

UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELLECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0127294>

DOI: 10.1371/journal.pone.0127294

Direitos autorais / Publisher's copyright statement:

©2015 by Public Library of Science. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>

RESEARCH ARTICLE

Brazilian Pediatric Reference Data for Quantitative Ultrasound of Phalanges According to Gender, Age, Height and Weight

Ezequiel Moreira Gonçalves¹*, Roberto Regis Ribeiro^{1,2}, Wellington Roberto Gomes de Carvalho^{1,3}, Anderson Marques de Moraes¹, Everton Paulo Roman^{1,2}, Keila Donassolo Santos¹, Pedro Augusto Rodrigues Medaets¹, Nélio Neves Veiga-Junior¹, Adrielle Caroline Lace de Moraes Coelho¹, Tathiane Krahenbühl¹, Leticia Esposito Sewaybricker^{1,4}, Antonio de Azevedo Barros-Filho^{1,4}, Andre Moreno Morcillo^{1,4}, Gil Guerra-Júnior^{1,4}

1 Growth and Development Lab—Center for Investigation in Pediatrics (CIPED)—School of Medical Sciences (FCM)—University of Campinas (UNICAMP), Campinas-SP, Brazil, **2** Department of Physical Education—Assis Gurgacz Faculty, Cascavel-PR, Brazil, **3** Department of Physical Education—Center for Biological Sciences and Health—Federal University of Maranhão (UFMA), São Luís-MA, Brazil, **4** Department of Pediatrics—FCM—UNICAMP, Campinas-SP, Brazil

* EMG and RRR made equal contributions to this manuscript.

* emaildozeique@gmail.com



OPEN ACCESS

Citation: Gonçalves EM, Ribeiro RR, Carvalho WRGd, de Moraes AM, Roman EP, Santos KD, et al. (2015) Brazilian Pediatric Reference Data for Quantitative Ultrasound of Phalanges According to Gender, Age, Height and Weight. PLoS ONE 10(6): e0127294. doi:10.1371/journal.pone.0127294

Academic Editor: Tuan Van Nguyen, Garvan Institute of Medical Research, AUSTRALIA

Received: October 1, 2014

Accepted: April 14, 2015

Published: June 4, 2015

Copyright: © 2015 Gonçalves et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This study was supported by the São Paulo Research Foundation (E.M.G and G.G.-J.) (FAPESP: Process n° 2006/01978-0 and 2011/23460-1 (E.M.G); 2002/13021-1 and 2012/16778-8 (G.G.-J)), (R.R.R. and W.R.G.C.) by the Coordination for the Improvement of Higher Education Personnel (CAPES) and (P.A.R.M., N.N.V.-J and A.C.L.M.C.) by National Council for Scientific and Technological Development (CNPq). The funders had no role in

Abstract

Aims

To establish normative data for phalangeal quantitative ultrasound (QUS) measures in Brazilian students.

Methods

The sample was composed of 6870 students (3688 females and 3182 males), aged 6 to 17 years. The bone status parameter, Amplitude Dependent Speed of Sound (AD-SoS) was assessed by QUS of the phalanges using DBM Sonic BP (IGEA, Carpi, Italy) equipment. Skin color was obtained by self-evaluation. The LMS method was used to derive smoothed percentiles reference charts for AD-SoS according to sex, age, height and weight and to generate the L, M, and S parameters.

Results

Girls showed higher AD-SoS values than boys in the age groups 7–16 ($p < 0.001$). There were no differences on AD-SoS Z-scores according to skin color. In both sexes, the obese group showed lower values of AD-SoS Z-scores compared with subjects classified as thin or normal weight. Age ($r^2 = 0.48$) and height ($r^2 = 0.35$) were independent predictors of AD-SoS in females and males, respectively.

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

AD-SoS values in Brazilian children and adolescents were influenced by sex, age and weight status, but not by skin color. Our normative data could be used for monitoring AD-SoS in children or adolescents aged 6–17 years.

Introduction

The adequate acquisition of bone mass during childhood and adolescence leads to a healthy bone structure throughout life [1, 2]. Therefore, bone mass assessment at those ages represents a useful tool to identify individuals who may have a greater risk of osteoporosis in adult life [3–4].

Quantitative ultrasound (QUS) of the proximal phalanges has been used for indirect assessment of bone tissue. Several experiments suggest that ultrasound parameters provide information not only on quantity, but also on the architecture and elasticity of bone [5–9]. Furthermore, this method has practical advantages compared to conventional methods which uses x-rays and photons, i.e. dual-energy X-ray absorptiometry (DXA), and peripheral quantitative computed tomography. It is safe, relatively cheap, takes less time to measure, the equipment is portable and ionizing radiation-free, which makes a good indication for its use with children and adolescents [5, 10–12].

Percentile reference charts are widely used in medical practice as a screening tool to identify how a value of an individual or a sample is positioned compared to the normal distribution of a reference population [13]. Most studies with children and adolescents to provide normative data for the QUS parameters, such as Amplitude Dependent Speed of Sound (AD-SoS), are based on European samples [14–20]. Those references may lead to contradictory results when used in populations belonging to other countries such as Brazil [21–23]. Reference growth charts are needed when any measurement depends strongly on co-variables such as sex, age, pubertal development, as it is observed for QUS parameters of phalanges [11, 15, 18, 20, 24].

Several factors influences bone mass mineralization such as sex, ethnicity, heredity, hormonal processes, diet, physical activity level and body weight. The latter has been an important concern and recent studies observed that, regardless of the method used to assess bone status (QUS or DXA), there is a negative influence of obesity on bone health in pediatric ages [25, 26]. Thus, the aims of the present study were to establish AD-SoS smoothed percentiles assessed by QUS of the phalanges in Brazilians students aged 6 to 17 years according to sex, age, height and weight, and to verify the influence skin color, and weight status on bone status of these children and adolescents.

Materials and Methods

Measurements were performed on 6,870 children and adolescents (3,688 girls and 3,182 boys), aged 6 to 17 years, recruited in schools from three cities located in the state of Paraná (*Cascavel*: $n = 3,504$; 2,016 females and 1,488 males; *Ceu Azul*: $n = 757$; 388 females and 369 males; and *Vera Cruz*: $n = 784$; 401 females and 383 males), and from two cities in the state of Sao Paulo (*Campinas*: $n = 1,296$; 633 females and 663 males; and *Francisco Morato*: $n = 529$; 250 females and 279 males). Paraná and São Paulo are located in the south and southeast regions of Brazil, respectively. The data were collected between 2006 and 2010 and results of each city have already been published [21, 22, 27, 28]. Exclusion criteria were the presence of physical

deficiencies (permanent or temporary) which made assessment impossible, use of drugs that could interfere with bone mass, non-agreement of parents or students, not turning up on the assessment day. According to those criteria 122 students were excluded. All subjects and their parents or guardians were informed about the possible risks of the investigation before giving written informed consent to participate. All procedures were approved by the ethics committee of the School of Medical Sciences, University of Campinas, and were conducted in accordance with the declaration of Helsinki for human studies.

Decimal age was calculated as the difference between date of birth and date of data collection. Each age group was categorized by the midpoint of an age range. For example, the group of children with 8 years old included all the children between 7.50 and 8.49 years, and so forth. The same calculation was used to determine the categories of height and weight (e.g. 150 cm = 145 to 154.9 cm and 50 kg = 45 to 54.9 kg).

Self-reported skin color was collected based on the Brazilian Institute of Geography and Statistics (IBGE) categories [29]: white, "mulatto" (lighter skinned black), and black. The results of those who reported as having yellow or indigenous skin color altogether accounted for less than 10% of the sample and therefore are not shown in tables but were included in the association analyses.

All anthropometric measurements were performed according to standardized procedures [30]. Weight was measured (kg) using portable digital scales to the nearest 0.1 kg. Height was measured (cm) using a vertical stadiometer to the nearest 0.1 cm. From those measurements, BMI was calculated using the formula (kg/m^2). Height and BMI values were converted to standard deviation scores (Z-scores) format by using international reference data [31,32]. We had used the International Obesity Task Force (IOTF) BMI cut-offs to assess the prevalence of thinness, overweight and obesity [32].

For the determination of AD-SoS, DBM Sonic Bone Profiler equipment (IGEA, Carpi, Italy) was used. This equipment is fitted with a probe that attaches two transducers (transmitter and receiver). The probe is positioned at the distal metaphysis of each of the last four proximal phalanges (II to V) in the non-dominant hand, as recommended by the manufacturer. The transducer transmitter emits a sound wave of 1.25 MHz, and the transducer receiver receives the signal and assesses the speed of propagation of sound through the phalange. AD-SoS was obtained automatically and represents the average of speed measurements of ultrasound (m/s) that track the trabecular bone tissue on the four proximal phalanges by transmission. This parameter depends on the amplitude of the electrical signal, obtained after the ultrasound has covered three types of bone in the phalanges (endosteal, trabecular and cortical). All assessments were performed using the same equipment and transducer, calibrated daily according to the manufacturer's recommendations. All the ultrasound measurements were performed by two evaluators (E.M.G. and R.R.R.) using the same equipment. *In vivo* short-term precision was assessed based on root mean square of coefficient of variation (RMS-CV) for 80 measurements made in 10 healthy young persons (6 males and 4 females measured 4 times each by each evaluator) calculated accordingly to Bonnick et al. [33]. The RMS-CV of AD-SoS ranged 0.55 to 0.62% and the inter-evaluator RMS-CV was 1.46%.

To calculate percentiles according to sex and age, the results of the QUS parameters were smoothed using the LMS method [13,34]. L, M and S values were calculated using the software LMSchartmaker Light version 2.42 [35]. Data obtained by the LMS method were converted to Z-scores using the equation: $Z = [(X/M)^L - 1]/(L \times S)$; where X is the measurement value (AD-SoS) and L, M and S are values from the smooth charts for age, height and weight, according to sex (S1, S2 and S3 Tables).

The SPSS version 16.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) was used for database and statistical analysis. The results were expressed as mean, standard

deviation (mean ± SD), range (minimum and maximum values) and absolute (n) and relative (%) frequencies. The normal distribution of the data was tested using the Kolmogorov-Smirnov test. We tested several transformations to achieve data normality and homogeneity. However, no approaches produced good outcomes. Therefore, Mann-Whitney U test was used to compare variables between sexes and Kruskal Wallis test was used to compare AD-SoS and Z-scores between age groups, skin color and weight status. Non parametric multiple comparison test and Bonferroni correction were performed when necessary. Pearson’s correlation coefficient was performed to determine correlation between AD-SoS and independent variables. Multiple regression analysis (stepwise method) was performed to identify which variable or combination of variables would best explain AD-SoS variance; for this analysis, data were transformed to reach the parametric tests criteria. The adjusted coefficient of determination (r^2) was estimated. For all tests, statistical significance was established at $p < 0.05$.

Results

General features of the sample are reported in [Table 1](#). Females were significantly older and heavier than males. However, height (cm and Z-scores) and BMI Z-scores were higher in boys. There was no sex related differences for BMI (kg/m^2) values. The majority of the sample was classified as white skin color (68%), and normal weight status (71%).

Females showed higher AD-SoS values than males the age groups 7–16 ($p < 0.001$). Significant increase in AD-SoS values was seen when comparing a specific age group with a one-year younger group. This was found for girls from age groups of 10, 12, 13 e 14 years and boys with 9 and 14 years ([Table 2](#)).

Table 1. General characteristics of the sample.

Variables	Girls		Boys		All Mean ± SD
	Mean ± SD	(range)	Mean ± SD	(range)	
Age (years) ^a	11.9 ± 2.7	(5.5 to 17.5)	11.1 ± 2.8	(5.5 to 17.5)	11.5 ± 2.75
Weight (kg) ^a	41.9 ± 13.2	(15.0 to 93.7)	41.0 ± 15.4	(16.0 to 115.5)	41.5 ± 14.3
Height (cm) ^a	145.0 ± 14.3	(105.0 to 183.5)	145.8 ± 17.3	(105.6 to 194.9)	147.0 ± 15.8
BMI (kg/m^2)	18.6 ± 3.5	(12.0 to 35.5)	18.6 ± 3.5	(12.5 to 37.7)	18.6 ± 3.5
Height Z-score (SDS) ^a	0.23 ± 1.03	(-3.76 to 5.11)	0.35 ± 1.09	(-5.57 to 4.82)	0.28 ± 1.06
BMI Z-score (SDS) ^a	0.23 ± 1.03	(-3.02 to 3.25)	0.45 ± 1.04	(-2.99 to 3.96)	0.33 ± 1.04
	n (%)		n (%)		n (%)
Skin color/race					
White	2,621 (71.1)		2,064 (64.9)		4,685 (68.2)
Black	324 (8.8)		343 (10.8)		667 (9.7)
Mulatto	702 (19.0)		713 (22.4)		1,415 (20.6)
Yellow/Indigenous	41 (1.1)		62 (1.9)		103 (1.5)
Weight status ^b					
Thinness	422 (11.4)		239 (7.5)		661 (9.6)
Normal	2,646 (71.7)		2,246 (70.6)		4,892 (71.2)
Overweight	492 (13.3)		533 (16.8)		1,025 (14.9)
Obese	128 (3.5)		164 (5.2)		292 (4.3)
Total	3,688 (53.7)		3,182 (46.3)		6,870 (100)

^aDifferences between sexes, $p < 0.001$, Mann-Whitney Test.

^bWeight status established according to BMI Z-score cut-offs defined by IOTF: Thinness: < -1.01 and < -0.98 ; Normal: between -1.01 to 1.30 and -0.98 to 1.23 ; Overweight: between 1.31 to 2.28 and 1.24 to 2.18 and Obese: > 2.28 and > 2.18 for boys and girls, respectively.

Table 2. AD-SoS values according to sex and age.

Age	Sample size n (%)	AD-SoS (m/s)				
		Girls Mean ± SD	(Range)	n (%)	Boys Mean ± SD	(Range)
6	84 (2.3)	1893 ± 66	(1651–2002)	103 (3.2)	1884 ± 64	(1636–1976)
7 ^c	150 (4.1)	1898 ± 63	(1630–2012)	239 (7.5)	1881 ± 57	(1663–1995)
8 ^c	167 (4.5)	1919 ± 52	(1714–2040)	331 (10.4)	1880 ± 64	(1638–1991)
9 ^c	334 (9.1)	1933 ± 53	(1730–2096)	308 (9.7)	1902 ± 60^a	(1650–2069)
10 ^c	421 (11.4)	1951 ± 56^a	(1767–2119)	388 (12.2)	1911 ± 61	(1644–2039)
11 ^c	489 (13.3)	1964 ± 50	(1788–2146)	386 (12.1)	1920 ± 64	(1635–2146)
12 ^c	515 (14.0)	1985 ± 60^b	(1818–2229)	350 (11.0)	1938 ± 57	(1743–2143)
13 ^c	487 (13.2)	2017 ± 62^b	(1818–2209)	361 (11.3)	1949 ± 71	(1627–2268)
14 ^c	370 (10.0)	2046 ± 66^b	(1738–2236)	307 (9.6)	1992 ± 76^b	(1767–2319)
15 ^c	310 (8.4)	2062 ± 57	(1856–2220)	203 (6.4)	2018 ± 78	(1842–2360)
16 ^c	226 (6.1)	2085 ± 52	(1926–2198)	136 (4.3)	2047 ± 73	(1868–2274)
17	135 (3.7)	2087 ± 53	(1920–2226)	70 (2.2)	2090 ± 64	(1968–2315)
All ^c	3688	1992 ± 80	(1630–2236)	3182	1,936 ± 83	(1,627–2,360)

^a Significant differences to previous age, $p < 0.05$.

^b Significant differences to previous age, $p < 0.001$.

^c Significant differences between sexes, $p < 0.001$.

doi:10.1371/journal.pone.0127294.t002

Figs 1 and 2 illustrate the LMS smoothed percentiles for AD-SoS according to age, height and weight for girls and boys, respectively. These results (LMS coefficients and percentiles) are presented in detail in the S1, S2 and S3 Tables. In both genders, AD-SoS increased with age and height. However, AD-SoS seems to decrease among girls with body weight above 60 kg and boys with more than 70 kg.

Table 3 shows the AD-SoS Z-scores according to skin color and weight status. There were no differences on AD-SoS Z-scores related to skin color in both sexes. The obese group showed lower values of AD-SoS Z-scores compared with subjects classified as thin and normal weight. Furthermore, overweight girls showed significantly lower AD-SoS Z-scores than thinness and normal weight girls but higher than obese individuals.

Fig 3 illustrates the relationship between AD-SoS and BMI for girls and boys.

Positive correlation was observed between AD-SoS and BMI both in girls and boys ($r = 0.21$ and 0.24 , respectively). However, the best relation between AD-SoS and BMI was obtained by the cubic model ($r^2 = 0.13$ for girls and $r^2 = 0.10$ for boys) instead of linear ($r^2 = 0.04$ and $r^2 = 0.06$, for girls and boys, respectively). Considering the results observed in AD-SoS centiles with weight, we subjectively decided to divide the sample of boys and girls in two groups according to body weight. The cut-off for the groups was set at 65 kg (< 65 kg and ≥ 65 kg) for girls and 75 kg (< 75 kg and ≥ 75 kg) for the boys, because these values are the boundaries between the categories 60–70 kg and 70–80 kg for girls and boys, respectively. Correlations between AD-SoS (m/s and Z-score) and age and anthropometric variables for total sample divided by sex and weight categories, < 65 kg and ≥ 65 kg for girls and < 75 kg and ≥ 75 kg for boys, are presented in detail in the S4 Table.

The results of multiple linear regression analysis for total sample and after division by weight categories are illustrated in Figs 4 (Girls) and 5 (Boys). For girls, age accounted for 48% ($\beta = 0.692$; $p < 0.001$) and 50% ($\beta = 0.707$; $p < 0.001$) of AD-SoS (m/s) variance, in whole-group and those with < 65 kg, respectively. For boys, height was the best predictor of

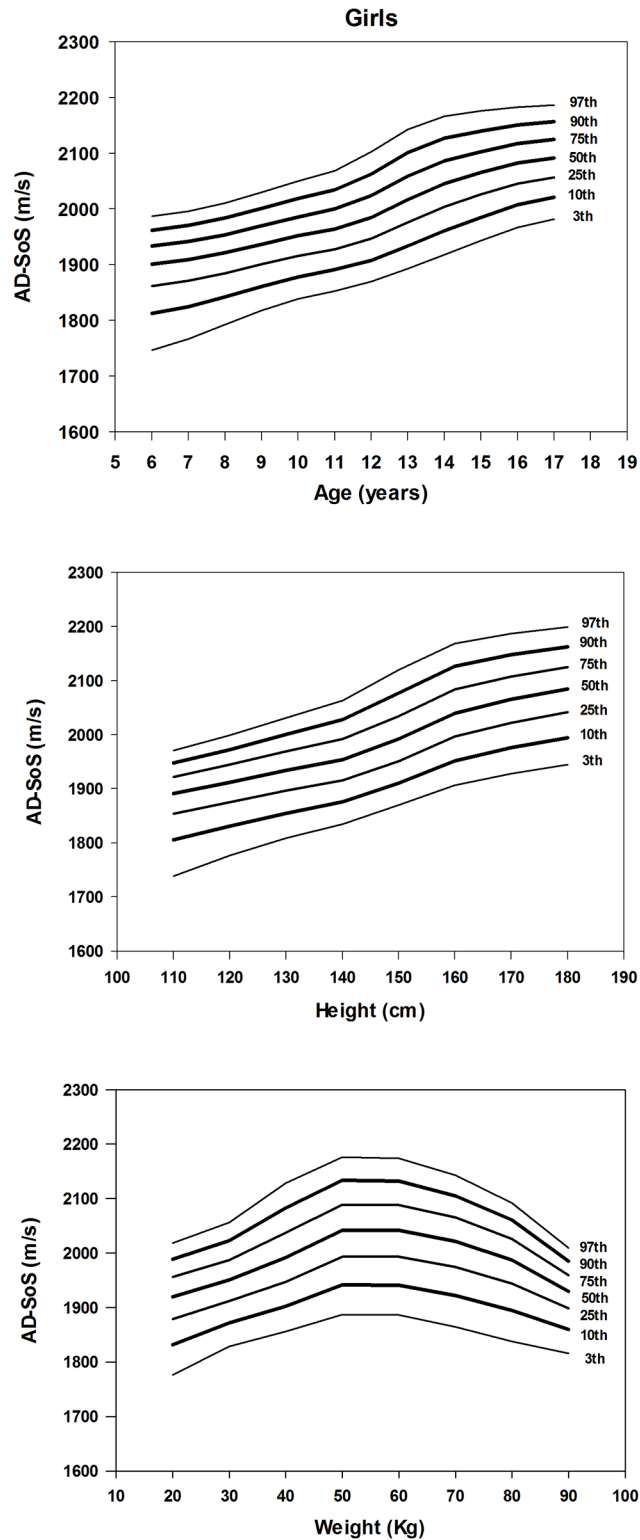


Fig 1. Smoothed percentile charts of AD-SoS for girls according to age (year), height (cm) and weight (kg).

doi:10.1371/journal.pone.0127294.g001

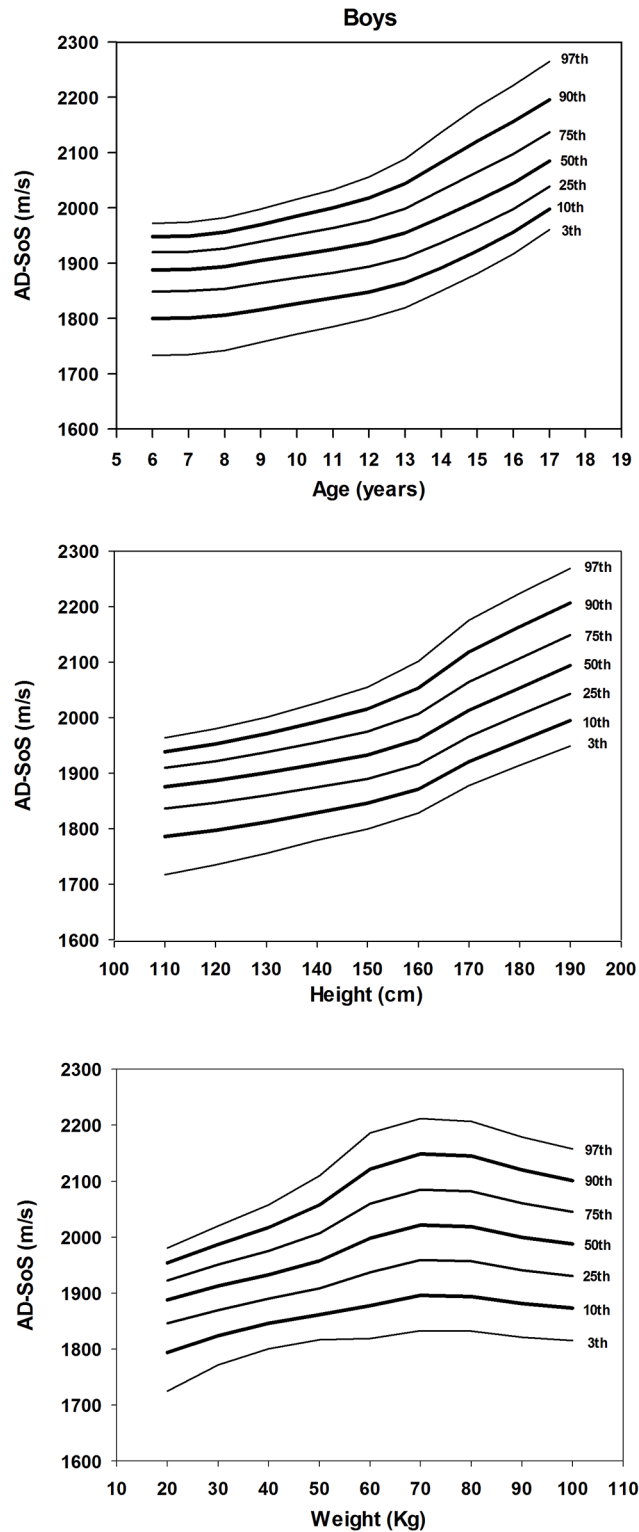


Fig 2. Smoothed percentile charts of AD-SoS for boys according to age (year), height (cm) and weight (kg).

doi:10.1371/journal.pone.0127294.g002

Table 3. AD-SoS Z-scores according to skin color and weight status.

	AD-SoS Z-score					
	N	Girls Mean ± SD	Median (range)	N	Boys Mean ± SD	Median (range)
Skin color						
White	2621	-0.01 ± 0.98	-0.03 (-3.29 to 3.55)	2,064	-0.02 ± 0.98	0.01 (-4.84 to 4.60)
Black	324	0.05 ± 1.06	-0.03 (-3.14 to 3.18)	343	0.05 ± 1.09	0.06 (-3.36 to 4.69)
Mulatto	702	0.02 ± 1.07	-0.01 (-4.61 to 4.14)	713	0.03 ± 1.01	0.01 (-3.39 to 3.14)
Yellow/Indigenous	41	-0.18 ± 0.89	-0.06 (-2.66 to 1.43)	62	0.09 ± 1.14	1.00 (-2.62 to 3.54)
Weight status						
Thinness	422	0.11 ± 1.00^{a,b}	0.11 (-3.14 to 3.05)	239	-0.02 ± 0.93^c	0.00 (-2.92 to 4.04)
Normal	2,646	0.07 ± 0.97^{a,b}	0.03 (-3.29 to 4.14)	2,246	0.04 ± 0.99^{a,d}	0.06 (-3.54 to 4.69)
Overweight	492	-0.25 ± 1.01^a	-0.28 (-4.61 to 3.55)	533	-0.08 ± 1.10	-0.11 (-4.84 to 4.02)
Obese	128	-0.88 ± 1.07	-0.92 (-3.21 to 3.17)	164	-0.28 ± 0.89	-0.29 (-2.68 to 2.49)

^a Significant differences from obese, p<0,001

^b Significant differences from overweight, p<0,001

^c Significant differences from obese, p<0,05

^d Significant differences from overweight, p<0,01.

doi:10.1371/journal.pone.0127294.t003

AD-SoS in whole-group (beta = 0.593; p < 0.001) and among boys with less than 75 kg (beta = 0.585; p < 0.001).

When analyzing the groups of individuals with lower body weight (< 65 kg and < 75 kg, for girls and boys, respectively) and total samples, in both sexes the independent variables tested showed low predictive power for the AD-SoS Z-score, with the value of r² ranging from 0.02 to 0.03. However, among heavier girls and boys (≥ 65 kg and ≥ 75 kg, respectively) similar results were observed: BMI Z-score and BMI (kg/m²) showed a significant negative correlation with AD-SoS (m/s) and AD-SoS Z-score, respectively, and independent predictors of AD-SoS (BMI Z-score: beta = -0.608; p < 0.001 for girls and -0.596; p < 0.001 for boys) and AD-SoS Z-score (BMI: beta = -0.499; p < 0.001 and -0.340; p < 0.001, for girls and boys, respectively).

Discussion

Several studies demonstrate that AD-SoS parameter in healthy children and adolescents increase with age [14–18,20,25,36]. In the present study, increases in AD-SoS from 6 to 17 years were approximately 10% in both sexes. Girls presented higher values of Ad-SoS in all age groups compared to boys, except for the age group of 6 and 17 years. These findings are probably due to the later manifestation of puberty in boys. These results are consistent with other studies that verify puberty and gender influence in AD-SoS [15,18]. Puberty changes body composition and at this stage sexual dimorphism becomes clear. Bone mass acquisition reaches its peak on puberty, earlier in females than males [1], influenced by sexual hormones, GH-IGF1 axis, insulin and leptin [37].

Besides considering weight, age and gender influences, bone mineral content also varies depend on race or skin color [23]. In Brazil, miscegenation is an important aspect and must always be considered in racial analyses. Since 1991 the country has officially adopted the proposal that these data should be collected based on self-declaration; i.e., each individual chooses from five race categories—white, black, mulatto, yellow, and indigenous—which he or she feels is appropriate [29]. Brazilians are one of the most heterogeneous populations in the world, as the result of more than five centuries of miscegenation of people from different ethnic

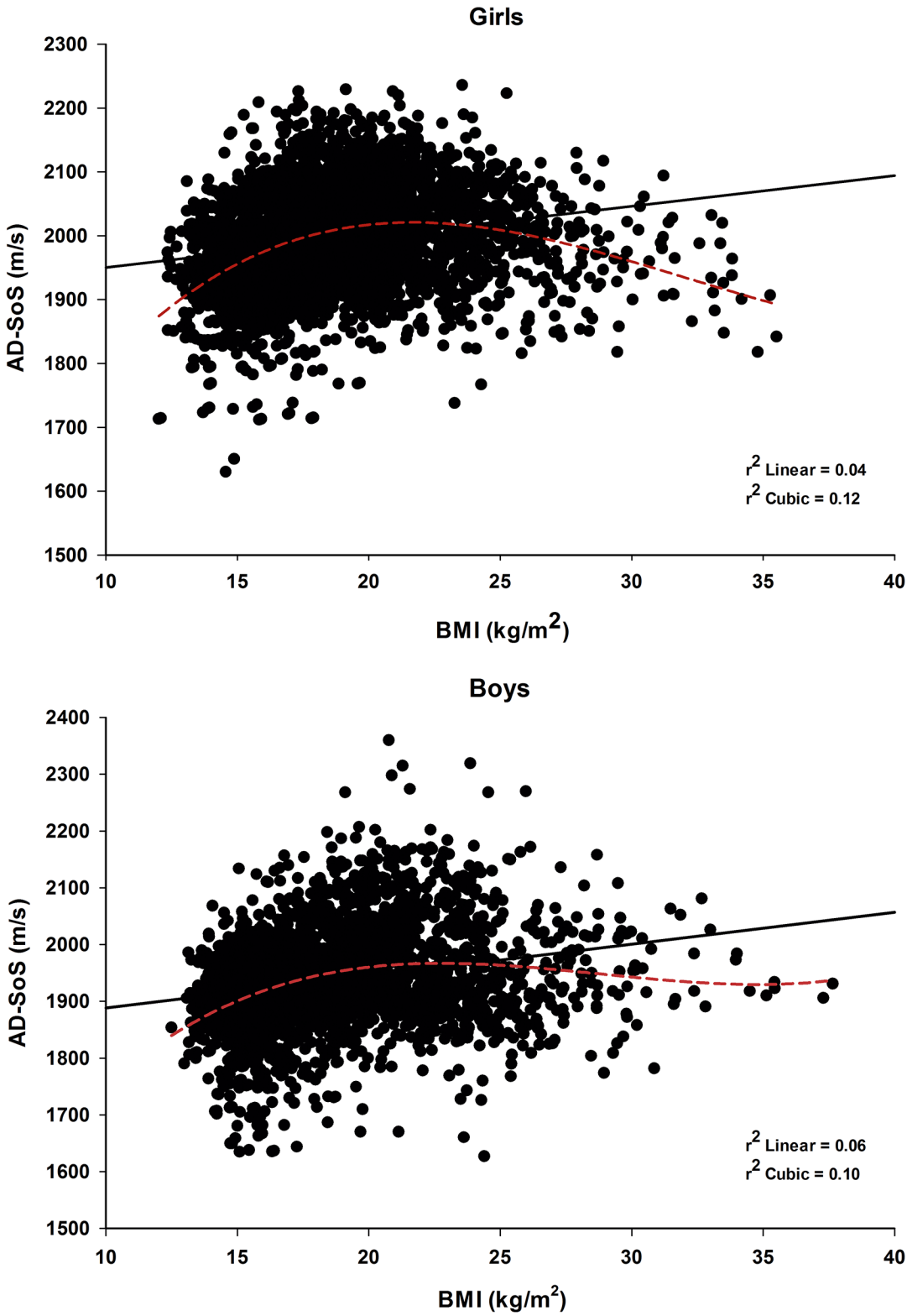


Fig 3. Linear (solid line) and cubic (dotted line) relationship between AD-SoS and BMI for girls and boys.

doi:10.1371/journal.pone.0127294.g003

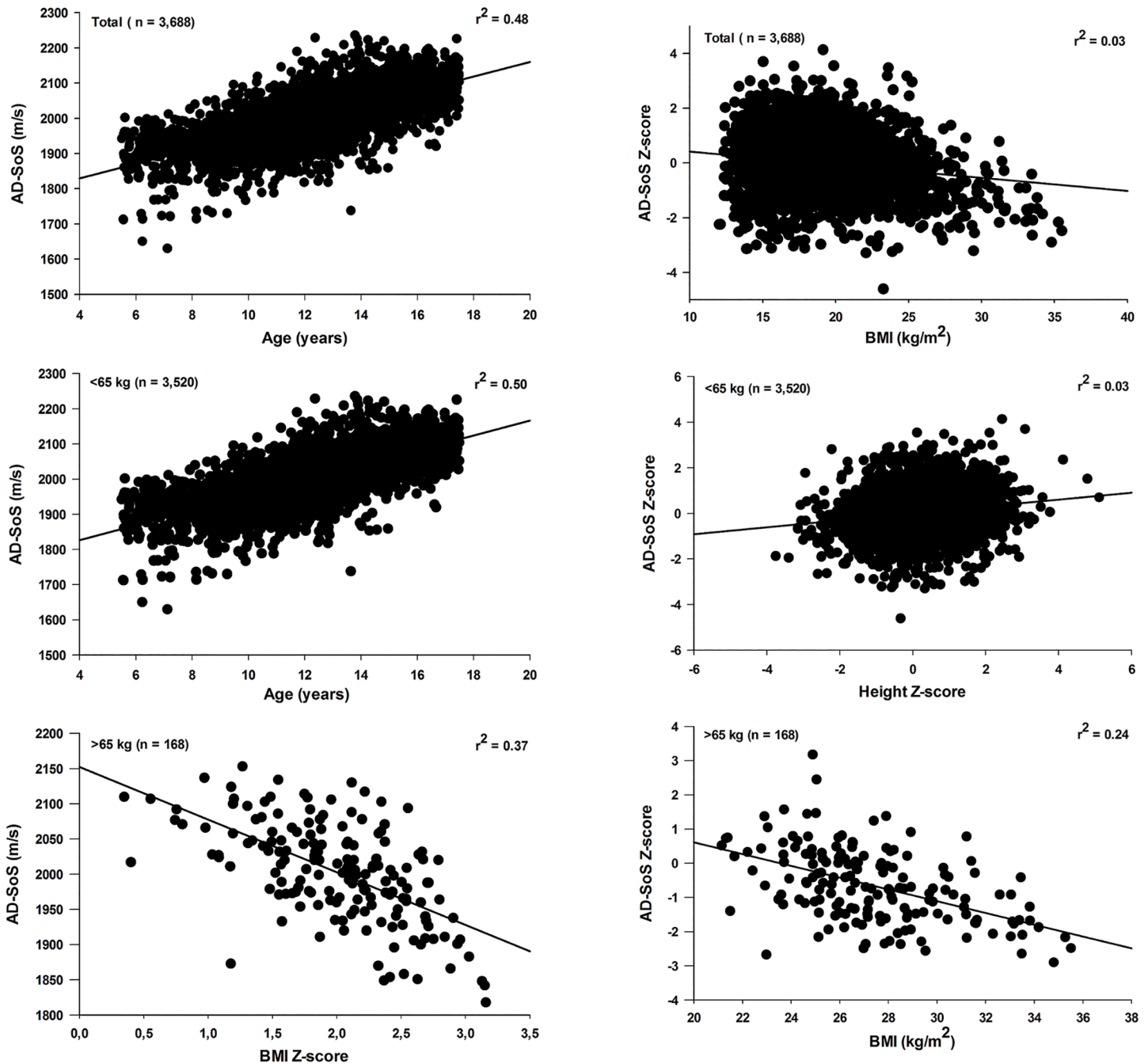


Fig 4. Relationship of AD-SoS (left panel) and AD-SoS Z-score (right panel) versus independent variables for total sample and divided by weight categories in girls.

doi:10.1371/journal.pone.0127294.g004

groups from several continents (Europe, Africa and Asia). In addition, around 2.5 million American Indians were already in the country. Probably, due to this great miscegenation, we did not find the influence of skin color on bone mass in this study.

Regarding the weight status, this study showed lower values of AD-SoS Z-scores in obese when compared to normal and thin boys and girls. These results are consistent with other studies using QUS of phalanges, in which obese children and adolescents showed low bone quality

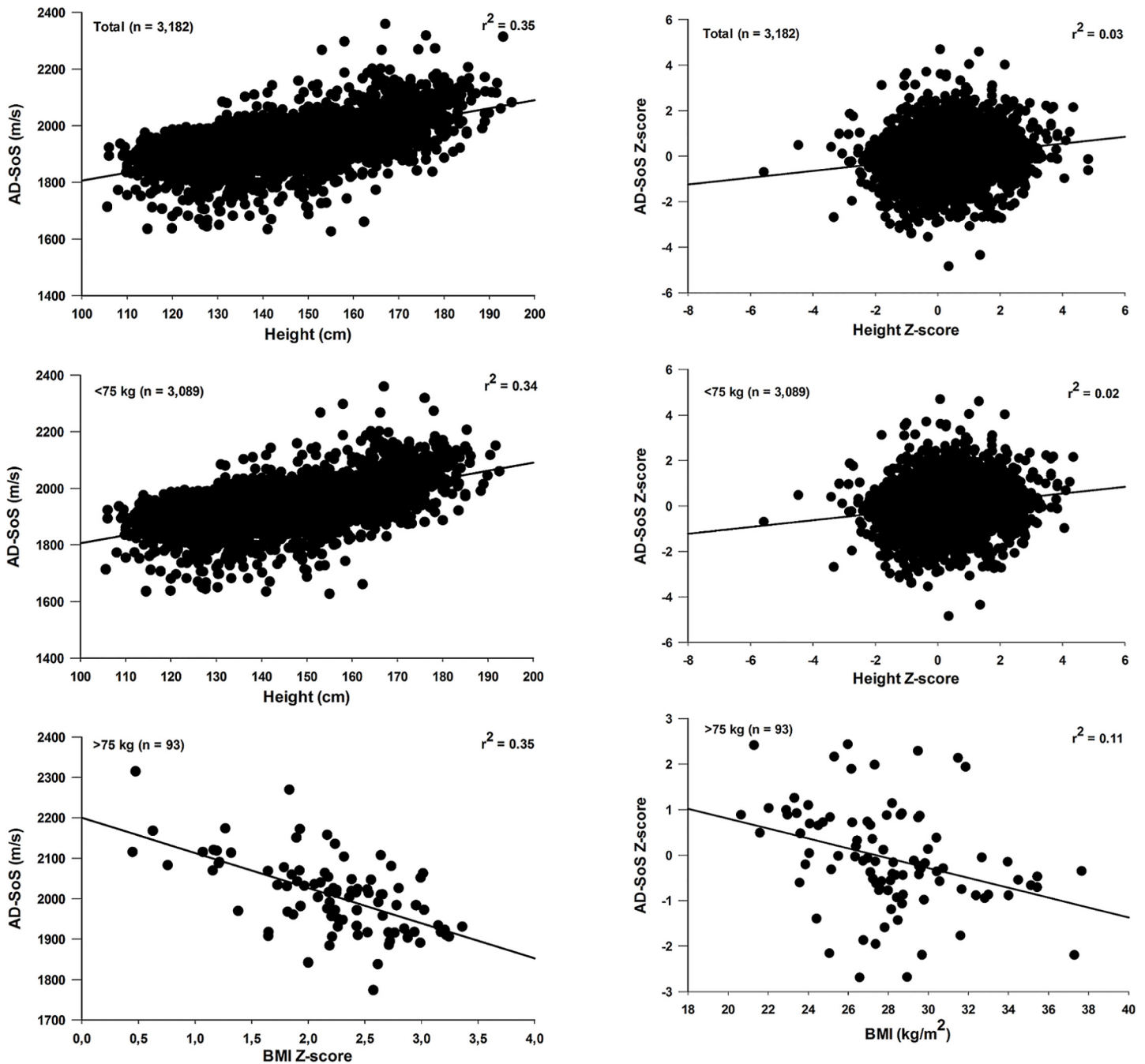


Fig 5. Relationship of AD-SoS (left panel) and AD-SoS Z-score (right panel) versus independents variables for total sample and divided by weight categories in boys.

doi:10.1371/journal.pone.0127294.g005

and reduced strength compared to controls [25], and a negative correlation between body fat and AD-SoS was found in healthy children and adolescents [24]. In our study, impairment in AD-SoS Z-scores was observed mainly among obese girls, and it was verified that in heavier children and adolescents BMI influenced negatively AD-SoS values (Figs 4 and 5 and S4 Table). The reason of the influence of obesity on bone status in children and adolescents is still unclear. Previous studies proposed a protective effect of overweight on low bone mass based on

the observation of decreased risk for fragility fractures and increased bone mineral density in obese adults. The same hypothesis was also considered for pediatric age-groups.

Obese children and adolescents may have several factors impairing bone mass. Results from DXA in obese adolescents demonstrated that, despite increased mechanical loading and independent of lean mass, adiposity is not beneficial to bone structure [38]. Recently, a negative effect of fat mass was observed in bone mass development in male adolescents, and in femur and spine bone mineral density among females [26].

However, several studies have demonstrated effect of soft tissue thickness on AD-SoS measurement [39–42]. The impairment in the speed of the ultrasound wave can be misinterpreted as a reduction in the quality of the bone, while it is likely just an artifact from increased subcutaneous fat [43]. In our study it is clear that obesity negatively influences AD-SoS values in children and adolescents, but to confirm this it would be necessary to have another method (i.e., DXA) or parameter independent of the thickness of soft tissue, such as bone transmission time (BTT), unlike AD-SoS, BTT is largely independent of ultrasound attenuation and soft tissue bias [44]. In our study, we are not able to establish if this occurred due to the higher attenuation of ultrasound wave velocity in these individuals or by the negative impact of obesity in bone mass. We decided to remove the BTT, in our study, because we lost about 30% of the data of this variable and we considered that this could interfere with the comparisons with AD-SoS results.

The new database provided in this study of QUS parameters of the phalanges can be a useful tool to assess the position of an individual, compared to a reference population and analyzing the trajectory of these parameters in longitudinal assessments [11]. However, it's important to highlight that we cannot claim that individuals with lower values of AD-SoS for age, height or weight (ie: < -2 or z-score < 10 th centile) may have increased risk of bone fracture than individuals considered "normal" (AD-SoS Z-scores values greater than 0). Our study did not evaluate the risk of fracture, until now the only validated skeletal site for the clinical use of QUS in the management of osteoporosis is the heel [45]. A recent prospective study demonstrated that AD-SoS is significantly independent predictor of osteoporotic fractures (hip and clinical vertebral fractures) in postmenopausal women and it was able to discriminate between fractured and non-fractured subjects in the group of women [46]. Moreover, previous studies indicated that QUS might be a useful method to identify bone fracture risk in pediatric patients with bone and mineral disorders. [4,47]. Considering this findings, our data could be used as an initial screen for low bone mass among children and adolescents.

Our study has some limitations, such as the lack of a reference method, such as DXA, to determine more accurately if the negative influence found by the analyzed ultrasound parameter (AD-SoS) reflects negative effect of obesity on bone mass or a limitation of this parameter in assessing these individuals. Additionally, there is lack of information on past bone fractures, sex hormones measurements and physical activity level, which could enhance the results. Nevertheless, the absence of such measures can be justified in part by the difficulties to obtain those informations in a large number of individuals, particularly in children and adolescents.

However, this study provides a large number of subjects investigated and the use of a relatively simple and portable tool for assessment of bone status. This can facilitate the reproducibility of our findings in groups of individuals with similar characteristics as schools around the world. Moreover, QUS assesses not only quantity but also the quality of bone based on the information of the micro-architecture and elasticity [9,10,12]. The relative simplicity, ease of transportation and non-exposure to radiation, presents advantages for the use of QUS in comparison to other methods in children and adolescents [5].

Conclusions

In our sample of girls and boys aged 7–17 years, age and height, respectively were main determinants of AD-SoS. The present study reports reference AD-SoS values in healthy children and adolescents based on a representative and the largest sample ever published. This parameter proved to be sensitive to changes influenced by sex, age and weight status, but not by skin color. These results indicate a negative influence of obesity on AD-SoS. In this sense, we recommend that the results of AD-SoS in obese children and adolescents should be interpreted with caution. However, our normative data could be used for monitoring bone status in individual or samples with age range from seven to 17 years.

Supporting Information

S1 Table. LMS coefficients and smoothed percentiles (3th, 10th, 25th, 75th, 90th and 97th) of AD-SoS (m/s) for Brazilian children and adolescents according to age (years) and sex. (DOCX)

S2 Table. LMS coefficients and smoothed percentiles (3th, 10th, 25th, 75th, 90th and 97th) of AD-SoS (m/s) for Brazilian children and adolescents according to height (cm) and sex. (DOCX)

S3 Table. LMS coefficients and smoothed percentiles (3th, 10th, 25th, 75th, 90th and 97th) of AD-SoS (m/s) for Brazilian children and adolescents according to weight (kg) and sex. (DOCX)

S4 Table. Correlation coefficients between AD-SoS and age and anthropometrics measurements for Brazilian children and adolescents according to weight categories and sex. (DOCX)

Acknowledgments

This study was supported by the São Paulo Research Foundation (FAPESP: Process n° 2006/01978-0 and 2011/23460-1 (E.M.G); 2002/13021-1 and 2012/16778-8 (G.G-J)), by the Coordination for the Improvement of Higher Education Personnel (CAPES: R.R.R. and W.R.G.C.) and by National Council for Scientific and Technological Development (CNPq: P.A.R.M., N.N. V-J and A.C.L.M.C.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: EMG RRR AA-BF GG-J. Performed the experiments: EMG RRR WRGC AMdM KDS EPR. Analyzed the data: EMG RRR AMM GG-J. Contributed reagents/materials/analysis tools: PARM NNV-J ACLMC. Wrote the paper: EMG RRR TK LES AA-BF AMM GG-J.

References

1. Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R (1991) Critical years and phases of puberty for spinal and femoral bone mass accumulation during adolescence. *J Clin Endocrinol Metab* 73: 555–563. PMID: [1874933](#)
2. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H (1992) Longitudinal monitoring of bone mass accumulation in healthy adolescents: Evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab* 75: 1060–1065. PMID: [1400871](#)

3. Fielding KT, Nix DA, Bachrach LK (2003) Comparison of calcaneous ultrasound and dual X-ray absorptiometry in children at risk of osteopenia. *J Clin Densitom* 6: 7–15. PMID: [12665697](#)
4. Baroncelli GI, Federico G, Bertelloni S, Sodini F, De Terlizzi F, Cadossi R, et al. (2003) Assessment of bone quality by quantitative ultrasound of proximal phalanges of the hand and fracture rate in children and adolescents with bone and mineral disorders. *Pediatr Res* 54: 125–136. PMID: [12700367](#)
5. Baroncelli GI (2008) Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. *Pediatr Res* 63: 220–228. doi: [10.1203/PDR.0b013e318163a286](#) PMID: [18287958](#)
6. Kaufman JJ, Einhorn TA (1993) Perspective: ultrasound assessment of bone. *J Bone Miner Res* 8: 517–525. PMID: [8511979](#)
7. Fuerst T, Glüer CC, Genant HK (1995) Quantitative ultrasound. *Eur J Radiol* 20:188–192. PMID: [8536746](#)
8. Njeh CF, Boivin CM, Langton CM (1997) The role of ultrasound in the assessment of osteoporosis: a review. *Osteoporos Int* 7: 7–22. PMID: [9102067](#)
9. Baroncelli GI, Battini R, Bertelloni S, Brunori E, de Terlizzi F, Vierucci F, et al. (2010) Analysis of quantitative ultrasound graphic trace and derived variables assessed at proximal phalanges of the hand in healthy subjects and in patients with cerebral palsy or juvenile idiopathic arthritis. A pilot study. *Bone* 46: 182–189. doi: [10.1016/j.bone.2009.09.010](#) PMID: [19772958](#)
10. Wüster C, Albanese C, De Aloysio D, Duboeuf F, Gambacciani M, Gonnelli S, et al. (2000) Phalangeal osteosonogrammetry study: age-related changes, diagnostic sensitivity, and discrimination power. *J Bone Miner Res* 15: 1603–1614. PMID: [10934660](#)
11. Halaba ZP (2008) Quantitative ultrasound measurements at hand phalanges in children and adolescents: a longitudinal study. *Ultrasound Med Biol* 34:1547–1553. doi: [10.1016/j.ultrasmedbio.2008.03.007](#) PMID: [18485570](#)
12. Albanese CV, Cepollaro C, De Terlizzi F, Brandi ML, Passariello R (2009) Performance of five phalangeal QUS parameters in the assessment of gonadal-status, age and vertebral fracture risk compared with DXA. *Ultrasound Med Biol* 35: 537–544. doi: [10.1016/j.ultrasmedbio.2008.09.027](#) PMID: [19097682](#)
13. Cole TJ, Green PJ (1992) Smoothing reference centile charts: the LMS method and penalized likelihood. *Stat Med* 11: 1305–1319. PMID: [1518992](#)
14. Halaba Z, Pluskiewicz W (1997) The assessment of development of bone mass in children by quantitative ultrasound through the proximal phalanxes of the hand. *Ultrasound Med Biol* 23: 1331–1335. PMID: [9428132](#)
15. Baroncelli GI, Federico G, Bertelloni S, De Terlizzi F, Cadossi R, Saggese G (2001) Bone quality assessment by quantitative ultrasound of proximal phalanxes of the hand in healthy subjects aged 3–21 years. *Pediatr Res* 49: 713–718. PMID: [11328957](#)
16. Gimeno Ballester J, Azcona San Julian C, Sierrasesumaga Ariznabarreta L (2001) Bone mineral density determination by osteosonography in healthy children and adolescents: normal values. *An Esp Pediatr* 54: 540–546. PMID: [11412400](#)
17. Barkmann R, Rohrschneider W, Vierling M, Troger J, De Terlizzi F, Cadossi R, et al. (2002) German pediatric reference data for quantitative transverse transmission ultrasound of finger phalanges. *Osteoporos Int* 13: 55–61. PMID: [11878455](#)
18. Vignolo M, Brignone A, Mascagni A, Ravera G, Biasotti B, Aicardi G (2003) Influence of age, sex, and growth variables on phalangeal quantitative ultrasound measures: a study in healthy children and adolescents. *Calcif Tissue Int* 72: 681–688. PMID: [14562996](#)
19. Halaba ZP, Pluskiewicz W (2004) Quantitative ultrasound in the assessment of skeletal status in children and adolescents. *Ultrasound Med Biol* 30: 239–243. PMID: [14998676](#)
20. Baroncelli GI, Federico G, Vignolo M, Valerio G, del Puente A, Maghnie M, et al. (2006) Cross-sectional reference data for phalangeal quantitative ultrasound from early childhood to young-adulthood according to sex, age, skeletal growth, and pubertal development. *Bone* 39: 159–173. PMID: [16473568](#)
21. Santos K, Petroski E, Ribeiro R, Guerra-Junior G (2009) Bone quantity and quality in Brazilian female schoolchildren and adolescents. *J Bone Miner Metab* 27: 507–512. doi: [10.1007/s00774-009-0067-5](#) PMID: [19326046](#)
22. Ribeiro RR, Guerra-Junior G, Barros-Filho AA (2009) Bone mass in schoolchildren in Brazil: the effect of racial miscegenation, pubertal stage, and socioeconomic differences. *J Bone Miner Metab* 27: 494–501. doi: [10.1007/s00774-009-0062-x](#) PMID: [19283337](#)
23. Ribeiro RR, Santos-Ribeiro KD, Guerra-Junior G, Barros-Filho AA (2010). Comparison of bone quantity by ultrasound measurements of phalanges between white and black children living in Paraná, Brazil, with Europeans. *Braz J Med Biol Res* 43: 976–981. PMID: [20802978](#)

24. Dib L, Arabi A, Maalouf J, Nabulsi M, El-Hajj Fuleihan G (2005) Impact of anthropometric, lifestyle, and body composition variables on ultrasound measurements in school children. *Bone* 36: 736–742. PMID: [15784188](#)
25. Longhi S, Pasquino B, Calcagno A, Bertelli E, Olivieri I, Di Iorgi N, et al. (2013) Small metacarpal bones of low quality in obese children. *Clin Endocrinol (Oxf)* 78: 79–85.
26. Mosca LN, Goldberg TB, da Silva VN, da Silva CC, Kurokawa CS, Bisi Rizzo AC, et al. (2014) Excess body fat negatively affects bone mass in adolescents. *Nutrition* 30: 847–852. doi: [10.1016/j.nut.2013.12.003](#) PMID: [24985003](#)
27. Carvalho WR, Gonçalves EM, Ribeiro RR, Farias ES, Carvalho SS, Guerra-Júnior J (2011) Influence of body composition on bone mass in children and adolescents. *Rev Assoc Med Bras* 57: 662–627. PMID: [22249546](#)
28. de Moraes AM, Gonçalves EM, Barbeta VJ, Guerra-Junior G (2013) Cross-sectional study of the association of body composition and physical fitness with bone status in children and adolescents from 11 to 16 years old. *BMC Pediatr* 13: 117. doi: [10.1186/1471-2431-13-117](#) PMID: [23937889](#)
29. INEP—Instituto Nacional de Estudos e Pesquisas Educacionais Anísio Teixeira. Mostre sua raça, declare sua cor. Available at: http://inep.gov.br/imprensa/noticias/censo/escolar/news05_05.htm.
30. Lohman TG, Roche AF, Martorell R (1988) Anthropometric standardization reference manual. Human Kinetics, Champaign.
31. de Onis M, Onyango A, Borghi E, Siyam A, Nishida C, Siekmann J (2007) Development of a WHO growth reference for school-aged children and adolescents. *Bull WHO* 85: 660–667. PMID: [18026621](#)
32. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 7: 284–294. doi: [10.1111/j.2047-6310.2012.00064.x](#) PMID: [22715120](#)
33. Bonnick SL, Johnston CC Jr, Kleerekoper M, Lindsay R, Miller P, Sherwood L, et al. (2001) Importance of Precision in Bone Density Measurements. *J Clin Densitom* 4: 105–110. PMID: [11477303](#)
34. Cole TJ (1990) The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 44: 45–60. PMID: [2354692](#)
35. Pan H, Cole TJ. LMSchartmaker, a program to construct growth references using LMS method. Version 2.42. 2000. Available at: <http://www.healthforallchildren.co.uk>. Accessed 10 January 2012.
36. Drozdowska B, Pluskiewicz W (2003) Skeletal status in males aged 7–80 years assessed by quantitative ultrasound at the hand phalanges. *Osteoporos Int* 14: 295–300. PMID: [12730791](#)
37. Loomba-Albrecht LA1, Styne DM (2009) Effect of puberty on body composition. *Curr Opin Endocrinol Diabetes Obes* 16: 10–5. PMID: [19115520](#)
38. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, et al. (2007) Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab* 92: 143–147. PMID: [17047019](#)
39. Guglielmi G, de Terlizzi F, Scalzo G, Battista C, Scillitani A (2010) Cortical thickness and medullary canal dimensions of the bone phalanx are predicted by quantitative ultrasound parameters. *J Clin Densitom* 13:219–227. doi: [10.1016/j.jocd.2010.01.002](#) PMID: [20435266](#)
40. Porta F, Spada M, Lala R, Mussa A (2008) Phalangeal quantitative ultrasound in children with phenylketonuria: a pilot study. *Ultrasound Med Biol* 34:1049–1052. doi: [10.1016/j.ultrasmedbio.2007.12.013](#) PMID: [18313203](#)
41. Mussa A, Bertorello N, Porta F, Galletto C, Nicolosi MG, Manicone R, et al. (2010) Prospective bone ultrasound patterns during childhood acute lymphoblastic leukemia treatment. *Bone* 46:1016–1020. doi: [10.1016/j.bone.2009.12.019](#) PMID: [20044045](#)
42. Mussa A, Porta F, Gianoglio B, Gaido M, Nicolosi MG, De Terlizzi F, et al. (2007) Bone alterations in children and young adults with renal transplant assessed by phalangeal quantitative ultrasound. *Am J Kidney Dis* 50:441–449. PMID: [17720523](#)
43. Bajaj M, Koo W, Hammami M, Hockman EM (2010) Effect of subcutaneous fat on quantitative bone ultrasound in chicken and neonates. *Pediatr Res* 68:81–83. doi: [10.1203/00006450-201011001-00155](#) PMID: [20357694](#)
44. Barkmann R, Lüsse S, Stampa B, Sakata S, Heller M, Glüer CC (2000) Assessment of the geometry of human finger phalanges using quantitative ultrasound in vivo. *Osteoporos Int* 11:745–755. PMID: [11148802](#)
45. Moayyeri A, Adams JE, Adler RA, Krieg MA, Hans D, Compston J, et al. (2012) Quantitative ultrasound of the heel and fracture risk assessment: an updated meta-analysis. *Osteoporos Int* 23: 143–153. doi: [10.1007/s00198-011-1817-5](#) PMID: [22037972](#)

46. Guglielmi G, Rossini M, Nicolosi MG, Ragno A, Lentini G, de Terlizzi F (2013) Three-year prospective study on fracture risk in postmenopausal women by quantitative ultrasound at the phalanges. *J Clin Densitom* 16: 341–346. doi: [10.1016/j.jocd.2012.07.006](https://doi.org/10.1016/j.jocd.2012.07.006) PMID: [22901551](https://pubmed.ncbi.nlm.nih.gov/22901551/)
47. Mussa A, Porta F, Baldassarre G, Tuli G, de Terlizzi F, Matarazzo P, et al. (2012) Phalangeal quantitative ultrasound in 1,719 children and adolescents with bone disorders. *Osteoporos Int* 23: 1987–1998. doi: [10.1007/s00198-011-1794-8](https://doi.org/10.1007/s00198-011-1794-8) PMID: [21947033](https://pubmed.ncbi.nlm.nih.gov/21947033/)