS) and/or broadband ultrasound attenuation (BUA) (mainly at the calcaneus, phalanges or tibia). Both parameters have been described as measurable predictors of bone status in children [6], and several studies have addressed the correlation between BUA and Ad-SoS measurements with anthropometric variables [7].

Thus QUS values could be related more to the architectural organization of bone (number of lamellars and secondary osteons) and trabecular orientation (changes in the arrangement of the trabecular struts reflecting the anisotropy of bone), as well as number, or thickness [8–11].

There is controversy around whether specific nutritional variables are beneficial for bone health in children and teenagers. In 10 randomized, controlled trials assessing calcium (Ca) supplementation, 9 demonstrated a 1–6% increase in BMD or bone mineral content (BMC) [12]. In adults, other nutrients have been postulated to have direct and/or indirect effects on Ca and bone metabolism or bone mineral status, including magnesium (Mg) [13], vitamin D [14] and zinc [15]. Thus, it is not yet known whether the standard dietary intake of Ca, phosphorus (P), vitamin D, Mg and potassium (K) affects the bone status in children or whether this ultimately affects the achievement of maximum peak bone mass.

Extensive studies characterizing bone mass acquisition and status during childhood and adolescence by means of QUS techniques have been attempted [9, 16–18]. However, current literature about the BMD of children is limited regarding the effects of anthropometric factors and body composition parameters, such as fat percentage (FP), fat mass (FM), lean mass (FFM) and total body water (TBWater) [19, 20]. To our knowledge, no study has investigated the association between body composition parameters and both Ad-SoS and BUA measurements in children from Spain.

The aims of this study were (a) to evaluate the pattern of Ad-SoS and BUA in a large cohort of Spanish school-aged children and relate these values to anthropometric, body composition and dietary factors, and (b) to present reference curves for easy, practical use.

Materials and methods

Subjects

A total of 245 healthy children, aged 4–16 years, attending primary schools in the health district of Cáceres, Spain were included (124 girls and 121 boys). Among those schools five schools were randomly selected to participate in the study. An invitation to participate in the study was sent out to parents and guardians of all children within the targeted age-group. Only healthy subjects were enrolled in the study. Parental consent and the child's assent were required from all subjects, and the investigation was approved by the Office for Protection from Research Risks of the Extremadura University and the Ethics Committee of the Hospital San Pedro de Alcantara in Cáceres.

Before a candidate was enrolled in the study, a complete medical history was taken and a physical examination was performed. Nutrient intake was quantified using dietary scales, measuring cups and spoons, as we have described elsewhere, based on 7 days of dietary records [21, 22].

Ultrasound studies

For all children, an ultrasound study was performed on the 2nd to 5th proximal phalanges of the nondominant hand using a DBM Sonic Bone Profiler (IGEA, Capri, Italy) equipped with a caliper that closes tangentially on the phalanx and measures the Ad-SoS through the phalanx in meters/second. Positioning and repositioning the instrument is easy because the prominences of the lower phalangeal epiphysis are used as reference, and the clip is placed just behind them.

The instrument precision was determined from 5 measurements carried out on 8 children at time intervals of up to 21 days. Measurements were always performed by the same operator and the coefficient of variation (CV) was 1.1%. The inter-observer CV was 0.77%.

BUA was measured in the left calcaneus using an ultrasonic bone analyzer (CUBA Clinical, McCue Ultrasonic Ltd, Winchester, UK). Precision was evaluated by performing five replicate scans on each subject.

Body composition and pubertal development assessment

A trained anthropometrist used standard techniques to take anthropometric measurements for all of the children. BMI, TBWater, FFM, FM and body fat (BF) were measured by bioelectrical impedance using a Holtain body composition analyzer (Holtain Ltd). The technique is simple to perform and highly acceptable to patients. Pubertal development was evaluated in girls and boys by a pediatrician according to Tanner's criteria.

Statistical studies

All values are expressed as the mean ± SD. The parameters studied in each group were compared using ANOVA, and statistical significance was defined by a $p < 0.05$. Relationships between continuous variables were examined using a single regression analysis when appropriate. Stepwise multiple linear regression (stepwise forward estimation) was used to identify predictors of Ad-SoS and BUA. To model the curvilinear nature of the relationship between age and calcaneal ultrasound parameters, we used natural cubic splines [23]. The Bonferroni correction was used where multiple comparisons were performed. Data was analyzed using the StatView 5.01 statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

General characteristics

The characteristics of the study group are shown in Table 1. The mean age of the study participants was 9.860 ± 3.310 years in boys and 10.097 ± 3.257 years in girls. Both genders were equally represented, and the mean age did not differ significantly between boys and girls. Similarly, no significant differences were found between groups in weight, height, BMI, TBWater and FFM (Table 1). Significant differences ($p < 0.001$) were found in FM and BF values, and both were increased in girls. The analysis of QUS parameters revealed no significant differences in the BUA measured between groups, but significant differences ($p < 0.05$) were found in the Ad-SoS value, which was higher in girls.

Table 1 Characteristics of the study group

	Boys	Girls	*p* value
Age (years)	9.860 ± 3.310	10.097 ± 3.257	NS
Weight (kg)	39.64 ± 15.57	39.60 ± 12.67	NS
Height (m)	1.423 ± 0.178	1.420 ± 0.161	NS
BMI (kg/m^2)	18.80 ± 3.84	19.07 ± 3.15	NS
FFM (kg)	37.84 ± 13.14	35.15 ± 9.36	NS
FM (kg)	1.948 ± 6.87	4.481 ± 6.39	0.0035
BF (%)	1.571 ± 16.23	9.121 ± 13.37	0.0001
TBWater (L)	27.64 ± 9.59	25.65 ± 6.83	NS
BUA (db/s)	57.73 ± 17.4	59.64 ± 19.60	NS
Ad-Sos (m/s)	1937.9 ± 72.6	1966.1 ± 75	0.0460

Table 2 Anthropometric data and pubertal status of the participants

Tanner stage	*n*		Age (years)		Weight (kg)		Height (m)		BMI (kg/m^2)	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Stage I	63	56	7.15 ± 1.79	7.25 ± 1.91	29.21 ± 8.45	28.73 ± 7.17	1.28 ± 0.09	1.28 ± 0.11	17.40 ± 3.49	17.10 ± 2.41
Stage II	27	29	11.48 ± 1.05	10.44 ± 1.02	40.42 ± 8.12	44.08 ± 6.61	1.48 ± 0.08	1.46 ± 0.08	18.20 ± 3.50	20.50 ± 2.59
Stage III	14	10	13.07 ± 0.91	12.70 ± 0.48	54.90 ± 7.57	48.63 ± 6.95	1.59 ± 0.08	1.58 ± 0.07	21.71 ± 2.57	19.43 ± 2.58
Stage IV	12	13	14.33 ± 0.65	13.61 ± 0.65	63.30 ± 9.80	50.87 ± 7.13	1.68 ± 0.04	1.58 ± 0.05	22.34 ± 3.08	20.34 ± 3.21
Stage V	5	16	15.40 ± 0.54	14.93 ± 0.57	67.38 ± 8.27	55.25 ± 8.26	1.71 ± 0.04	1.58 ± 0.07	22.79 ± 2.29	22.08 ± 2.45

Table 2 shows the number of subjects enrolled in each age by gender, as well as the anthropometric data and pubertal status of the participants. Tanner stages revealed that 76.23% of the participants in the boys group had a Tanner stage of 1. Similarly, 69.44% of the girls were in stage 1.

Correlation between Ad-SoS or BUA and anthropometric or body composition variables

The sex- and age-specific mean values of ultrasound parameters are presented in Table 3. In both genders, Ad-SoS and BUA increased with age. Ad-SoS and BUA showed significant positive correlations with age, weight, height, BMI, TBWater and FFM in boys ($R = 0.309–0.753$, $p < 0.001$) (Table 4). No significant correlation in boys was found between Ad-SoS and BMI, FM, or BF. FM and BF showed moderate positive correlations with BUA in boys ($R = 0.249–0.258$, $p < 0.01$) (Table 4).

In girls, Ad-SoS and BUA showed significant positive correlations with age, weight, height, BMI, TBWater, FFM and FM ($R = 0.219–0.866$, $p < 0.01$) (Table 5). BF was not significantly correlated with Ad-SoS in girls but was positively correlated with BUA ($R = 0.380$, $p < 0.0001$).

Table 3 Ad-SoS and BUA parameters measured by age and gender

Years	Boys		Girls	
	Ad-SoS (m/s)	BUA (db/s)	Ad-SoS (m/s)	BUA (db/s)
4	1857 ± 12	39.5 ± 11.2	1836 ± 34	45.80 ± 11.41
5	1860 ± 26	36.10 ± 11.68	1858 ± 37	36.57 ± 11.50
6	1877 ± 22	43.81 ± 11.62	1876 ± 21	39.77 ± 11.46
7	1923 ± 42	48.18 ± 6.88	1900 ± 27	50.22 ± 13.39
8	1934 ± 31	52.00 ± 15.93	1928 ± 28	44.44 ± 7.07
9	1912 ± 32	57.93 ± 12.41	1929 ± 35	53.14 ± 9.55
10	1909 ± 43	62.20 ± 13.03	1995 ± 46	60.84 ± 14.75
11	1940 ± 26	60.60 ± 1400	1944 ± 40	59.69 ± 16.39
12	1942 ± 41	63.36 ± 14.78	1995 ± 46	64.71 ± 13.58
13	1949 ± 73	65.20 ± 13.97	2027 ± 46	74.53 ± 13.48
14	2034 ± 60	71.41 ± 13.22	2052 ± 31	65.77 ± 12.57
15	2059 ± 61	71.75 ± 13.99	2053 ± 46	87.50 ± 14.81

Table 4 Correlation studies (Fisher *r*-to-*z* test) in the boys group

	Ad-SoS (m/s)	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	BUA (db/s)
Ad-SoS (m/s)		0.734 $p < 0.0001$	0.753 $p < 0.0001$	0.652 $p < 0.0001$	0.309 $p = 0.0006$	0.590 $p < 0.0001$
Age (years)	0.734 $p < 0.0001$		0.953 $p < 0.0001$	0.833 $p < 0.0001$	0.466 $p < 0.0001$	0.674 $p < 0.0001$
Height (m)	0.753 $p < 0.0001$	0.953 $p < 0.0001$		0.885 $p < 0.0001$	0.495 $p < 0.0001$	0.670 $p < 0.0001$
Weight (kg)	0.652 $p < 0.0001$	0.833 $p < 0.0001$	0.885 $p < 0.0001$		0.824 $p < 0.0001$	0.636 $p < 0.0001$
BMI (kg/m^2)	0.309 $p = 0.0012$	0.466 $p < 0.0001$	0.495 $p < 0.0001$	0.824 $p < 0.0001$		0.410 $p < 0.0001$
BUA (db/s)	0.590 $p < 0.0001$	0.674 $p < 0.0001$	0.670 $p < 0.0001$	0.636 $p < 0.0001$	0.410 $p < 0.0001$	
TBWater (l)	0.753 $p < 0.0001$	0.846 $p < 0.0001$	0.923 $p < 0.0001$	0.900 $p < 0.0001$	0.571 $p < 0.0001$	0.621 $p < 0.0001$
BF (%)	0.752 $p = 0.7239$	0.272 $p = 0.0028$	0.237 $p = 0.0095$	0.524 $p < 0.0001$	0.776 $p < 0.0001$	0.258 $p = 0.0047$
FM (kg)	0.042 $p = 0.6518$	0.273 $p = 0.0027$	0.245 $p = 0.0074$	0.553 $p < 0.0001$	0.781 $p < 0.0001$	0.249 $p = 0064$
FFM (kg)	0.752 $p < 0.0001$	0.845 $p < 0.0001$	0.923 $p < 0.0001$	0.900 $p < 0.0001$	0.570 $p < 0.0001$	0.620 $p < 0.0001$

Table 5 Correlation studies (Fisher *r*-to-*z* test) in the girls group

	Ad-SoS (m/s)	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	BUA (db/s)
Ad-SoS (m/s)		0.866	0.824	0.688	0.291	0.629
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.0012$	$p < 0.0001$
Age (years)	0.866		0.908	0.834	0.470	0.723
	$p < 0.0001$		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Height (m)	0.824	0.908		0.883	0.462	0.652
	$p < 0.0001$	$p < 0.0001$		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Weight (kg)	0.688	0.834	0.883		0.807	0.683
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$		$p < 0.0001$	$p < 0.0001$
BMI (kg/m^2)	0.291	0.470	0.462	0.807		0.491
	$p = 0.0012$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$		$p < 0.0001$
BUA (db/s)	0.629	0.723	0.625	0.683	0.491	
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	
TBWater (l)	0.798	0.883	0.942	0.893	0.536	0.640
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
BF (%)	0.140	0.309	0.335	0.614	0.818	0.380
	$p = 0.1281$	$p = 0.0006$	$p = 0.0002$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
FM (kg)	0.245	0.420	0.441	0.725	0.835	0.455
	$p = 0.0066$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
FFM (kg)	0.798	0.883	0.942	0.893	0.536	0.639
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Nutrient intake

The nutrient intake for each group is shown in Table 5. Significant differences were found in the intake of Ca ($p < 0.001$), P ($p < 0.05$), total protein ($p < 0.001$), fat ($p < 0.001$), glucides ($p < 0.001$), iodine ($p < 0.001$), energy ($p < 0.001$), Mg ($p < 0.05$) and fluoride ($p < 0.05$) between the two groups. In all studied nutrients, the intake in boys was significantly higher than in girls. No significant differences were found between boys and girls for the intake of vitamins D and E, iron, zinc, copper, selenium and the Ca/protein ratio.

Correlation between Ad-SoS or BUA and nutrient intake

Ad-SoS was negatively correlated ($R = -0.204$ to (-0.267)) with Ca ($p < 0.001$), iron ($p < 0.05$), Mg ($p < 0.05$) and the Ca/protein ratio ($p < 0.01$) in girls (Table 6). Similarly, BUA was also negatively correlated ($R = -0.209$ to (-0.289)) with Ca ($p < 0.05$), iron ($p < 0.05$), Mg ($p < 0.001$) and the Ca/protein ratio ($p < 0.001$) in girls (Table 6). No significant correlation between nutrient intake and ultrasound parameter values was observed in boys.

Table 6 Nutrient intake by gender

	Boys	Girls	*p* value
Vit D (UI)	327 ± 447	280 ± 365	NS
Vit E (mg)	2407 ± 0.749	2233 ± 0.804	NS
Calcium (mg)	1836 ± 736	1556 ± 632	0.0017
Phosphorous (mg)	2010 ± 751	1884 ± 680	0.0146
Iron (mg)	26.8 ± 11.4	25.4 ± 10.2	NS
Zinc (mg)	24.5 ± 11.4	15.3 ± 62.7	NS
Protein (g)	107.0 ± 32.5	93.6 ± 31.1	0.0012
Fat (g)	110.9 ± 32.3	98.0 ± 33.1	0.0023
Carbohydrates (g)	245 ± 70	219 ± 62	0.0025
Calories/day	2322 ± 638	2052 ± 644	0.0024
Iodum (μg)	251 ± 255	160 ± 201	0.0024
Magnesium (mg)	400 ± 160	358 ± 137	0.0283
Fluoride (μg)	627 ± 286	540 ± 254	0.0127
Copper (mg)	1033 ± 0.703	0.917 ± 0.484	NS
Selenium (mg)	97.1 ± 33.7	103.8 ± 123.2	NS
Ca/protein (mg/g)	17.7 ± 6.7	17.3 ± 6.8	NS

Multiple regression analysis

Significant variables identified by the univariate analysis were assessed in a stepwise multiple regression model using the backward elimination method**.** Separate models were developed for Ad-SoS and BUA. The models included gender, height, BMI, FFM, TBWater, BF, vitamin D, Ca, calories and Ca/protein index. In the Ad-SoS model, BF in boys and height, and BF and calories in girls were identified as significant independent variables ($p < 0.0001$). In the BUA model, height in boys and weight in girls were identified as significant independent variables ($p < 0.0001$). The results are shown in Tables 7 and 8.

Reference curves of bone ultrasound measurements in Spanish children

A total of 245 children, aged 4–16 years, were studied (124 girls and 121 boys) to provide normative data for Spanish children. For the graphical presentation of normative data, age was plotted against Ad-SoS and BUA. Natural cubic splines were used to create the mean curves. Values for the mean ± 2 SDs for age are shown in Figs. 1 and 2.

Discussion

Our study provided gender-specific values of the QUS parameters assessing bone status (Ad-SoS and BUA) by age in Spanish school-aged children. It showed the anticipated continuous increase in these parameters with age in both genders. Age, height and weight were consistent independent predictors of QUS parameters for both genders. The effect of age on ultrasound parameters was in agreement with the results obtained by DEXA, which showed an increase of BMC and BMD with age in both genders [9, 24].

Females have previously been shown to have higher mean Ad-SoS values than males between the ages of 11–15 years, and this has been attributed to the earlier onset of pubertal development in girls compared to boys [9]. The ultrasound device measures the distal portion of the proximal phalanges, which are rich in trabecular bone. Girls have higher mean BMD than boys at trabecular sites, as measured by DEXA, suggesting the effect of estrogen on trabecular bone [25, 26]. We did not find differences between BUA parameters in either gender. Similar results have been found by others [27, 28]. However, Zhu et al. [29] addressed gender differences measured by BUA from age 13 in a cohort of healthy Chinese children, but the conflict between the studies can be explained by the effects of ethnicity on BMD [30]. Additionally, BUA measured at the calcaneus is strongly influenced by anthropometric factors, such as body height and to a lesser degree by body weight, and we did not find significant gender differences between those factors in our cohort.

QUS parameters were not related to dietary factors in boys, which is in agreement with other studies [31]. Surprisingly, after a simple regression analysis, the QUS parameters showed moderate negative correlations with the intake of nutrients, such as Ca, iron, Mg, and the Ca/protein ratio in girls, but this result could not be confirmed after

Table 7 Variables identified by stepwise regression analysis as predictors of Ad-SoS

Outcome variables	Independent variables	Unstandardized coefficients		Standardised coefficients Beta	F value	p value
Boys						
		B	Standard error			
Ad-SoS	BF (%)	−0.699	0.276	−0.155	6.425	$p < 0.0001$
Girls						
		B	Standard error			
Ad-SoS	Height	4.111	0.250	0.880	270.799	$p < 0.0001$
	BF (%)	−0.846	0.296	−0.152	8.157	
	Calories	0.13	0.006	0.115	5.226	

Table 8 Variables identified by stepwise regression analysis as predictors of BUA

Outcome variables	Independent variables	Unstandardized coefficients		Standardised coefficients Beta	F value	p value
Boys						
		B	Standard error			
BUA	Height	0.653	0.068	0.665	91.825	$p < 0.0001$
Girls						
		B	Standard error			
BUA	Weight	1.053	0.105	0.678	100.549	$p < 0.0001$

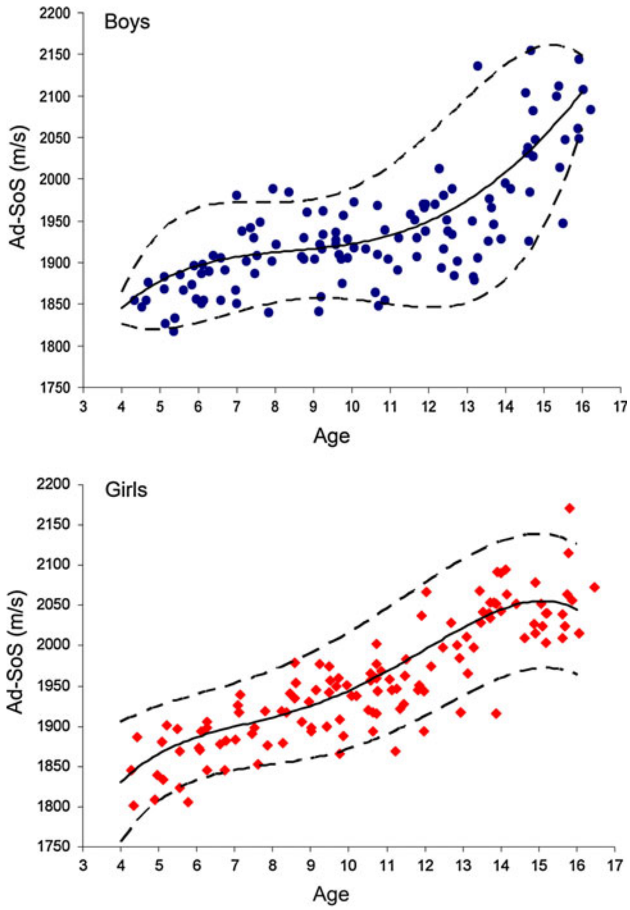

Fig. 1 Ad-SoS by age for Spanish boys and girls. Natural cubic splines were used to create the mean curves. The *solid* and *dashed lines* represent the mean value ± 2 SDs for age

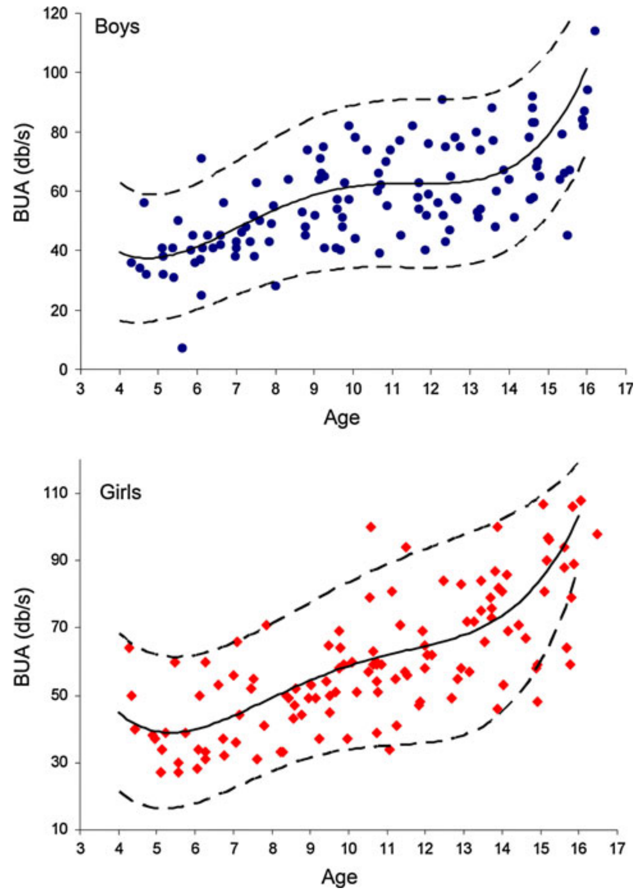

Fig. 2 BUA by age for Spanish boys and girls. Natural cubic splines were used to create the mean curves. The *solid* and *dashed lines* represent the mean value ± 2 SDs for age

multiple regression analysis. Mg is involved in bone and mineral homeostasis and is important in bone crystal growth and stabilization. It also plays a role in the vitamin D/parathyroid hormone axis. The influence of Mg intake has been reported to be positively associated with BMD in adults [32], but its role in children requires further clarification. Protein intake is a determinant of urinary Ca excretion [33]. There are concerns, therefore, that high protein intake, especially those rich in animal protein, are inadvisable for long-term bone health and could be assessed by QUS parameters [21, 22]. In our cohort, iron intake was very close to the European Union Recommendations for children.

QUS variables, Ad-SoS and BUA were related to growth variables, such as height and weight, as these also reflected bone size. These findings agree with those of earlier studies using phalangeal and calcaneal QUS in both adults and children [9, 24] and are in accordance with others (the coefficients of correlations were stronger with height than with weight) suggesting that QUS parameters are affected more by bone size than by body thickness [19, 24].

To our knowledge, our study is the first to assess the respective contribution of FP, FM, FFM and TBWater to QUS parameters in a healthy pediatric population of Spanish children. The powerful impact of body composition parameters on DEXA-derived BMD in children [25, 34] has been well characterized. In growing children, it has been estimated that only 6% of Ad-SoS values can be related to finger width [10]. Thus, bone width is only a minor confounder of Ad-SoS measurements. It has been previously shown that the thickness of the soft tissues surrounding the finger in phalangeal QUS measurements is inversely correlated with Ad-SoS values [35], suggesting that soft tissue thickness could affect the assessment of bone mineral status. Because our cohort of girls had a significantly higher amount of FM and BF than boys, the lack of correlation observed with the Ad-SoS parameter could be explained by a larger amount of soft tissue present in the phalanx. This effect was previously reported by others in adult females, where a lack of correlation between SOS parameters and FM [36] or percent FM [37] was observed. In this sense, assessing BUA using calcaneal QUS devices can accurately assess bone mineral status, thereby minimizing the effect of soft tissue thickness variations [38]. Thus, in our cohort, this could explain why BUA values significantly correlated with FM and BF in girls. Although BUA and Ad-SoS correlate differently with FM and BF, there was a strong correlation between them, and both correlated with the anthropometric variables, confirming previous data [39].

Our study provides a large reference database for phalangeal and calcaneus QUS variables, Ad-SoS and BUA, according to the main anthropometric findings from early childhood to young adulthood. Data for both Ad-SoS and BUA are reported to examine the longitudinal changes of bone mineral status according to age and gender. Phalangeal and calcaneal QUS may be a useful tool for assessing bone mineral status in children and adolescents with a very small confounding effect and without exposing the subjects to a source of radiation. Moreover, our study has established a normative reference database for male and female Spanish children. This set of data will allow for more accurate determinations of Ad-SoS and BUA. It acts as an impetus to encourage the use of QUS, thereby allowing more children to be screened for bone status.

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