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ORIGINAL ARTICLE

Characteristics of healthy German children and adolescents across tertiles of calcaneal stiffness index

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Abstract

Aim Identifying risk factors for low bone stiffness index (SI) might be one crucial strategy for osteoporosis prevention. Purpose was to characterize healthy schoolchildren across tertiles of SI.

Subject and methods In 248 girls (13.4 ± 1.9 years, BMI: 20.2 ± 4.8 kg/m^2) and 231 boys (13.6 ± 1.7 years, BMI: 19.3 ± 3.3 kg/m^2), the following parameters were assessed: calcaneal SI (quantitative ultrasound), body composition (bioelectrical impedance analysis), Bone Healthy Eating Index (BoneHEI; food frequency questionnaire), and physical activity level (PAL; activity questionnaire). Participants were classified according to age- and sex-specific SI tertiles (low, medium, and high). Between-group comparisons were achieved by Kruskal–Wallis-*H*-tests ($\alpha = 0.05$).

Results Girls with low SI had significantly lower body mass (49.2 ± 16.7 vs 54.8 ± 12.2 kg; $p < 0.01$), BMI (19.6 ± 5.4 vs 21.3 ± 3.9 kg/m^2; $p < 0.0001$), fat-free mass (36.3 ± 8.3 vs 39.5 ± 6.0 kg; $p < 0.01$), and fat mass (23.7 ± 9.1 vs 26.8 ± 7.2%; $p < 0.05$) compared to those with high SI. In boys, significant differences between low and high SI were obtained for PAL (1.49 ± 0.12 vs 1.56 ± 0.14; $p < 0.01$). BoneHEI was not significantly different between tertiles in both sexes.

Conclusion Girls with low body mass and boys with low PAL have a higher risk for low SI. Schoolchildren should strive for normal body mass and perform regular physical activity.

Keywords Bone health · Bone healthy eating index · Physical activity level · Body composition

Introduction

Skeletal growth and mineralization develop through the first 2 decades of life and reach a plateau (denoted as *peak bone mass*) in the late-teen or young-adult years (Faulkner and Bailey 2007). A high peak bone mass reduces the risk of fractures and osteoporosis later on (Heaney et al. 2001). Nearly 60% of the osteoporosis risk can be explained by the amount of bone mineral acquired by early adulthood (Hui et al. 1990). Therefore, an essential strategy for osteoporosis prevention is attaining a high peak bone mass during adolescence and young-adult life respectively.

In addition to genetic factors, several other factors, such as body weight and its composition, and lifestyle factors, influence adult peak bone mass (Weaver et al. 2016). Some of the most critical variables are anthropometric status and body composition. It has been shown that body mass (which is itself a composition of environmental and hereditary factors) is the largest single determinant of adult bone mass variability (Heaney et al. 2001), and a very low body mass index (BMI) is related to a higher risk of osteoporosis (Munhoz et al. 2018). In contrast, high fat-free mass levels are favorable for bone status due to the mechanical and biochemical coupling between muscle and bone (Ho-Pham et al. 2014).

Another crucial factor for bone health and bone mineral density is regular physical activity. Increased bone loading due to higher amounts and intensity of physical activity improves bone metabolism and leads to a higher bone density (Rubin and Lanyon 1984). Repeated activities with high intensity and rapid changes of direction, accelerations, and/or jumps are recommended to increase bone mineral density in children and adolescents (Witzke and Snow 2000).

Furthermore, the individual diet influences bone metabolism. It was shown that calcium and vitamin D

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deficiencies can decrease bone mineral density and lead to a predisposition to osteoporosis (Heaney et al. 2001). Finally, positive associations between sunlight exposure (Farrar et al. 2016; Alghadir et al. 2018), early-life breastfeeding (Jones et al. 2013), and bone status have been reported in adolescents. Other risk factors for osteoporosis include smoking, excessive alcohol intake, chronic diseases, and the use of medications (Ferrari 2005).

One strategy for prevention of osteoporosis in later life is the identification of children and adolescents with low bone mineral density, because of their high risk for low peak bone mass. In this context, it is important to point out risk factors for low bone mass in children and adolescents. The identification of these risk factors can help to develop prevention programs specifically targeted to the needs of girls and boys with low bone mass. In the literature, numerous risk factors for low bone parameters in children and adolescents are reported (Jones et al. 2013; Farrar et al. 2016; Alghadir et al. 2018; Heydenreich et al. 2020). However, the authors often used correlation analysis for the identification of a few risk factors. In contrast, characterization of numerous anthropometric and lifestyle factors of participants across tertiles of certain bone parameters has not yet been performed. Therefore, the purpose of this study was to characterize healthy girls and boys with low, medium, and high calcaneal stiffness index (SI), as a measure of bone mass, and to identify risk factors for low bone parameters of participants from the lowest tertile of SI.

Materials and methods

Participants

Four hundred and eighty-six schoolchildren from six secondary schools of different school types in or close by to Schwäbisch Gmünd, Germany, and their respective parents participated in the study. Further information about recruitment process can be found elsewhere (Heydenreich et al. 2020). Measurements were conducted between June 2010 and July 2011.

The participants were tested in a discrete room provided by the respective school. Measurements took place on one testing day in the following order: 1) anthropometry and body composition and 2) bone status (see details below). Furthermore, participants were asked to fill out a standardized questionnaire to assess their lifestyle habits and dietary intake (see details below). In addition, before investigations, parents of the students filled out a standardized questionnaire.

Anthropometric data and body composition

Body mass was measured to the nearest 0.1 kg using a calibrated beam scale (Seca 877, Seca, Hamburg, Germany), with participants in light clothing and without shoes. After that, depending on the estimated weight the clothing participants were wearing, examiners subtracted 0.5–1.0 kg from the measured body mass. Participants were standing in an upright position without wearing shoes and looking straight ahead when height was assessed to the nearest 0.5 cm using a measuring tape. The BMI was calculated and classified according to age- and sex-specific percentile curves for German children and adolescents (Kromeyer-Hauschild et al. 2001).

Body composition was assessed using an impedance analyzer (BIA 2000 – S, Data Input GmbH, Pöcking, Germany) by taking whole-body bioelectrical impedance measurements (resistance; R, and reactance; Xc, at 50 kHz and 800 µA). Participants were in light clothing, bladder-voided, and all metal artifacts were removed when measurements were performed in the morning. During the measurement, participants were in a supine position with legs and arms abducted 45° from the body. Two pairs of detector and injector electrodes were placed on the right alcohol-cleaned hand and foot. Participants were requested not to move during the measurement. Afterwards, the absolute fat-free mass was calculated (Plachta-Danielzik et al. 2012).

Bone status

Quantitative ultrasound (QUS) measurements with the Lunar Achilles InSight Ultrasonometer (GE Healthcare, Milwaukee, WI, USA) were performed to assess bone status. Obtained data were further analyzed using the accompanying software. QUS measurements use ultrasound waves to measure broadband ultrasound attenuation (BUA; $\text{dB} \bullet \text{MHz}^{-1}$) and speed of sound (SOS; $\text{m} \bullet \text{s}^{-1}$). The SI was then calculated using the following equation provided by the manufacturer:

$$\text{SI} = (0.67 \bullet \text{BUA}) + (0.28 \bullet \text{SOS}) - 420. \quad (1)$$

The Z-score values of BUA and SOS were computed using the reference sample of a healthy, pediatric German sample stratified by age and sex (Wünsche et al. 2000) using the following equation: Z-score = (measured values – matched mean values)/matched SD. A Z-score of −2.0 standard deviations or lower was defined as “below the expected range for age” and a Z-score above −2.0 as “within the expected range for age” (Lewiecki et al. 2008).

Trained researchers performed and analyzed QUS measurements, and standardization was achieved according to the manufacturer's recommendations. Participants were in a comfortable seated position directly in front of the Achilles device. They placed their left leg in the device so that foot, calf, and thigh aligned with the calf support and the positioner. Participants were requested not to move during the measurement. Calibration of the Achilles device was performed every week using a calibration phantom.

The precision of the QUS device which was used was reported to be < 2% for SI in children and adolescents (Xu et al. 2014). Furthermore, the SI measured by QUS significantly correlated with outcomes measured by dual energy X-ray absorptiometry ($r = 0.69$; $p < 0.001$) (Xu et al. 2014).

Participants were classified into age- and sex-specific tertiles (lowest, medium, and highest) according to their individual SI, where age was treated as a range (i.e., tertiles for 10- to < 11-year-old girls, tertiles for 10- to < 11-year-old boys, tertiles for 11- to < 12-year-old girls, etc.) (Online Resource 1). We assumed that children and adolescents in the lowest tertile are at higher risk for low peak bone mass than the other groups.

Lifestyle questionnaire and socio-demographic data

Participants were asked to fill out a standardized questionnaire in German, including questions about demographical data, physical activity, staying outside during summer/winter and weekdays/weekend ($h \bullet d^{-1}$), and their smoking status (see Supplementary Material S1; translated into English). Participants documented their average time spent for low-, medium-, and high-intensity physical activities during 1 week (Krems et al. 2004). The average time spent for sporting activities was calculated as the sum of low-, medium-, and high-intensity sporting activities. By use of the equation of Müller et al. (Müller et al. 2004) the resting metabolic rate (RMR) was calculated:

$$\text{RMR}\left(\text{MJ} \bullet \text{d}^{-1}\right) = 0.02606 \bullet \text{weight (kg)} + 0.04129 \bullet \text{height (cm)} \\ +0.311 \bullet \text{sex (female} = 0, \text{male} = 1)–0.08369 \bullet \text{age (years)}–0.808. \tag{2}$$

For calculation of total energy expenditure, specific factors (coefficients) for each activity [World Health Organization (WHO) 1985] were multiplied by the estimated RMR (Müller et al. 2004). Lastly, PAL was calculated in accordance with the WHO's approach (total energy expenditure/estimated RMR) (World Health Organization (WHO) 1985).

Participants had to estimate their physical development using a validated questionnaire consisting of five items (growth in height, body hair, skin changes, facial hair, and voice changes for boys; breast development and menses for girls) (Carskadon and Acebo 1993). By use of an adapted 4-point-scale [1 = not yet started, 2 = barely started, 3 = definitely started, 4 = seems completed] each item had to be estimated (Petersen et al. 1988). Afterwards, puberty category scores were calculated with the three preeminent sexual maturation characteristics of the pubertal development scale (girls: menarche, breast and body hair growth, boys: deepening-voice, body hair and facial hair growth). By use of this score, the children were categorized into one of the five pubertal development stages (pre-, early, midpubescent, advanced, and postpubescent) designed to be similar to Tanner staging categories (Crockett 1988).

Parents of the schoolchildren were also asked to fill out a standardized questionnaire containing questions about the parents´ educational and migration status. The educational status of the parents was calculated according to a modified version of the international standard classification of education (ISCED) provided by UNESCO (Online Resource 2) (United Nations Educational Scientific and Cultural Organization (UNESCO) 2006; Schroedter et al., 2006). Since the education status was determined for both parents individually, the higher value was chosen to classify the children into low, medium, and high parental educational background. Participants were classified as having a migration background if they and at least one parent were not born in Germany or if both parents immigrated to Germany. Furthermore, parents were asked to respond to questions about their child (e.g., vitamin D supplementation in the first year of life, regular drug intake, and the history of fractures). The parental questionnaire was available for all school children.

Food intake

By using an adapted version of the standardized food frequency questionnaire "What do you eat?"(Mensink and Burger 2004) the intake of 13 food items was assessed. Participants estimated the average frequency and the usual quantity of food consumed within the last weeks. A Bone Healthy Eating Index (BoneHEI) was then computed based on the Healthy Nutrition Score for Kids and Youth (HuSKY) (Kleiser et al. 2009), taking into account the actual guidelines for an optimized mixed diet for children and adolescents (Alexy et al. 2008; Kersting and Alexy 2009). In short, the food items in the questionnaire were summarized into eight different food groups [fruits and vegetables, fish, bread, milk and dairy products, meat and sausages, tolerated food (sweets and snacks), soft drinks, and caffeinated beverages] (Schweter 2015). The individual intake was then related to the recommended intake of these food groups (Alexy et al. 2008; Kersting and Alexy 2009; Kleiser et al. 2009). A maximum score of 100 points could be reached for each category when the individual intake corresponded to the recommended intake. The BoneHEI was then calculated as the average of the points earned for each category [range 0–100 points]. A higher BoneHEI reflects a healthier diet for the bone. Detailed information about the calculation of the BoneHEI can be found elsewhere (Schweter 2015; Heydenreich et al. 2020).

Statistics

Statistical analyses were performed with SPSS statistics version 26 for MS-Windows (IBM Corp., Chicago, IL, USA). Data was checked for normality using the Shapiro–Wilk test, and mean values and standard deviations (SD) were calculated. Between-group comparisons were tested by Mann–Whitney *U* tests and Kruskal–Wallis tests for continuous variables and Fisher's exact test for categorical variables. Cramér's *V* was calculated to quantify the strength of association for the output of Fisher's exact tests. The relationship between bone parameters and age were investigated using Spearman's rank correlation analysis. The correlation coefficients (*r*) and Cramér's *V* were classified according to Cohen (Cohen 1988). An *r* and *V* between 0.10–0.29 was considered a small, between 0.30–0.49 a moderate, and between 0.50–1.0 a strong association. The statistical significance was set at $p < 0.05$.

Results

Participants

Out of 486 initial participants, seven school children were excluded from the final analysis (two boys with missing questionnaire, one growth-restricted girl, two girls and one boy with implausible nutrition data, one girl with missing bone parameters). In total, data of 231 boys and 248 girls were analyzed. In Table 1, socio-demographic and anthropometric data and lifestyle factors, as well as further characteristics for all participants, are shown. Boys had significantly higher values for height, fat-free mass, absolute and relative RMR. In addition, boys spent more time on sports, had a higher PAL, and stayed longer outside than girls. The absolute and relative fat mass and the puberty category score were higher in girls than boys. Fisher's exact test revealed that the puberty category score distribution significantly differed by sex. The association between puberty category score and sex was highly significant and can be regarded as "moderate" (Cramér's *V*: 0.36, $p < 0.001$).

The mean bone status parameters were not significantly different between girls and boys ($p > 0.05$; Table 2). Whereas none of the children had SI and BUA values "below the expected range for age", six individuals

Table 1 Characteristics of study participants. Data are presented as mean ± SD and percentage

Variables	Girls (*n* = 248)	Boys (*n* = 231)	*P* value [a]
Socio-demographic data			
Age (years)	13.4 ± 1.9	13.6 ± 1.7	0.190
Educational background			
Low (%)	57.7	58.8	
Medium (%)	15.4	19.5	
High (%)	26.8	21.7	0.299
Migration background (%)	16.0	16.9	0.901
Anthropometric data			
Body mass (kg)	51.3 ± 14.8	51.0 ± 13.7	0.894
Height (cm)	158 ± 10	161 ± 13	0.017
BMI			
kg • m^{-2}	20.2 ± 4.8	19.3 ± 3.3	0.166
Underweight (%)	8.5	10.8	
Normal weight (%)	73.4	77.5	
Overweight (%)	18.1	11.7	0.120
Fat-free mass (kg)	37.6 ± 7.2	41.6 ± 10.4	< 0.001
Fat mass (kg)	13.5 ± 7.9	9.6 ± 5.6	< 0.001
Fat mass (%)	24.9 ± 8.1	18.0 ± 7.3	< 0.001
Lifestyle factors			
Sports (min • d^{-1})	61 ± 95	85 ± 98	< 0.001
PAL [b]	1.4 ± 0.1	1.5 ± 0.2	< 0.001
Stay outside (h • d^{-1})	3.6 ± 2.1	5.0 ± 5.6	0.003
Breastfeeding			
Prevalence (%)	87.4	83.8	0.295
Duration (months)	7.0 ± 4.2	7.8 ± 5.1	0.183
Vitamin D supplementation			
Frequently (%)	64.0	58.9	
Often (%)	8.7	14.6	
Rarely (%)	5.6	7.6	
Never (%)	21.7	19.0	0.325
Smoking [c] (%)	8.9	5.2	0.154
Further characteristics			
RMR			
(MJ • d^{-1})	5.9 ± 0.6	6.4 ± 0.8	< 0.001
(kcal • kg^{-1} • d^{-1})	29.3 ± 5.1	31.2 ± 5.3	< 0.001
Puberty category score [d]	8.4 ± 2.9	7.0 ± 2.5	< 0.001
Prepubescent (%)	3.5	10.2	
Early (%)	10.5	20.9	
Midpubescent (%)	18.6	36.2	
Advanced (%)	57.0	31.1	
Postpubescent (%)	10.5	1.7	< 0.001
History of fractures (%)	22.6	26.6	0.338
Use of medication (%)	6.5	7.9	0.596

BMI = body mass index, RMR = resting metabolic rate, PAL = physical activity level. [a] Mann–Whitney *U* tests were used for differences in participants´ characteristics across sexes, and Fisher's exact test for categorical variables. [b] Data shown for 220 girls and 174 boys. [c] Smoking and occasionally smoking. [d] Data shown for 172 girls and 177 boys.

Table 2 Bone status of the study participants across age groups. Data are presented as mean ± SD

Age groups (years)	Girls (n = 248)				Boys (n = 231)			
	BUA (dB • MHz^{-1})	SOS (m • s^{-1})	SI	P value [a]	BUA (dB • MHz^{-1})	SOS (m • s^{-1})	SI	P value [a]
10 to < 11	96 ± 10	1552 ± 16	79 ± 10	< 0.001	99 ± 10	1551 ± 22	80 ± 11	< 0.001
11 to < 12	103 ± 13	1561 ± 23	86 ± 13		101 ± 10	1558 ± 27	84 ± 9	
12 to < 13	106 ± 17	1562 ± 24	88 ± 17		102 ± 9	1554 ± 23	84 ± 11	
13 to < 14	115 ± 15	1579 ± 29	100 ± 16		108 ± 12*	1563 ± 28*	90 ± 15**	
14 to < 15	117 ± 18	1580 ± 31	101 ± 18		114 ± 15	1578 ± 38	98 ± 19	
15 to < 16	121 ± 15	1579 ± 24	103 ± 16		122 ± 16	1587 ± 38	106 ± 20	
≥ 16	128 ± 15	1585 ± 33	109 ± 17		126 ± 21	1611 ± 33*	115 ± 20	
Total	111 ± 18	1570 ± 28	94 ± 18		110 ± 16	1571 ± 34	94 ± 19	

BUA = broadband ultrasound attenuation, SOS = speed of sound, SI = stiffness index. [a] Level of significance for BUA, SOS, and SI between age groups (Kruskal–Wallis test). *Significantly different from girls of the same age group ($p < 0.05$). ** Significantly different from girls of the same age group ($p < 0.01$).

(five girls, one boy) had SOS values below this range. In both sexes, for BUA, SOS, and SI significant differences between age groups were obtained. There were significant positive relationships between BUA, SOS, SI, and age in both sexes ($r = 0.38$–0.57; $p < 0.001$), classified as moderate–strong associations. In the age group 13 to ≤ 14 years, girls had significantly higher values for BUA, SOS, and SI than boys, whereas boys ≥ 16 years had significantly higher values of SOS than girls.

Classification of SI tertiles

In Table 3, the characteristics of study participants across tertiles of SI are shown. Girls allocated in the highest tertile of SI had significantly higher body mass, BMI, fat-free mass, absolute and relative fat mass, absolute RMR, and a lower relative RMR than girls from the other two tertiles ($p < 0.05$). Furthermore, the classification of BMI was significantly different by SI tertiles (Fisher's exact test). The association between BMI and SI was highly significant and can be classified as "strong" (Cramér's V: 0.78, $p < 0.001$). In boys, the Fisher's exact test revealed that the educational status of the parents significantly differed by SI tertiles. Parents of boys allocated in the lowest and medium tertile of SI had more often a lower educational status than those of boys from the highest tertile of SI. However, the association between parental educational status and SI was non-significant and can be classified as "small" (Cramér's V: 0.14, $p = 0.053$). Also, the duration of sports and PAL were significantly different between tertiles, with lower values in the lowest compared to the highest tertile of SI ($p < 0.05$).

In Table 4, the food intake and the BoneHEI of included study participants across SI tertiles is shown. There were no significant group differences in either food intake or BoneHEI in girls and boys.

Discussion

The study aimed to characterize healthy German children and adolescents across tertiles of calcaneal SI, and to identify risk factors for low bone parameters of participants from the lowest tertile of SI. We assumed that children and adolescents in the lowest tertile are at higher risk for low peak bone mass, as well as for fractures and osteoporosis later in life.

In general, the bone status of participants of this study can be classified as relatively good. Compared to other studies, our research found a higher mean value of SI (girls and boys: SI ~94). For example, the SI of 177 school children (56 girls and 121 boys; age range 11–18 years) from a German college of physical education can be estimated to be ~69 when applying the equation used in the present study for calculation of SI (Mentzel et al. 2005). Furthermore, when the SI of our participants was compared with an age- and sex-matched German reference sample (1623 girls and 1676 boys; age range 6–18 years) (Wünsche et al. 2000), we found ~47% and ~60% higher values of SI for girls and boys of the present study respectively. One explanation for the observed differences of our participants´ bone parameters might be that there are methodological differences between the studies. Specifically, in the two mentioned studies, a different QUS device was used. It has been shown that the type of scanner used strongly influences the validity of QUS measurements (Wang et al. 2014). However, it also might be that participants of the present study have a more advantageous lifestyle for bone health than study participants of the above-mentioned studies. Unfortunately, there is no data available

Table 3 Characteristics of study participants across tertiles of calcaneal stiffness index (SI). Data are presented as Mean ± SD and percentage

Variables	Girls				Boys			
	Lowest (n = 82)	Medium (n = 83)	Highest (n = 83)	*P* value [a]	Lowest (n = 77)	Medium (n = 77)	Highest (n = 77)	*P* value [a]
Bone parameters								
BUA (dB • MHz^{-1})	97 ± 11	110 ± 13	127 ± 14	< 0.001	99 ± 11	108 ± 10	123 ± 16	< 0.001
SOS (m • s^{-1})	1548 ± 18	1569 ± 17	1593 ± 26	< 0.001	1544 ± 17	1568 ± 21	1560 ± 35	< 0.001
SI	78 ± 9	93 ± 11	111 ± 15	< 0.001	79 ± 9	92 ± 10	110 ± 19	< 0.001
Socio-demographic data								
Age (years)	13.3 ± 1.9	13.4 ± 1.9	13.4 ± 1.9	0.962	13.6 ± 1.7	13.6 ± 1.7	13.6 ± 1.8	0.993
Educational background								
Low (%)	63.4	57.3	52.4		67.5	60.0	48.6	
Medium (%)	8.5	15.9	22.0		19.5	13.3	25.7	
High (%)	28.0	26.8	25.6	0.213	13.0	26.7	25.7	0.047
Migration background (%)	19.5	9.9	18.8	0.168	18.4	17.3	14.9	0.857
Anthropometric data								
Body mass (kg)	49.2 ± 16.7	48.8 ± 11.1	54.8 ± 12.2	0.001	50.4 ± 14.3	50.3 ± 13.3	52.3 ± 13.5	0.651
Height (cm)	157 ± 12	158 ± 9	160 ± 8	0.328	160 ± 13	161 ± 14	162 ± 12	0.688
BMI								
kg • m^{-2}	19.6 ± 5.4	19.4 ± 3.6	21.3 ± 3.9	< 0.001	19.3 ± 3.6	19.1 ± 3.0	19.6 ± 3.1	0.526
Underweight (%)	12.2	10.8	2.4		11.7	11.7	9.1	
Normal weight (%)	73.2	77.1	69.9		76.6	77.9	77.9	
Overweight (%)	14.6	12.0	27.7	0.013	11.7	10.4	13.0	0.974
Fat-free mass (kg)	36.3 ± 8.3	36.6 ± 5.9	39.5 ± 6.0	0.003	40.4 ± 10.3	41.1 ± 10.6	43.2 ± 10.3	0.204
Fat mass (kg)	12.9 ± 9.6	12.2 ± 6.3	15.4 ± 7.3	0.001	10.0 ± 6.1	9.5 ± 5.3	9.2 ± 5.3	0.669
Fat mass (%)	23.7 ± 9.1	23.7 ± 7.0	26.8 ± 7.2	0.003	18.8 ± 8.0	18.2 ± 7.4	16.9 ± 6.5	0.317
Lifestyle factors								
Sports (min • d^{-1})	70 ± 148	59 ± 55	56 ± 49	0.260	69 ± 82	92 ± 99	93 ± 110	0.027
PAL [b]	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	0.115	1.5 ± 0.1	1.5 ± 0.2	1.6 ± 0.1	0.010
Staying outside (h • d^{-1})	3.7 ± 2.4	3.4 ± 1.7	3.7 ± 2.2	0.929	5.3 ± 6.1	5.1 ± 6.9	4.4 ± 2.9	0.568
Breastfeeding								
Prevalence (%)	89.0	90.2	83.1	0.384	81.8	86.8	82.9	0.728
Duration (months)	7.2 ± 4.6	6.9 ± 3.8	6.7 ± 4.2	0.770	7.4 ± 5.5	8.7 ± 4.9	7.4 ± 4.7	0.110
Vitamin D supplementation								
Frequently (%)	65.4	62.7	63.8		55.7	67.3	52.4	
Often (%)	5.8	7.8	12.1		11.5	14.5	19.0	
Rarely (%)	7.7	5.9	4.4		8.2	3.6	11.9	
Never (%)	21.2	23.5	20.7	0.899	24.6	14.5	16.7	0.437
Smoking [c] (%)	7.3	8.4	10.8	0.787	2.6	7.8	5.2	0.405
Further characteristics								
RMR								
(MJ • d^{-1})	5.9 ± 0.7	5.9 ± 0.5	6.1 ± 0.5	0.013	6.3 ± 0.8	6.3 ± 0.8	6.4 ± 0.7	0.648
(kcal • kg^{-1} • d^{-1})	30.4 ± 6.0	30.0 ± 4.6	27.6 ± 4.3	0.001	31.5 ± 5.9	31.5 ± 5.1	30.6 ± 4.8	0.690
Puberty category score [d]	8.1 ± 3.1	8.1 ± 2.8	9.0 ± 2.7	0.211	6.6 ± 2.5	7.1 ± 2.4	7.2 ± 2.4	0.281
Prepubescent (%)	3.6	1.6	5.5		7.4	9.8	12.9	
Early (%)	17.9	9.8	3.6		33.3	19.7	11.3	
Midpubescent (%)	16.1	26.2	12.7		35.2	34.4	38.7	
Advanced (%)	51.8	55.7	63.6		20.4	34.4	37.1	
Postpubescent (%)	10.7	6.6	14.5	0.137	3.7	1.6	0	0.092
History of fractures (%)	25.6	21.7	20.5	0.726	24.7	31.6	23.7	0.499
Use of medication (%)	8.5	7.2	3.6	0.428	10.4	6.6	6.6	0.708

BMI = body mass index, = resting metabolic rate, PAL = physical activity level. [a] Kruskal–Wallis *H* tests were used for differences in participants´ characteristics across SI tertiles, and Fisher's exact test for categorical variables. [b] Data shown for 220 girls and 174 boys. [c] Smoking and occasionally smoking. [d] Data shown for 172 girls and 177 boys.

Table 4 Intake of components of the Bone Healthy Eating Index (BoneHEI) of included study participants across tertiles of calcaneal stiffness index (SI). Data are presented as mean ± SD

Food group	Girls				Boys			
	Lowest (*n* = 82)	Medium (*n* = 82)	Highest (*n* = 83)	*P* value [a]	Lowest (*n* = 77)	Medium (*n* = 77)	Highest (*n* = 77)	*P* value [a]
Fruits and vegetables ($g \bullet d^{-1}$)	440 ± 575	450 ± 680	521 ± 724	0.770	410 ± 635	350 ± 401	401 ± 488	0.505
Fish ($g \bullet d^{-1}$)	7 ± 12	7 ± 12	8 ± 17	0.936	13 ± 21	14 ± 23	23 ± 62	0.885
Bread ($g \bullet d^{-1}$)	117 ± 126	109 ± 107	120 ± 168	0.598	187 ± 202	163 ± 186	160 ± 178	0.512
Milk and dairy products ($g \bullet d^{-1}$)	333 ± 367	325 ± 357	351 ± 409	0.983	422 ± 463	283 ± 279	440 ± 472	0.067
Meat and sausages ($g \bullet d^{-1}$)	76 ± 101	68 ± 80	82 ± 124	0.766	147 ± 185	163 ± 200	188 ± 209	0.305
Tolerated food ($g \bullet d^{-1}$)	85 ± 210	57 ± 72	87 ± 206	0.225	125 ± 307	158 ± 339	71 ± 92	0.948
Soft drinks ($ml \bullet d^{-1}$)	371 ± 736	295 ± 556	374 ± 776	0.371	506 ± 819	615 ± 852	570 ± 909	0.725
Caffeinated beverages ($ml \bullet d^{-1}$)	108 ± 254	88 ± 310	124 ± 389	0.678	87 ± 230	76 ± 232	148 ± 478	0.398
Total BoneHEI	60 ± 13	60 ± 13	59 ± 14	0.864	57 ± 15	55 ± 17	56 ± 16	0.709

[a] Kruskal–Wallis tests for differences between SI tertiles.

for either PAL or dietary intake in these studies. However, when we look in detail at our study participants´ characteristics, we do have to state that most of them were healthy, normal weight, breastfed, and vitamin D supplemented during infancy, and stayed outside on a regular basis. Their diet can be regarded as adequate [further details about their diet can be found elsewhere (Heydenreich et al. 2020)]. Therefore, it can be supposed that their lifestyle is favorable for bone health. This statement is supported by the fact that the mean SI of the lowest tertile of SI of our girls and boys were still ~22 and ~ 35% higher than the age- and sex-specific mean of the German reference sample (Wünsche et al. 2000). Nevertheless, we identified six individuals (five girls, one boy) with SOS values “below the expected range for age”.

Unsurprisingly, a positive association between age and bone parameters was obtained in the present study. This finding is in accordance with results published in the literature. For example, in 250 Egyptian children and adolescents (8–18 years), age was also significantly related to bone health (Alghadir et al. 2018). It is estimated that approximately 35% of the bone mineral content is acquired during the first 3 years of life, between 4 years of age and the start of puberty 20%, and during adolescence the remaining 45% (del Rio et al. 1994; Muñoz and Argente 2002). Since bone density and bone metabolism necessarily change during adolescence, it is essential to avoid disturbances in bone metabolism which interfere with peak bone mass achievement.

We found that the factors most associated with bone status in girls were anthropometric and body composition variables. Girls with low SI had a significantly lower body mass, BMI, fat-free mass, and absolute and relative fat mass than girls with high SI. These findings support the literature, where it is clearly stated that body weight is the largest single determinant of the variability in adult bone mass (Heaney et al. 2001). One explanation is that body mass imposes a greater mechanical load on the bone, and bone mass increases to accommodate the more significant load (Zhao et al. 2007). Therefore, it is obvious that higher levels of fat-free mass and fat mass, which are two components of body weight, are associated with higher bone mass. However, the impact of fat-free mass on bone health has also been attributed to the influence of biomechanical usage on bone development (Rauch and Schoenau 2001). In addition, hormones and nutrition influence the mechanical loads on bone; however, they cannot replace the guiding effect of mechanical strain on bone (Rauch and Schoenau 2001). Therefore, children and adolescents should perform regular physical activity to increase the mechanical loading on the bone and increase their amounts of fat-free mass. Also, very low body weight and BMI should be avoided, since this is associated with a higher risk for osteoporosis in later life (Munhoz et al. 2018). The finding of low body mass in relation to poor bone health is especially pertinent in adolescents, who may have distorted body image and therefore strive to achieve low body weight. For example, of 230 female and male German school children (8–12 years), 42% of the boys and 53% of the girls preferred a thin ideal body image, with 18% of the boys and 19% of the girls trying to lose body mass at the time of the investigations (Berger et al. 2005). The authors also found out that almost one third (32%) of the normal weight children expressed the wish to be thinner. However, distorted body image and weight-related factors are important individual-level risk factors for developing eating disorders. For example, in girls from the UK, body dissatisfaction in childhood was associated with later eating disorders (Micali et al. 2015). Eating disorders are associated with multiple neuroendocrine disruptions and low bone mineral density (Muñoz and Argente 2002). Long-term skeletal complications can result when excessive food restrictions, particularly during adolescence, can disrupt peak bone mass attainment (Muñoz and Argente 2002). It was shown that 50% of adolescents suffering from anorexia nervosa had bone mineral densities 1 SD below the mean at one or more skeletal sites (Misra et al. 2004). Therefore, not only with regard to better bone health, it is essential to improve body satisfaction in adolescents to reduce the prevalence of severe dietary restrictions and the risk of development of an eating disorder.

In girls with low SI, we also found that the absolute RMR was lower, and the relative RMR was higher than in those with high SI. However, RMR is mainly determined by anthropometric data. Since we observed a significant difference in body mass between groups, it is not surprising that the group with the highest body mass (highest tertile of SI) also has a higher absolute RMR than the other groups. The finding that the relative RMR was higher in girls with low SI is not contradictory; indeed, it results from of the low body mass in the denominator.

In boys, the duration of sports and PAL were significantly different between SI tertiles, with lower values in boys with low compared to those with high SI. This finding supports the theory that increased bone loading due to higher amounts and intensity of physical activity enhances bone metabolism and leads to a higher bone density (Rubin and Lanyon 1984). It was also shown that in very active German children and adolescents, higher values of bone factors were found compared to a normal-active reference sample (Mentzel et al. 2005). Again, these results support the importance of regular physical activity for optimal bone development during childhood and adolescence.

We also obtained the finding that in boys, parental educational status significantly differed across SI tertiles. One explanation is that higher education typically correlates with higher socioeconomic status and better living conditions. In adolescents, a positive association between higher socioeconomic status and physical activity is reported (Hanson and Chen 2007). This might be because adolescents with low socioeconomic backgrounds spend more time indoors due to unsafe neighborhoods or lack of green spaces, and lack of financial resources to participate in sports clubs or fitness centers (Estabrooks et al. 2003). Also, adolescents with low socioeconomic background usually report inadequate nutritional habits (Hanson and Chen 2007). Reasons might be that nutritious food cannot be afforded and nutrition knowledge is lower (Nabhani-Zeidan et al. 2011). In the present study, we also found that girls and boys with low educational background had a significantly lower BoneHEI than those with high or medium educational background ($p < 0.05$; data not shown). However, the duration of sports and PAL did not differ depending on educational background.

Surprisingly, in the present study, we did not observe significant differences in food intake and BoneHEI between SI tertiles. In contrast, a recent systematic review found that dietary patterns do relate to bone mineral density across different age groups (Denova-Gutiérrez et al. 2018). The authors found that in children and adolescents, the lowest category of a “prudent/healthy” dietary pattern (i.e., poorest diet) was associated with low bone mineral density. Furthermore, Korean adolescents in the highest tertile of “milk and cereal” dietary pattern score had a significantly reduced likelihood of having a low bone mineral density compared with those from the lowest tertile (Shin et al. 2013). One explanation for the missing association between dietary intake and bone status in participants of the present study might be that their dietary intake, in general, can be regarded as adequate. Therefore, dietary intake may not add substantially to other determinants of bone status at this age. It also might be that the selected food items may not capture the variables of the diet that are truly important for the determination of adolescent bone quality.

Limitations and strengths

The following limitations of the present study have to be discussed. Firstly, QUS measurements were applied to assess bone parameters in the study population, which is not the gold standard for assessing bone quality. Nevertheless, QUS measurements can represent an acceptable alternative method compared to the gold standard dual-energy X-ray absorptiometry to assess bone health in adolescents (Torres-Costoso et al. 2018). However, it has been shown that bone mineral density varies between measuring sites (Arlot et al. 1997). In the present study, the bone status was determined at the calcaneal, which is obviously a weight-bearing site, and thus demonstrates probably higher bone mineral densities than at other sites. Therefore, when comparing the results of the present study with those from studies assessing bone mineral densities at other sites, one should take into account the variation of bone parameters across different measuring sites. We classified the participants into low, moderate, and high SI using age- and sex-specific tertiles. Using another method for SI classification might have led to different results.

Secondly, we have to admit that a direct link between low levels of SI (and other bone parameters) during childhood and adolescence, and a higher risk for osteoporosis in later life is not reported in the literature, since there do not exist longitudinal studies studying bone parameters of participants across a wide life-span. However, there is a universal consensus that the amount of peak bone mass during early adulthood is an essential factor with regard to osteoporosis development in later life (Heaney et al. 2001). Therefore, we wanted to characterize healthy children and adolescents across SI tertiles and identify risk factors for low bone parameters in participants allocated in the lowest SI tertile.

Another limitation of the results of our study is that the relative importance of SI compared to other factors influencing peak bone mass cannot be estimated. To our knowledge, in the literature there exists only one longitudinal study aiming to assess bone parameters during childhood, adolescence, and young-adult life to determine peak bone mass (Baxter-Jones et al. 2011). Unfortunately, in this study neither food intake nor physical activity behavior was reported. In addition, bone parameters were assessed by dual-energy X-ray absorptiometry, limiting the transferability of these results to our study. Therefore, at this moment it is impossible to quantify the impact of SI in relation to other factors on peak bone mass.

The assessment of physical activity represents another limitation. Physical activity was estimated based on a structured questionnaire (Krems et al. 2004). However, self-reported physical activity measures demonstrate a greater level of variability than objective measures. Also, we used estimated values to calculate PAL (Müller et al. 2004). It was shown that equation formulas often under- or overestimate real energy costs at rest at an individual level (Heydenreich et al. 2019). We tried to minimize the effect of false estimation of RMR by using an equation developed explicitly for German children and adolescents aged 5–17 years (Müller et al. 2004).

In addition, to evaluate the relationship of food intake and bone health, an adapted version of a standardized food frequency questionnaire was applied, where intake of 13 food items was questioned (Mensink and Burger 2004). However, it might be that other food items also linked to bone health were not considered.

Lastly, we have to address the limitation that further information, apart from education and immigration status, about the parents were not assessed. Therefore, it is impossible to evaluate the relationship between further parent-related risk factors (e.g., prevalence of fractures, body composition status) and bone mass in girls and boys from the present study.

In addition to the limitations described above, the present study has several strengths worth noting. First of all, we assessed and analyzed multiple parameters in many girls and boys ($n = 479$). Also, we questioned numerous lifestyle factors, such as physical activity habits, dietary factors, habitual outdoor physical activity, use of medication, history of fractures, and breastfeeding during infancy. In comparison to other studies, where mostly correlation analysis was performed for the detection of risk factors for low bone status in adolescents (Jones et al. 2013; Farrar et al. 2016; Alghadir et al. 2018; Heydenreich et al. 2020), we differentiated our participants into tertiles of SI. Thereby, we were able to characterize healthy girls and boys with a lower SI (i.e., lowest tertile of SI) in comparison to those from the other tertiles, and to identify risk factors for low bone parameters of participants from the lowest tertile of SI.

Conclusions

The present study results show that girls and boys from the lowest SI tertile had a lower body mass and a lower level of physical activity than those in the other tertiles. Therefore, we recommend girls to strive for a normal body mass and boys to increase the level of physical activity to improve bone strength and reduce the risk for fractures and osteoporosis in later life. Adequate prevention programs should consider these findings.

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