UNIVERSITEIT GENT

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Scientific Article

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Master in de Verpleegkunde en de Vroedkunde

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Influence of birth weight on calcaneal bone stiffness in Belgian pre-adolescent children

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Keywords: birth weight; children; calcaneal quantitative ultrasound; bone stiffness

Running title: Birth weight and bone stiffness in children

Abstract

Purpose: The aim of this study was to investigate the relation between birth weight and calcaneal bone stiffness in a large sample of Belgian healthy pre-adolescent children.

Methods: Participants were 827 children (3.6–11.2 y, 51.6% boys) from the Belgian cohort of the IDEFICS study. Birth weight was obtained using a parental questionnaire and quantitative ultrasound (QUS) measurements were performed to determine the calcaneal Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS) and Stiffness Index (SI) using Lunar Achilles Device.

Results: The average birth weight was 3435.7 ± 512.0 g for boys and 3256.9 ± 471.1 g for girls. The average calcaneal QUS measurements were equal to 89.6 ± 24.0 (23.3 to 153.9) dB/MHz for BUA, 1621.4 ± 49.6 (1516.3 to 1776.5) m/sec for SOS and 92.8 ± 15.6 (49.0 to 163.0) for SI. Birth weight was positively associated with BUA ($r = 0.128$; $p = 0.002$) and with SOS ($r = -0.157$; $p < 0.001$). The associations remained after correcting for age and sex in multiple regression analyses, but disappeared after correcting for anthropometric covariates.

Conclusion: Our findings suggest that birth weight, as a rough proxy indicator for genetic and environmental influences during intrauterine life, is associated with BUA and SOS in pre-adolescent children and may therefore influence the risk of osteoporosis later in life. Further studies using QUS are needed to investigate the consistency of the results of this study.

Introduction

Osteoporosis is one of the most widespread, costly and debilitating diseases in Europe [1,2]. The World Health Organization (WHO) defined osteoporosis as a progressive systemic skeletal disease that is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and in fracture risk [3]. This skeletal disease most often appears acutely in the form of a fracture at high age, but the origin can be found at much younger age, with two major factors determining the increased fracture risk: achieved peak bone mass during the third life decade and the rate of bone loss thereafter [4, 5]. Peak bone mass is almost entirely achieved in the first two decades of life and the amount is determined by heredity, calcium and vitamin D intake through nutrition, hormones, physical activity and other lifestyle factors (e.g. tobacco and alcohol use) [6-12]. To attain the maximal peak bone mass in healthy children and adolescents, and prevent osteoporosis later in life, public health strategies should address on all fronts starting at very early age. Recent studies investigated other early determinants of osteoporosis of which birth weight is one of them. At this stage, several studies have shown associations between birth weight and adult bone mass [13-17]. It is uncertain whether that influence of birth weight is already visible in childhood. Several studies with varying sample sizes (from 64 till 6876) investigated birth weight and bone health in children between the ages of 6 and 10 years old; most of them analyzed different bone sites with dual energy X-ray absorptiometry (DXA) [18-23] and one study used quantitative ultrasound (QUS) in this age group [24]. The study results are hard to compare and are not conclusive. Moreover, no literature is available for children at pre-school age. Generally, no recent studies investigating the influence of birth weight on bone health in young Belgian children are available. To provide more in depth knowledge on the relation between birth weight and bone health in young children, this study investigated a large sample of Belgian, healthy pre-school and primary school children.

To determine the bone strength in these children, QUS was used in this study, an easy to use, radiation free, portable and cost-effective tool [25]. Two associated parameters Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) were determined. These QUS parameters are related to trabecular bone, the most metabolic active bone tissue, with a higher variation rate compared to

cortical bone tissue. QUS can be measured at different sites (e.g. at the phalangeal or the tibia) of which the calcaneus is the most popular site since it consists almost entirely of trabecular bone [25, 26]. So far, the number of studies using QUS to investigate the relationship between birth weight and bone strength in pre- and primary school children are limited [24].

Therefore, the purpose of this research is to further investigate the influence of birth weight as independent variable on bone strength assessed by calcaneal bone stiffness in a large sample of healthy children at prepubertal age.

Materials and methods

Subjects

The subjects are participants of the Belgian cohort of the EU 6th Framework Programme IDEFICS study (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS; www.idefics.eu). The IDEFICS study is a unique longitudinal and multicenter study investigating factors that influence the health, growth and development of European children with emphasis on obesity and its co-morbid conditions. The study was conducted in eight European countries and included two measurement periods: a baseline survey in 2007 – 2008 and a follow-up survey in 2009-2010. In this paper, only the data of the Belgian IDEFICS participants are used and the bone parameters used in this paper were collected in 2009 – 2010. The participating Belgian children are residents from two regions in the Northern Dutch-speaking part of Belgium: the city of Aalter (51°05'N, March-June 2010) and the city of Geraardsbergen (50°46' N, October 2009 – February 2010). At baseline, the children were contacted using random cluster sampling (all children from a selection of classes from all schools in the control and intervention cities) [27-30]. For the purpose of this analysis, data of 827 children aged 3 – 11 years old (427 boys and 400 girls, mean age 7.7 ± 1.5 years) were available (227 children from Geraardsbergen en 600 children from Aalter) in which QUS measurements were performed and data on birth weight was available. Twins or triplets were excluded since multiple birth influences the birth weight. The study was conducted according to the guidelines laid down in the Helsinki Declaration of the World Medical Association. The project protocol was approved by the Ethical Committee of the Ghent University Hospital. Written informed consent was obtained from all parents of the participating children.

Measurements

1. Questionnaire

A self-administered parental questionnaire was used to obtain information on the following variables: sex of the child, birth date, birth length and birth weight. The age of the child at time of examination was calculated using date of birth and date of examination.

2. Quantitative Ultrasound

QUS measurements were performed with a Lunar Achilles Insight (GE Healthcare, Milwaukee, WI). This portable device measures bone stiffness using ultrasound waves. The first outcome parameter BUA reflects the absorption of sound waves and is expressed as decibels per megahertz (n = 596). The second parameter SOS expresses the stiffness of a material by the ratio of the traversed distance to the transit time, in meter per second (n = 600). The more complex the bone structure, the more sound waves will be absorbed. The Stiffness Index (SI) is a third, derived, parameter (n = 827). SI is calculated by a linear combination of BUA and SOS (SI= (0.67 x BUA) + (0.28 x SOS – 420)) [31, 32]. The real time image of the calcaneus and the Region of Interest (ROI) ensures that the measurement is precise. During the entire study period, daily calibration was done and measurements were made according to the standard procedure provided by the manufacturer. The use of an adapter for the children's feet ensured the proper position of the calcaneus. The main heel bones (os calcis) of both feet were measured once and the mean of both measurements was calculated and used in the statistical analyses. The overall QUS measurement required about ten minutes per child.

3. Anthropometric measurements and body composition

Anthropometric measurements were performed by two trained researchers. Height was measured with a standard clinical Seca 224 stadiometer (Seca GmbH & Co. KG., Hamburg, Germany) to the nearest 0.1 cm. Weight was determined with a standard balance (Tanita BC 420 SMA; Tanita, Amsterdam, The Netherlands) to the nearest 0.1 kg, without shoes and in light clothing. The two measurement instruments did not need further calibration or maintenance except the daily verification of the degree of horizontality. The Tanita balance (adapted to the small foot size of children) also measured leg-to-leg impedance (ohm). The Tyrrell formula was used to calculate the fat-free mass (FFM, in kg) based on this impedance value [33]. To take weight into account, %FFM was computed using the formula %FFM = (FFM/weight)*100.

Body mass index (BMI) was computed according to the following formula: BMI = weight (kg) /height^2 (m^2) [34]. Z-scores of each child's weight, height and BMI was determined using the LMS method (with British reference population) which summarizes the distribution of this variables at each age by its median and coefficient of variation, plus a measure of skewness based on the Box-Cox

power required to transform the data to normality [35]. Waist and hip circumferences were measured using a Seca 200 inelastic tape (Seca GmbH & Co. KG., Hamburg, Germany, precision 0.1 cm, range 0-150 cm), which did not need any calibration. The ratio of waist/hip circumference was calculated and used in the analyses. A Holtain skinfold caliper (Holtain Ltd., Crosswell, UK, range 0-40 mm) was used to measure skinfold thickness at the previously marked points. The calipers were calibrated every morning and additionally when dropped by means of a calibration block of 20 mm. Skinfold measurements were obtained at two sites (triceps and subscapular) according to the International standards for anthropometric assessment (ISAK) [36]. Skinfold thickness was measured twice at each site and the mean of both measurements was calculated. If both measurements differed more than 2 mm, a third measurement was performed and the mean was calculated between the two closer values differing less than 2 mm. Additionally, the sum of both skinfold thicknesses were computed and used as indicator of the fat distribution in the upper limbs.

Statistical analysis

After logarithmic transformation of the tricipital skinfold thickness, the subscapular skinfold thickness, the sum of both and the waist/hip ratio, all the residuals showed a satisfactory pattern (normal distribution). Descriptive data by sex were examined with independent samples T-tests (for normally distributed variables) and Mann-Whitney U tests (for non-normally distributed variables). Since an interaction between sex and the bone variables was not observed ($p > 0.05$), boys and girls were analysed together. Pearson correlation coefficients were performed to define potential confounders in the association bone – birth weight. Stepwise multiple regression analysis was used to find the best models predicting the dependent variables BUA, SOS and SI respectively. Birth weight was included as an independent variable and sex and age were included as confounders in all analyses, since sex had an influence on birth weight and age on anthropometric variables. Different variables were included as covariates in multiple models, separate for BUA, SOS and SI. All statistical measurements were obtained using the PASW Statistics Program version 20.0.0 (SPSS Inc, IBM, IL, USA) and statistical results with $p < 0.05$ were considered as statistically significant.

Results

Subject characteristics

Information of the boys and girls early life factors, body composition and current characteristics are summarized in table 1. The mean birth weight, birth length and the mean %FFM were slightly higher in boys compared to girls ($p < 0.001$). Tricipital, subscapular and the sum of both skinfold thicknesses were higher in girls compared to boys ($p < 0.001$). No sex differences in bone parameters were found.

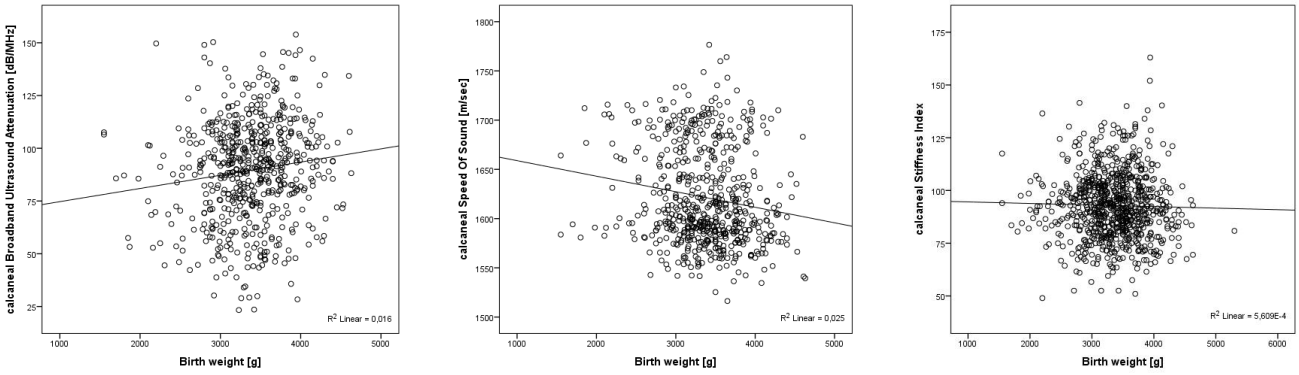
Table 1. Descriptive characteristics of the studied children by sex Mean ± standard deviation (SD)

	Boys (n = 427)	Girls (n = 400)	P-value (sex difference)
Early life factors			
Birth weight (g)	3435.7 ± 512.0	3256.9 ± 471.1	< 0.001
Birth height (cm)	50.6 ± 2.3	49.7 ± 2.6	< 0.001
Current subject characteristics			
Calcaneal BUA (dB/MHz)	91.4 ± 25.3	87.7 ± 22.6	0.066
Calcaneal SOS (m/sec)	1621.2 ± 47.3	1621.6 ± 51.9	0.925
Calcaneal SI	93.7 ± 15.5	91.9 ± 15.6	0.097
Age (years)	7.8 ± 1.5	7.7 ± 1.6	0.244
Height z-score	0.4 ± 1.0	0.3 ± 1.1	0.203
Height (cm)	128.6 ± 10.7	127.3 ± 10.5	0.087
Weight z-score	0.2 ± 1.0	0.1 ± 1.1	0.765
Weight (kg)	26.5 ± 6.0	26.3 ± 6.5	0.555
BMI z-score	-0.1 ± 1.0	-0.1 ± 1.2	0.684
Waist/hip ratio*	0.9 – 0.05	0.85 – 0.06	0.066
Tricipital skinfold thickness (mm)*	8.75 – 3.5	10.9 – 4.9	< 0.001
Subscapular skinfold thickness (mm)*	5.4 – 1.5	6.4 – 2.9	< 0.001
Sum skinfolds (mm)*	14.1 – 4.4	17.2 – 7.7	< 0.001
Fat-free mass (%)	75.9 ± 5.4	69.7 ± 10.1	< 0.001

SI: Stiffness Index; BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound; IOTF: International Obesity Task Force; * Mann-Whitney U test. Median – Interquartile range (IQR)

Correlation coefficients

Correlation analyses between birth weight and bone measurements are presented in Figure 1. Significant correlations were found between birth weight and SOS ($r = -0.157$; $p < 0.001$) as well as between birth weight and BUA ($r = 0.128$; $p = 0.002$). No association has been observed between birth weight and SI. Table 2 shows the correlation coefficients between the calcaneal bone parameters (BUA, SOS and SI), age and anthropometric variables. BUA and SOS were significantly associated with age and all the anthropometric variables (all $p < 0.05$), except for the association between SOS and %FFM ($p = 0.316$). SI was significantly correlated with height, weight, FFM and %FFM (all $p < 0.05$). Generally, the skinfolds and BMI z-score had a weak association with the bone parameters in contrast to the variables height, weight and waist z-score. FFM was strongly associated with BUA, SOS and SI (all $p < 0.001$) but that association weakened when using the %FFM. The anthropometric variables height, weight and fat-free mass were further analyzed in stepwise multiple regression analysis due to high correlation coefficients. The variables birth length, BMI z-score, waist z-score, waist/hip ratio and the skinfolds thicknesses were not retained because of low correlation coefficients.



$r = 0.128$; $p = 0.002$; $n = 596$ $r = -0.157$; $p < 0.001$; $n = 600$ $r = -0.024$; $p = 0.496$; $n = 827$

Figure 1. Correlation (Pearson) of birth weight and calcaneal BUA, SOS and SI

Table 2. Results of Pearson's correlations between bone parameters and anthropometric variables

	BUA (dB/MHz) n = 596	SOS (m/sec) n = 600	SI n = 827
Age (years)	0.489***	-0.451***	0.171***
Birth length (cm)	0.156***	-0.151***	0.003
Height z-score	0.303***	-0.356***	0.016
Height (cm)	0.574***	-0.571***	0.153***
Weight z-score	0.293***	-0.377***	0.005
Weight (kg)	0.527***	-0.574***	0.111***
BMI z-score	0.163***	-0.257***	-0.014
Waist z-score	0.236***	-0.343***	0.005
Waist/hip ratio (log)	-0.171***	0.163***	-0.010
Tricipital skinfold thickness (log)	0.117**	-0.254***	-0.060
Subscapular skinfold thickness (log)	0.146***	-0.257***	-0.030
Sum skinfolds (log)	0.139***	-0.273***	-0.048
Fat-free mass (kg)	0.570***	-0.552***	0.164***
Fat-free mass (%)	0.083*	0.041	0.094**

***≤ 0.001; **< 0.010; *<0.050; BMI: Body Mass Index; (log): log-transformed variables

Stepwise multiple regression

Multiple regression analyses were performed to explore independent variables (including birth weight) influencing BUA, SOS and the calculated parameter SI. Table 3 shows the association between *calcaneal BUA* and birth weight. Model A shows raw data, Model B = Model A + sex and age (confounders), Model C = Model B + weight z-score, Model D = Model C + %FFM and Model E = Model C + height z-score. Height z-score and %FFM could not be together in the model due to multicollinearity, a high correlation between both covariates. Birth weight retained a positive association with BUA after controlling for age and sex (model B) but showed no significant associations after controlling for anthropometric variables (model C, D and E). Sex was not associated with BUA in any model. Age influenced BUA in four models except in model C where weight z-score was added. Finally, weight z-score, %FFM and height z-score were significantly associated with BUA. The adjusted R^2 was low for model A ($R^2 = 0.015$) but higher after controlling in the other models. Table 4 shows the association between *calcaneal SOS* and birth weight. Model A shows raw data, Model B = Model A + sex and age (confounders), Model C = Model B + weight z-score, Model D = Model C + height z-score. Birth weight retained a negative association with SOS, also after controlling

for age and sex (model B) but showed no significant associations after adjusting for weight z-score (model C) and height z-score (model D). Age, weight z-score and height z-score were independently associated with SOS. As was the case for calcaneal BUA, sex had no influence on calcaneal SOS. The unadjusted model A explained less of the variation in SOS than the adjusted models. Table 5 shows the association between *calcaneal SI* and birth weight. Model A shows raw data, Model B = Model A + sex and age (confounders) and Model C = Model B + %FFM. In line with the insignificant univariate correlation between birth weight and SI, birth weight did not predict SI in any of the regression models. Age was the only predictor of SI ($p < 0.001$). The variable %FFM did not show significant associations with SI. The adjusted R^2 was very low in all models.

Table 3. Results of stepwise multiple regression analyses using calcaneal BUA (n = 596) as a dependent variable and various other variables as independent variables

	Model A (R^2§ = 0.015)		Model B (R^2 = 0.252)		Model C (R^2 = 0.338)		Model D (R^2 = 0.351)		Model E (R^2 = 0.346)	
Variables	B*	P-value	B	P-value	B	P-value	B	P-value	B	P-value
Birth weight (g)	0.006	**0.002**	0.006	**0.001**	0.002	0.230	0.002	0.339	0.002	0.994
Age (years)			7.422	< **0.001**	7.616	0.510	7.442	< **0.001**	7.566	< **0.001**
Sex			-1.565	0.367	-2.165	0.185	-0.013	0.994	-1.824	0.262
Weight z-score					7.166	< **0.001**	8.255	< **0.001**	4.534	< **0.001**
FFM (%)							0.374	< **0.001**		
Height z-score									3.537	**0.003**

Model A shows raw data, Model B = Model A + sex and age (confounders), Model C = Model B + weight z-score, Model D = Model C + %FFM and Model E = Model C + height z-score; *B: Unstandardized regression coefficients; § R^2: Adjusted R square; FFM (%): percentage fat-free mass (lean mass); BUA: Broadband Ultrasound Attenuation; P-values < 0.05 are indicated in bold

Table 4. Results of stepwise multiple regression analyses using calcaneal SOS (n = 600) as a dependent variable and various other variables as independent variables

	Model A (R^2§ = 0.023)		Model B (R^2 = 0.224)		Model C (R^2 = 0.359)		Model D (R^2 = 0.366)	
Variables	B*	P-value	B	P-value	B	P-value	B	P-value
Birth weight (g)	-0.016	< **0.001**	-0.016	< **0.001**	-0.006	0.073	-0.005	0.117
Age (years)			-14.217	< **0.001**	-14.699	< **0.001**	-14.607	< **0.001**
Sex			-4.132	0.254	-2.395	0.468	-3.068	0.351
Weight z-score					-18.488	< **0.001**	-13.603	< **0.001**
Height z-score							-6.577	**0.007**

Model A shows raw data, Model B = Model A + sex and age (confounders), Model C = Model B + weight z-score, Model D = Model C + height z-score; *B: Unstandardized regression coefficients; § R^2: Adjusted R square; SOS: Speed of Sound; P-values < 0.05 are indicated in bold

Table 5. Results of stepwise multiple regression analyses using calcaneal SI (n = 827) as a dependent variable and various other variables as independent variables

	Model A (R^2§ = -0.001)		Model B (R^2 = 0.029)		Model C (R^2 = 0.032)	
Variables	B*	P-value	B	P-value	B	P-value
Birth weight (g)	-0.001	0.496	-0.001	0.344	-0.001	0.391
Age (years)			1.694	< **0.001**	1.649	< **0.001**
Sex			-1.770	0.104	-0.935	0.423
FFM (%)					0.123	0.067

Model A shows raw data, Model B = Model A + sex and age (confounders) and Model C = Model B + %FFM; *B: Unstandardized regression coefficients; § R^2: Adjusted R square; FFM (%): percentage fat-free mass (lean mass); SI: Stiffness Index; P-values < 0.05 are indicated in bold

Discussion

This study investigated the relationship between birth weight and bone strength assessed as calcaneal BUA, SOS and SI–values measured by QUS in 827 healthy children, aged 3 to 11 years. The main findings were that birth weight was significantly positively correlated with BUA and significantly negatively correlated with SOS even after correction for age and sex in multiple regression analyses. When adjusting for other covariates such as weight and height, the association with birth weight did not persist. No significant correlation was found between birth weight and SI. Sex had no influence on bone strength and age was positively related with BUA and SI and negatively related with SOS.

Inconsistent results exist on the effect of birth weight on bone strength in pre-adolescent populations. Both Liao et al. [37] and Micklesfield et al. [24] investigated the influence of birth weight on bone properties using QUS measurements at the tibia and calcaneus, respectively. Liao et al. concluded that birth weight had a positive influence on bone strength at the age of three months in 542 Chinese children [37], while no significant correlations were found between birth weight and BUA and SOS in 109 South-African children between the ages of 7 and 9 years [24]. The first study is not comparable with our study sample due to differences in nationality and age of the population, while the second study examined the same bone site as in our study but the mixed ancestral origin, smaller sample size or the use of the nondominant calcaneus could possibly explain the lack of evidence supporting this association [24].

Four studies investigated the influence of birth weight, with correlation analyses, on bone properties using total body DXA measurements [18-21]. Ganpule et al. assessed total body bone mass density (BMD) in 698 Indian children aged 6 years old and concluded that birth weight was positively correlated with increased total body BMD [18]. Macdonald-Wallis et al. assessed different bone properties at spine bone and total body in 6877 nine year old children and found similar positive correlations with birth weight [20]. Micklesfield et al. examined 64 children between 7 and 9 years old with DXA and did not found any positive correlations with birth weight whereby the dissimilarity in sample size and method of measurement could explain the different results [21]. Finally Jones & Dwyer investigated BMD of the lumbar spine and femoral neck in 330 Australian children at the age

of 8 and concluded that birth weight has an influence on femoral neck BMD but not on lumbar spine BMD [19].

Summarized, six studies examined correlations between birth weight and different bone parameters in prepubertal children, without taking potential confounders into account. Half of the studies draw similar conclusions as we did. The other half did not found any relationship between birth weight and bone health which could be explained by differences in study population and design.

Only four studies investigating the relation between birth weight and bone health in pre-adolescent children considered also the role of potential covariates [19, 22, 23, 38]. In these studies, bone properties were measured using DXA: at the lumbar spine and femoral neck by Jones & Dwyer [19], the total body by Steer & Tobias [22], the lumbar spine, femoral neck and total body by Vidulich et al. [23] and total body and lumbar spine by Ay et al. [38]. Ay et al. examined 252 Dutch children at the age of 6 months and found an influence of birth weight on BMD and bone mineral content (BMC) after correcting for sex, gestational age and current age [38]. Unlike our study, Ay et al. did not add other potential covariates such as anthropometric variables in the regression model. Steer & Tobias found similar results in 109 nine year old children when only correcting for sex, age and gestational age. However, after additionally correcting for parental weight and height in a second model and the child's weight and height in a third model, this association decreased but was still significant with exception of BMC and bone area (BA) in the third model [22]. Similar effects were found in 330 Australian eight year old children: a relationship between birth weight and BMD when correcting for sex and growth variables and even when additionally correcting for breastfeeding, maternal smoking during pregnancy, calcium intake, sunlight exposure and sports participation [19]. However, the association disappeared after additionally correcting for maternal BMD. Also, in 476 ten year old South-African children with mixed ancestral origin no association between birth weight and bone health was found after adjusting for bone age, sex, race, socio-economic status, current height and weight [23]. Taken together, our research – having the advantage that radiation-free QUS was used – gave similar results as literature using bone health parameters assessed by DXA: an association between birth weight and bone health parameters after correcting for age and sex, but less or no association after correcting for additional confounders.

Strengths and limitations of the study

The strength of this study is the availability of a large representative population-based sample of both boys and girls from Belgian pre-school and primary school. Compared to other studies investigating birth weight and bone health in childhood, this cohort has a large sample from a previously unexplored age group (from 3 till 11 years). Data on early life factors, QUS measurements and a large battery of anthropometric measurements were complete for all 827 children. Consequently, the analyses could be corrected for potential confounders (age, sex and anthropometric variables). To ensure quality, trained researchers performed the QUS and anthropometric measurements. For the QUS measurement both feet (os calcis) were measured which has the advantage of showing bone metabolic changes first since it consist for 90% of trabecular bone and has a high turnover rate [39]. The average of the parameters measured at the left foot and at the right foot was calculated. In literature, different approaches can be found: some measure the right foot [40], the left foot [41, 42], twice the dominant leg [43] or scan both but choose the left foot when no difference in results [44]. Taking two feet into account could increase the accuracy.

Nevertheless, this study has some drawbacks as well. First important limitation is the absence of an exact gestational age per child as this could be an important potential confounder [18, 20, 37, 38]. A second limitation is the difference between the number of primary school children (n = 685) and pre-school children (n = 142). Therefore, analyses were not divided in age groups but age was added as a confounder in all analyses. Finally, a few remarks on the bone measurements with the QUS method. First, with this method light inaccurate measurements can occur due to difficult positioning and immobilization of the small feet in children. Therefore trained and only a limited number of researchers were used to obtain the QUS data. Second, the original BUA and SOS values measured by the QUS were not available for all participants in BUA (n = 596) and SOS (n = 600) compared to SI (n = 827), due to different registration settings of the measuring device. Finally, the QUS method is a practical device, but is not yet accepted as a standard measurement method in children [45]. Nevertheless, using the radiation-free QUS could increase the participation ratio in a child population, which is necessary in this kind of research.

Conclusion

The present findings support the hypothesis that birth weight, a proxy indicator of genetic intrauterine environmental factors, may have long-term impact on bone health and may be associated with the risk of osteoporosis much later in life. As a result, this study points towards the importance of a normal birth weight even in healthy children (including preschool children). Public health strategies should insist also on the importance of a normal birth weight as a basis for prevention of chronic diseases later in life. Guaranteeing optimal birth weight helps attaining maximal peak bone mass and could even prevent osteoporosis later in life. Further research could investigate whether the findings of this study are consistent in large study samples when using the QUS method as well.

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Influence of birth weight on calcaneal bone stiffness in Belgian pre-adolescent children

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Samenvatting in het Nederlands

Doel: Het doel van deze studie was het onderzoeken van de relatie tussen geboortegewicht en botstijfheid ter hoogte van de hiel in een grote steekproef gezonde Belgische pre-adolescente kinderen.

Methoden: De participanten zijn 827 kinderen (3.6–11.2 j, 51.6% jongens) van de Belgische cohorte van de Europese IDEFICS studie. Het geboortegewicht was verkregen via een vragenlijst ingevuld door de ouders. De ultrasone botmetingen (QUS) werden uitgevoerd met behulp van het Lunar Achilles toestel om de parameters Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS) en de Stiffness Index (SI) te bepalen.

Resultaten: Het gemiddelde geboortegewicht was 3435.7 ± 512.0 g voor jongens en 3256.9 ± 471.1g voor meisjes. Het gemiddelde van de ultrasone hielmeting was gelijk aan 89.6 ± 24.0 (23.3 tot 153.9) dB/MHz voor BUA, 1621.4 ± 49.6 (1516.3 tot 1776.5) m/sec voor SOS en 92.8 ± 15.6 (49.0 tot 163.0) voor SI. Geboortegewicht was significant positief geassocieerd met BUA ($r = 0.128$; $p = 0.002$) en significant negatief met SOS ($r = -0.157$; $p < 0.001$). De associaties bleven na correctie voor leeftijd en geslacht in de regressie analyses, maar verdwenen na correctie voor antropometrische covariaten.

Conclusie: Onze bevindingen suggereren dat geboortegewicht, als een ruwe indicator voor de genetische en omgevingsinvloeden tijdens het intra-uteriene leven, wordt geassocieerd met BUA en SOS bij pre-adolescente kinderen en kan daardoor ook het risico op osteoporose op latere leeftijd beïnvloeden. Verdere studies met behulp van QUS zijn nodig om de consistentie van de resultaten van deze studie te onderzoeken.

UNIVERSITEIT GENT

Faculteit Geneeskunde en Gezondheidswetenschappen

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Additional Report

Masterproef voorgelegd tot het behalen van de graad van

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Door Karen Van den Bussche

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Introduction

Osteoporosis is one of the most widespread, costly and debilitating diseases in Europe [1, 2]. It affects more than 75 million people in Europe, Japan and the USA and causes a large amount of fractures in elderly. An estimated 3 million hip fractures is expected by 2025. The burden of the disease arises from the associated factors, which are influenced by the severity of bone loss and the risk of falling. The total cost of osteoporosis is very high but difficult to calculate because there are different types of costs: e.g. the costs of acute hospital care, loss of working days for family carers, long-term care and medication [3]. The World Health Organization (WHO) defined osteoporosis as a progressive systemic skeletal disease that is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and in fracture risk [4]. Two major factors determining the low bone mass are the gain of bone and the loss of bone. The gain of bone or the 'peak bone mass' is the amount of bone tissue present at the end of skeletal maturation, mainly gained in the first two decades of life. The mass of bone tissue at any time during adult life is the difference between the amount accumulated at maturity and that lost with ageing [3]. Therefore there is a significant interest in exploring ways to increase peak bone mass. The amount of peak bone mass is determined by heredity, calcium and vitamin D intake through nutrition, hormones, physical activity, other lifestyle factors (e.g. tobacco and alcohol use) or impaired by various disorders (e.g. delayed puberty and anorexia nervosa) [3, 5-9]. The second major determinant of low bone mass is the loss of bone. The beginning of substantial bone loss is different for men (around 65 years) compared to women (around 50 years). Endocrine factors (mainly estrogen) are necessary for maximizing peak bone mass, but also for maintaining it. Besides that, physical activity and nutritional factors such as deficiencies in calcium, vitamin D and protein showed an association with accelerated bone loss or deficient skeletal growth [3, 10].

Osteoporosis is a silent epidemic whose foundation lies in the early years of childhood, but its manifestation lies later in life. Prevention should begin early and the question occurs if prevention should start before new life [11]. Recent studies investigated early determinants of osteoporosis of

which birth weight is one of them. Birth weight is determined by the duration of the pregnancy, 'the gestational age', and the prenatal growth rate. These determinants are influenced by genetic factors (e.g. ethnicity), the health of the mother (e.g. alcohol or tobacco use), environmental factors, socioeconomic status, fetal abnormalities (e.g. congenital diseases) and finally multiple births [12].

At this stage, several studies have shown associations between birth weight and adult bone mass [13-17]. It is uncertain whether that influence of birth weight is already visible in childhood. Seven studies with varying sample sizes (from 64 till 6876) investigated the influence of birth weight on bone health in children of the same age as in this study, between six and ten years old. Most of these studies analyzed different bone sites with dual energy X-ray absorptiometry (DXA) [18-23] and one study used quantitative ultrasound (QUS) within this age group [24]. The last-mentioned study explored bone health in 109 South-African children and used correlation analysis, without taking covariates into account. Summarized, literature on this subject in this pre-adolescent age group is hard to compare and inconclusive. Moreover, no literature is available for children at pre-school age. Besides the studies investigating bone health in primary school children, other studies investigating bone health in babies and adolescents have been carried out. Two studies explored the influence of birth weight on bone health in three and six months old babies with one study using DXA in 252 Dutch children [25] and the other study used QUS in 542 Chinese children [26]. The latter found a correlation between birth weight and bone health in children born appropriate for gestational age. Eight studies investigated the influence of birth weight on adolescents and young adults with varying sample sizes (from 40 till 496) [27-33]. The studies were conducted around the world and all used the DXA measurement technique.

Summarized, at this moment no conclusive research consists about the relationship between birth weight and bone health assessed by QUS in healthy children. Generally, no recent studies investigating the influence of birth weight on bone health in young Belgian children are available. To provide more in depth knowledge on the relation between birth weight and bone health in young children, this study investigated a large sample of healthy Belgian pre-school and primary school children.

The main purpose of this research is to further investigate the influence of birth weight as an independent variable on bone strength assessed by calcaneal bone stiffness in a large sample of healthy children at prepubertal age.

To achieve this goal, a quantitative approach based on the data of the follow-up survey of the Belgian cohort of the EU 6th Framework Programme IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS; www.idefics.eu) was used. This unique, longitudinal study investigates factors influencing health, growth and development of European children with emphasis on obesity and its co-morbid conditions. To determine the bone strength in these children, QUS was used in this study, an easy to use, radiation free, portable and cost-effective tool [34]. Two associated parameters were determined. First parameter is the Speed of Sound (SOS) that reflects the stiffness of a material by the ratio of the traversed distance to the transit time and is expressed as meter per second (m/sec). Second parameter is the Broadband Ultrasound Attenuation (BUA) that reflects the absorption of sound waves and is expressed as decibels per megahertz (dB/MHz). The more complex the bone structure, the more sound waves will be absorbed. The Stiffness Index (SI) is a third, derived, parameter and is calculated by a linear combination of BUA and SOS. These three QUS parameters are related to trabecular bone, the most metabolic active bone tissue, with a higher variation rate compared to cortical bone tissue. QUS can be measured at different sites (e.g. at the phalangeal or the tibia) of which the calcaneus is the most popular site since it consists almost entirely of trabecular bone [34, 35]. As previously cited, the number of studies using QUS to investigate the relationship between birth weight and bone strength in pre- and primary school children are limited [24].

For this study a thorough research of the literature was performed to determine the lack of evidence on the relationship between birth weight and bone health in children. Next, the research question was analyzed using quantitative statistical methods with the analysis of potential covariates and subsequently testing those results in various multiple regression models. The data used in this study

was not collected by the author because the data was already gathered in 2009 – 2010. Since it is very important to gain experience in all areas of scientific research through the master's thesis, the author participated in data collection of the same children two years later. In the following survey of the Belgian IDEFICS cohort, the author took several measurements in the pre-school and primary school children: blood collection, blood handling, measurements of the heel bones with QUS and the whole body scans with DXA.

This master's thesis consists of two large parts: a scientific article and an additional report. The latter is presented here and contains four parts. First a thorough review of the literature about the influence of birth weight on bone health in children and adolescents is presented. In addition an introduction on bone measurement techniques is given with the comparison of the three main bone measurement techniques. Second, additional analyses and results are given including the analysis of covariates and multiple regression analysis. Pre-final multiple regression models are presented in this additional report while the final models are presented in the scientific article. Additional points of discussion and the conclusion of this additional report are respectively the third and fourth part of this master's thesis.

Review of the literature

Methodology

The search for relevant literature was based on the following research question: 'What is the influence of birth weight on bone mass in children and adolescents?' The databases PubMed, ISI Web of Knowledge, the Cochrane Library and Google Scholar were searched. According to pre-established keywords and after reading title and abstract, 140 articles were found in the Cochrane Library (n = 11), Google scholar (n = 33), PubMed (n = 63), Web of Science (n = 24) and by hand searches (n = 9). However, the articles needed to fulfill the following criteria. The subjects had to be defined as healthy children who were born at term and who had no (congenital) diseases or conditions that may affect bone metabolism. The ancestral origin of the subjects did not matter. All articles written in English were eligible. The studies must have been published in the last ten years, because of the importance to use the most recent measurement techniques. After analyzing for the following inclusion criteria, 17 articles remained. After evaluating the quality [36, 37], each of these 17 articles was included.

Introduction on bone measurement techniques

In order to make the literature review comprehensive for those who are not familiar with bone measurement techniques, a small introduction is included here before presenting the results of the literature review. Most of the adult bone mass is gained in childhood and experts believe that optimizing bone mass early in life has a great importance. First there would be an influence on fractures in childhood and second it would have a deal in the delay of developing osteoporosis later in life. To measure bone health, three different techniques are commonly used.

The first and most used technique is **the dual energy X-ray absorptiometry** (DXA). Many bone sites can be measured such as the total body, the spine, several hip regions (total hip, femoral neck) and the forearm. The measurements result in four parameters: bone area (BA), bone mineral content (BMC), areal bone mineral density (aBMD) and derived bone mineral apparent density (BMAD). DXA has three main advantages: the low cost, minimal radiation exposure and the fact that this technique is relatively fast. A few disadvantages are present as well: DXA cannot separate cortical and trabecular

bone, no measures of bone geometry can be given and the bone size will influence aBMD. The reason is the two-dimensional image of a DXA measurement compared with a pQCT measurement that adds a third dimension. A second technique is the **peripheral quantitative computed tomography** (pQCT) which can measure the tibia and the radius as bone sites. It differs from DXA in its parameters whereby bone geometry and bone strength (CSMI, BSI, pSSI) can be measured, as well as BMC and volumetric density (vBMD). pQCT measures true vBMD and can differentiate bone tissue (cortical vs. trabecular) because it measures the bone in three dimensions. There is also a minimal radiation exposure. The disadvantages are the underestimation of cortical vBMD when cortical shell thickness is small (<2 mm). Another disadvantage is the fact that repeated measurements in longitudinal studies are difficult due to variations in longitudinal bone growth rates. A third technique is the **quantitative ultrasound** (QUS). Four possible bone sites can be measured: the calcaneus, the tibia, the radius and the phalanx (hand). QUS results in two possible parameters: Speed of Sound (m/sec) (SOS) and Broadband Ultrasound Attenuation (dB/MHz) (BUA). Three very important advantages are the low cost, the portability of the scanning device and the non-existing radiation exposure. One disadvantage is the influence of bone size (cortical thickness) on SOS [38, 39].

Results of the literature review

The first part of this literature review handles about the influence of birth weight on bone mass in children of all ages. Because childhood is a quite long period, we distinguish the following sub-periods: infancy (from birth till ± 12 year), adolescence (from ±13 till ±18 year) and young adulthood (from ±18 till ±22 year) [40]. Within this literature review, the results were divided into two parts: prepuberty and postpuberty as puberty is marked by specific hormonal changes possibly influencing bone growth and development. The prepuberty contains the infancy: only two articles were found from birth till one year, the other studies consider subjects between the range of six to ten years old. The postpuberty contains the adolescence and young adulthood. The last life stage is included here because of the wide age range of the subjects in some articles that would otherwise be excluded (e.g. from 16 till 20 year [41]). In the second part, a short overview is given of other factors influencing bone health in children.

Relation between birth weight and bone health

1. Bone mass at prepubertal age

1.1. From birth till one year

Two studies investigated the influence of birth weight on bone health at a very young age. The researchers of the generation R study, **Ay et al.**, found associations between birth weight and bone parameters after adjusting for gender, age and gestational age at birth in 6 months old children [25]. Positive associations between birth weight and total body bone mineral density (BMD) ($\beta = 0.002$ (95% CI = 0.001, 0.003), $p < 0.01$) and total body BMC ($\beta = 0.02$ (95% CI = 0.01, 0.02), $p < 0.01$) were found [25]. **Liao et al.** studied 542 infants divided into three groups according to birth weight (< 1,500g, n = 11; 1,500 – 2,500g, n = 60; > 2,500 g, n = 86). SOS of the tibia of the infants with a birth weight of < 1,500 g was significantly lower than SOS in infants with birth weight of > 2,500 g ($p < 0.042$) [26]. However, no significant differences were found among the three groups after accounting for the influences of gestational age and birth season. Moreover, Liao et al. found a positive correlation between birth weight and SOS measured from the tibia ($r = 0.232$; $p < 0.015$) in children appropriate for gestational age (AGA). This significant correlation was not found in children small or large for gestational age (SGA or LGA) [26].

Table 1: Two studies on the relation between birth weight and bone health in children between birth and one year of age, sorted alphabetically

Author	Sample	Method	Results
Ay et al. (2010)	n = 252 6 months old The Netherlands	DXA: BMD – BMC – BMAD - Total body - Lumbar spine	Regression (adj. for gender, gestational age and age) + total body BMD + total body BMC ○ lumbar spine
Liao et al. (2005)	n = 542 3 months old China	QUS: SOS (m/sec) - Tibia	Correlation + SOS tibia for AGA ○ SOS tibia for SGA – LGA

+ : the results show a significant and positive relationship; - : the results show a significant and inverse relationship; ○ : no relationship was found

1.2. From 6 till 10 years

Seven studies that examined children between the ages of 6 and 10 years were found.

Steer & Tobias found significant positive associations between birth weight and bone mass obtained in total body scans of English children after adjusting for gestational age, gender and current age of the child: BMD (β = 0,292 (95% CI = 0.241, 0.343), p < 0.001, r = 0.134), bone area (BA) (β = 0.518 (95% CI = 0.468, 0.567), p < 0.001, r = 0.240) and bone mineral content (BMC) (β = 0.459 (95% CI = 0.409, 0.509), p < 0.001, r = 0.213), with r being the partial correlation [22]. An inverse association between birth weight and area adjusted BMC (aBMC) was seen (β = - 0,216 (95% CI = - 0.268, - 0.136), p < 0.001, r = 0.097). A second model takes shared genetic factors into account by adjusting for parental height and weight. Similar, but attenuated, positive associations were found in BMD (β = 0,137 (95% CI = 0.074, 0.200), p < 0.001, r = 0.064), BA (β = 0.0.282 (95% CI = 0.225, 0.340), p < 0.001, r = 0.140) and BMC (β = 0.242 (95% CI = 0.183, 0.300), p < 0.001, r = 0.120). The inverse relationship between birth weight and aBMC persisted (β = - 0,159 (95% CI = - 0.225, - 0.092), p < 0.001, r = 0.070). But in a third model that adjusted for height and weight in nine year old children, an inverse association was observed in aBMC and in BMD. The researchers suggest that birth weight had a negative influence on bone mass after bone and body size were considered [22]. **Macdonald-Wallis et al.** found significant positive correlations between birth weight and bone measurements for total body (BMC, BA and BMD) and birth weight and bone measurements at the spine (BMC, BA and BMD) without adjusting for confounders: total body BMC (r = 0.197, p < 0.0001), total body BA (r = 0.216; p < 0.001), total body BMD (r = 0.139; p < 0.001), spine BMC (r = 0.158; p < 0.001), spine BMD (r = 0.095; p < 0.001) and spine BA (r = 0.185; p < 0.001). An inverse significant relationship was found in total body aBMC (r = - 0.065; p < 0.001) whereby no significant relationship was found in spine aBMC (r = -0.017; p NS) [20]. **Jones & Dwyer** examined the correlation between growth variables at birth and bone density in prepubertal Australian children. Significant positive correlations between birth weight and BMD femoral neck (r = 0.26; p < 0.0001) and bone mineral apparent density (BMAD) femoral neck (r = 0.11; p = 0.04) were found. But no correlations were found with BMD lumbar spine (r = 0.09; p = 0.22) and BMAD lumbar spine (r = -0.07; p = 0.22). In addition, the researchers conducted multivariate regression analyses. Firstly, BMD and BMAD were adjusted for

gender and growth variables with only BMD showing significant associations (femoral neck $\beta = 0.11$; $p = 0.041$ and lumbar spine $\beta = 0.12$; $p = 0.041$). Femoral neck and lumbar spine BMAD showed no significant associations. Second, the researchers added environmental factors (breastfeeding, maternal smoking, sports participation, sunlight exposure and calcium intake) as confounders in a subsequent model. The positive associations that were found in the first model remained significant at the femoral neck BMD ($\beta = 0.12$; $p = 0.033$) and the lumbar spine BMD ($\beta = 0.13$; $p = 0.029$). Thirdly, the study added maternal bone density as an additional confounder, but no significant associations were found [19].

The research of **Ganpule et al.** did not take place in a Western country like the three studies above, but examined subjects from a developing country, India. The focus of this study was the influence of the maternal nutritional status and diet during pregnancy on bone mass in offspring, but analysis using body size and bone mass measurements (DXA), without adjusting for covariates, took place as well. They found a positive association between birth weight and all bone outcomes ($p < 0.001$) [18].

The three other studies took place in South-Africa using subjects of mixed ancestral origin [21, 23, 24, 24]. **Vidulich et al.** measured BA and BMC of the total body, femoral neck and lumbar spine in black and white South African children. After categorizing birth weight into tertiles, an ANCOVA analysis was used to determine differences in means of bone parameters. Significant differences were only seen in boys, not in girls. In black boys, total body BA ($p < 0.001$) and BMC ($p < 0.001$), femoral neck BA ($p < 0.05$) and BMC ($p < 0.001$), lumbar spine BA ($p < 0.001$) and BMC ($p < 0.001$) were significantly different between the birth weight tertiles, while in white boys, total body BA ($p < 0.001$) and BMC ($p < 0.001$), femoral neck BA ($p < 0.01$) and BMC ($p < 0.001$), lumbar spine BA ($p < 0.05$) and BMC ($p < 0.01$) differed. For black and white girls no significant differences in the bone parameters were found. Multiple regression analysis was performed to calculate the predictive power of birth weight. After adjusting BA and BMC for many variables on which BA and BMC in children are dependent (e.g. race, gender and age), birth weight was not a significant predictor of total body BA and BMC, femoral neck BA and BMC and lumbar spine BA and BMC. However, when BMC was additionally corrected for BA, birth weight became predictive for femoral neck BMC ($\beta = 0.07 \pm 0.03$;

$p < 0.05$). The researchers concluded that low birth weight and small size at 1 year resulted in smaller bones and/or bones of lower BMC at the femoral neck [23]. Both studies by **Micklesfield et al.** investigated subjects of mixed ancestral origin from a working class community in South-Africa. Using QUS in the first study, neither BUA nor SOS were significantly correlated with any of the early life parameters, including birth weight, gestational age or whether the child was breastfed or not [24]. Using DXA in the smallest birth cohort (n = 64), Micklesfield et al. did not found significant correlations between birth weight and total body BMC with DXA (r = 0.25; p NS) [21].

Table 2: Seven studies on the relation between birth weight and bone health in children between 6 and 10 years old, sorted alphabetically

Author	Sample	Method	Results
Ganpule et al. (2006)	n = 698 6 yrs. India	DXA: BMD – BMC - Total body - Total spine	Correlation + for all parameters
Jones & Dwyer (2000)	n = 330 8 yrs. Australia	DXA: BMC – BMD – BMAD - Lumbar spine - Femoral neck	Correlation + BMD – BMAD f.n. ○ BMD – BMAD l.s. Regression (adj. for growth/ gender/environmental factors) + BMD f.n. & l.s. ○ BMD f.n. & l.s. after add. adj. for maternal bone density ○ BMAD f.n. & l.s.
Macdonald-Wallis et al. (2010)	n = 6876 9 yrs. UK	DXA: BMC – BMD – BA – aBMC - Total body minus head - Spine bone	Correlation + t.b. BMC – BMD – BA + spine BMC – BMD – BA - t.b. aBMC ○ spine aBMC
Micklesfield et al. (2006)	n = 109 7 – 9 yrs. South-Africa	QUS: BUA (dB/MHz) – SOS (m/sec) - Both calcanei	Correlation ○ BUA – SOS
Micklesfield et al. (2007)	n = 64 9 yrs. South-Africa	DXA: BMC - Total body	Correlation ○ BMC
Steer & Tobias (2011)	n = 6876 9 yrs. UK	DXA: BMD – BMC – BA – aBMC - Total body	Regression (adj. gestation, gender & age) + BMD – BMC – BA - aBMC +. adj. weight & height parents + BMD – BMC – BA - aBMC + adj. height and weight child ○ BMC – BA - BMD – aBMC

Vidulich et al. (2007)	n = 476 10 yrs. South-Africa	DXA: BA – BMC - Total body - Femoral neck - Lumbar spine	Ancova (age) + black and white boys for all ○ black and white girls for all Regression analysis (Adj. for bone age, gender, race, SES, height & weight) ○ BA & BMC f.n. – l.s. – t.b. ○ BMC t.b. – l.s. (+ adj. for BA) + BMC f.n. (+ adj. for BA)

+ : the results show a significant and positive relationship; - : the results show a significant and inverse relationship; ○ : no relationship was found. T.b. total body; f.n. femoral neck; l.s. lumbar spine; adj. adjusted for; UK: United Kingdom

2. Bone mass at postpubertal age

Eight studies investigated the relation between birth weight and bone health in subjects between 13 and 23 years old.

The study of **de Bono et al.** was performed in Gambia and had one main interest: the influence of birth weight on bone mass in adolescents. The division in bone mass parameters that is used in the study of de Bono et al. was applied in the following chapters to report also the results of the seven other studies. de Bono et al. divided the investigated bone parameters into four different segments (2.1) pQCT bone area, (2.2) DXA bone area, (2.3) DXA and pQCT-derived BMC and (2.4) pQCT and DXA-derived bone density. Importantly, de Bono et al. performed measurements on both cortical (compact bone tissue, situated in the outer layer) and trabecular sites (porous bone tissue, situated in the interior of the bone), a seldom researched combination [27].

2.1. The results from pQCT bone area

Using pQCT measurements for BA at cortical sites, **de Bono et al.** found that birth weight was a significant predictor of total cross-sectional area (CSA) at the tibial ($\beta = 0.33$; $p < 0.05$) and radial shaft ($\beta = 0.47$; $p < 0.01$) in males in unadjusted models. The association remained significant after adjustment for current height, weight and age ($\beta = 0.35$; $p < 0.01$ and $\beta = 0.29$; $p < 0.05$). Birth weight was a significant positive predictor of medullary CSA but not of cortical CSA at both the tibial shaft ($\beta = 0.90$; $p < 0.01$) and the radial shaft ($\beta = 0.87$; $p < 0.001$) in males. These relationships were independent of age at the radius and independent of weight at the tibia ($\beta = 0.93$; $p < 0.01$ and $\beta = 0.70$; $p < 0.01$ respectively). In females, birth weight was not a significant independent predictor of

total, cortical or medullary CSA at any site. Using pQCT measurements for BA at trabecular sites, birth weight was a significant positive predictor of total CSA at the distal tibia ($\beta = 0.49$; $p < 0.01$ and β adj. $= 0.31$; $p < 0.05$) and radius ($\beta = 0.61$; $p < 0.001$ and β adj. $= 0.46$; $p < 0.01$) in females with and without corrections for confounders. In males significance was only found in unadjusted models (distal tibia $\beta = 0.23$; $p < 0.05$) [27]. The Finnish study of **Wang et al.** is the second study that used the pQCT technique. Correlation coefficients between bone traits and weight at birth were explored. Influence of birth weight on bone strength index (BSI) was significant for the tibia ($r = 0.23$; $p \leq 0.05$) but not significant for the radius ($r = 0.06$; p NS). Bone total cross-sectional area was significant for both the tibia ($r = 0.21$; $p \leq 0.05$) and the radius ($r = 0.18$; $p \leq 0.05$) in unadjusted analyses [41]. **Jensen et al.** investigated in a third, three-step, analysis the relationship between birth weight and BA with adjusting for height but no significant association was observed ($\beta = 0.03$; 95% CI (-0.17, 0.23); $p = 0.76$) [31].

2.2. The results from DXA measurements of bone area

de Bono et al. investigated bone area using DXA in Gambian adolescents. Birth weight was a significant positive predictor of bone area at the radial shaft in both males ($\beta = 0.27$; $p < 0.01$) and females ($\beta = 0.21$; $p < 0.05$), in unadjusted and adjusted models. The significant positive results from the pQCT measurements agree with DXA findings at the ultra-distal radius (males $\beta = 0.27$; $p < 0.01$ and females $\beta = 0.31$; $p < 0.001$) in unadjusted models. After adjusting only ultradistal radius BA in females remained significant ($\beta = 0.22$; $p < 0.001$). At other DXA sites significant positive associations were found in the spine BA in males ($\beta = 0.25$; $p < 0.05$), femoral neck in both males ($\beta = 0.26$; $p < 0.01$) and females ($\beta = 0.34$; $p < 0.001$), total body BA in both males ($\beta = 0.23$; $p < 0.01$) and females ($\beta = 0.20$; $p < 0.05$) and total hip BA in both males ($\beta = 0.16$; $p < 0.05$) and females ($\beta = 0.33$; $p < 0.001$) in the unadjusted models. After adjusting, only radial shaft BA in males (β adj. $= 0.18$; $p < 0.05$) and females (β adj. $= 0.21$; $p < 0.05$), femoral neck BA in males (β adj. $= 0.20$; $p < 0.05$) and females (β adj. $= 0.34$; $p < 0.001$) and total hip BA in females (β adj. $= 0.22$; $p < 0.05$) remained significant [27].

Among males, **Schlüssel et al.** found significant positive associations between birth weight tertiles and bone area at the spine ($p < 0.001$), femoral neck ($p < 0.001$) and proximal femur ($p < 0.001$) in linear regression. Among females, BA tended to be greater at the spine ($p = 0.024$), femoral neck ($p = 0.017$) and proximal femur ($p = 0.028$) for those in the highest birth weight tertile. The association between birth weight and BA was explored in multiple linear regression models with adjusting for gender, gestational age, skin color and lifestyle variables. Bone area at the spine was not associated but the femoral neck ($\beta = 0.06$; SE = 0.03; $p = 0.046$) and the proximal femur ($\beta = 0.81$; SE = 0.25; $p = 0.001$) were. Wang et al. found significant correlations for the total body ($r = 0.19$; $p \leq 0.05$), lumbar spine ($r = 0.16$; $p \leq 0.05$) and the femoral neck ($r = 0.18$; $p \leq 0.05$) without adjusting for confounders [33]. The Danish study of **Mølgaard et al.** did not found significant correlation coefficients after adjusting for gender between birth weight and total body BA ($r = 0.14$ $p = 0.16$), while the lumbar spine BA was significant ($r = 0.35$; $p < 0.001$) [32]. **El Hage et al.** found a significant positive correlation between birth weight and bone mineral area in adolescent girls ($r = 0.46$; $p < 0.01$) [42].

2.3. The results of DXA and pQCT-derived BMC

Birth weight did not predict pQCT-derived BMC at any site in males (cortical and trabecular BMC) but it did predict the cortical BMC of the radial shaft in females in both models ($\beta = 0.40$; $p < 0.05$ and β adj. = 0.33; $p < 0.05$) in the research of **de Bono et al.** [27]. These findings were consistent with DXA-derived BMC at corresponding sites in females ($\beta = 0.30$; $p < 0.05$ and β adj. = 0.28; $p < 0.05$). The trabecular BMC at the distal radius was significant in females ($\beta = 0.53$; $p < 0.05$) but did not remain significant after adjusting. At other DXA sites, no significant relationships with birth weight were found in males (e.g. total body $\beta = 0.15$; p NS) but the femoral neck BMC in females was significant in both models ($\beta = 0.47$; $p < 0.05$ and β adj. = 0.40; $p < 0.05$) [27]. Unlike de Bono et al., **El Hage et al.** found at the on hand significant associations between birth weight and total body BMC ($\beta = 162.6$; SE = 45.3; $p < 0.01$) after adjusting for weight and maturation index and at the other a significant correlation between BMC and birth weight ($r = 0.58$; $p < 0.001$) [42]. Among males, **Schlüssel et al.** found significant differences between birth weight in tertiles and BMC at all three studied bone sites among males: spinal ($p < 0.001$), femoral neck ($p = 0.008$) and proximal femur ($p =$

0.003). Among females, a significant difference was found between birth weight in tertiles and BMC at the spine (p = 0.044). In multiple linear regression models, the association between birth weight and BMC was explored with adjusting for gender, gestational age, skin color and lifestyle variables. BMC at the spine was not associated with birth weight, but significant associations were found in the femoral neck BMC (β = 0.15; SE = 0.06; p = 0.014) and proximal femur BMC (β = 1.39; SE = 0.49; p = 0.005) [33]. The Danish study of **Jensen et al.** examined the association between birth weight and BMC as well. Significant associations were found for total body BMC (β = 0.24, 95% CI (0.04-0.45); p = 0.02) and lumbar spine BMC (β = 0.30; 95% CI (0.13, 0.48); p = 0.001). After correction for adolescent height and weight, no associations remained significant. Adjusting for other confounders such as BMI, smoking habits, physical activity and calcium intake did not alter the results. In a second, size-adjusted, model birth weight was not significantly associated with birth weight (β = - 0.002; 95% CI (-0.08, 0.08); p = 0.97). In a third, three-step, analysis BMC was adjusted for BA and showed a significant negative association with birth weight (β = - 0.27; 95% CI (-0.45, -0.09); p = 0.004) [31]. **Wang et al.** examined correlation coefficients between bone traits and BMC without adjusting for confounders. Significant correlations were found in BMC of the total body (r = 0.17; $p \leq 0.05$), lumbar spine (r = 0.13; $p \leq 0.05$) and of the femoral neck (r = 0.10; $p \leq 0.05$) [41]. **Mølgaard et al.** found no significant correlation between birth weight and total body BMC (r = 0.073 p = 0.47) and lumbar spine BMC (r = 0.20; p = 0.048) after adjusting for gender [32]. The Japanese study of **Saito et al.** used both Pearson's correlation and stepwise multiple regression analysis to investigate birth weight and BMC. Significant correlation coefficients were found for lumbar spine BMC (r = 0.30; $p < 0.01$), femoral neck (r = 0.25; $p < 0.05$) and total hip (r = 0.32; $p < 0.01$). With the multiple regression analysis the researchers tried to find the best model predicting BMC. Both BMC lumbar spine (β = 3.48, SE = 1.72, $R^2 = 0.042$, p = 0.0474) and the total hip (β = 2.25, SE = 1.05, R^2=0.039, p = 0.0352) were significantly associated with birth weight [28].

2.4. The results of bone density (BMD, vBMD, aBMD and BA adjusted BMC)

de Bono et al. found that birth weight was a significant negative predictor of pQCT-derived vBMD of one cortical site (tibial shaft β = - 0.01; $p<0.05$) and both trabecular sites in adjusted models (distal

tibia β = - 0.27; $p < 0.05$ and distal radius β = -0.34; $p < 0.05$) in males. In contrast, birth weight was not a significant predictor of vBMD at any site in females and of aBMD or BA adjusted BMC at the majority of sites in males and females in both adjusted and unadjusted models. Only in the β adjusted aBMD of the radius in males (β = -0.33; $p < 0.05$) was found significant [27]. In multiple linear regression analysis, **El Hage et al.** found a significant association between birth weight and total body BMD (β = 0.042; SE = 0.001; $p < 0.01$) after controlling for weight [42]. Among males, **Schlüssel et al.** found a significant association between birth weight in BMD at the spine site ($p = 0.015$) but not at the femoral neck ($p = 0.160$) or the proximal femur ($p = 0.205$). Among females, no significant associations with BMD were found at the spine ($p = 0.332$), the femoral neck ($p = 0.799$) or the proximal femur ($p = 0.999$). In multiple linear regression models, the association between birth weight and BMD was explored after adjusting for gender, gestational age, skin color and lifestyle variables whereby no significant associations were found between birth weight and the three bone sites [33]. One of the two Danish studies, **Jensen et al.**, examined the association between birth weight and BMC. Significant associations were found for lumbar spine BMD (β = 0.16, 95% CI (0.004, 0.31); $p = 0.04$), but not for total body BMD ($p = 0.41$). After correction for adolescent height and weight, no association remained significant [31]. The second Danish study, **Mølgaard et al.**, did not found significant associations between birth weight and total body BMD ($r = 0.000$; $p = 0.998$) and lumbar spine BMD ($r = -0.036$; $p = 0.72$) after adjusting for gender [32]. **Leunissen et al.** used multiple regression analysis and did not found significant influences of birth weight on the four models on total body BMD: model A was adjusted for age, gender, birth length, height and birth weight (β = –0.38, $p = 0.471$), model B added weight (β = –0.24, $p = 0.607$), model C added fat mass and lean body mass (β = –0.66, $p = 0.145$) and model D adjusted for delta weight and height during childhood (β = 1.17, $p = 0.068$). A significant inverse association between birth weight and BMD lumbar spine was found in three out of four models: model A (β = –1.8, $p = 0.026$), model B (β = –1.65, $p = 0.037$) and model C (β = –2.11, $p = 0.007$) [29]. Using Pearson's correlation, **Saito et al.** found that birth weight was significantly correlated with femoral neck ($r = 0.23$; $p < 0.05$), but not with BMD lumbar spine ($r = 0.21$; p NS) and the total hip ($r = 0.15$; p NS) [28]. **El Hage et al.** found no significant correlation coefficients between birth weight and BMAD in adolescent females ($r = 0.14$; p NS) [42].

Table 3: Eight studies on the relation between birth weight and bone health in children between 13 till 23 years old, sorted alphabetically

Author	Sample	Method	Results
de Bono et al. (2010)	n = 120 17 – 21 yrs. Gambia	DXA: BA – BMC – aBMD – BA adj BMC - Lumbar spine - Femoral neck - Total body - Total hip - Radial shaft pQCT: CSA – BMC – vBMD - Radial shaft - Tibial shaft	<u>DXA (adj.= current weight, height & age)</u> + BA (unadjusted males and females) *Except: ○ BA l.s. (females)* ○ BA (adjusted males) *Except:+ BA r.s – f.n. (males)* + BA (adjusted females) *Except: ○ l.s. – t.b. (females)* ○ BMC (adj. & unadj. males and females) *Except: + r.s. – f.n. (females)* ○ aBMD – BA adj BMC (adj. & unadj. males and females) *Except: + aBMD r.s. (adj. males)* <u>pQCT cortical sites</u> ○ CSA t.s. – r. s. (adj. & unadj. females) *Except: + cortical r.s. (unadj. females)* + total & medullary CSA t.s. – r.s. (adj. & unadj. males) ○ cortical CSA t.s. – r.s. (adj. & unadj. males) ○ BMC all (adj. & unadj. males) ○ BMC t.s. (adj. & unadj. females) + BMC r.s. (adj. & unadj. females) - vBMD t.s. (adj. males) ○ vBMD t.s. (adj. & unadj. females) <u>pQCT trabecular sites</u> ○ CSA d.t. – d.r. (adj. & unadj. males) *Except: + total CSA d.t. (unadj.)* + CSA d.t. – d.r. (adj. & unadj. females) ○ BMC all (adj. & unadj. males and females) *Except: + d.r. (unadj. females)* ○ vBMD all (adj. and unadj. females) - vBMD all (adj. & unadj. males) Except : ○ vBMD d.t. (unadj. males)
El Hage et al. (2010)	n = 40 13 – 20 yrs. All females Lebanon	DXA: BMC – BMD – BMA – BMAD - Total body	+ BMC (regression and correlation) + BMD (regression and correlation) ○ BMAD (correlation) + BMA (correlation)
Jensen et al. (2008)	n = 123 16 – 19 yrs. Denmark	DXA: BMC – BMD - Total body - Lumbar spine	<u>Regression analysis</u> + BMC t.b. – l.s. ○ after correction + BMD l.s. ○ after correction <u>Size-adjusted model</u> ○ BMC <u>Three-step analysis</u> ○ BA adj for height - BMC adj for BA

Leunissen et al. (2008)	n = 312 18 – 24 yrs. The Netherlands	DXA: BMD - Lumbar spine - Total body	4 models – regression analysis ○ BMD t.b. (all models) - BMD l.s. model A – B – C ○ BMD l.s. model D
Mølgaard et al. (2011)	n = 109 17 yrs. Demark	DXA: BMC – BA – BMD - Total body - Lumbar spine	○ BA t.b. + BA l.s. ○ BMC l.s. – t.b. ○ BMD t.b. – l.s.
Saito et al. (2005)	n = 86 18 – 21 yrs. Japan	DXA: BMC – BMD - Lumbar spine - Left hip	Correlation + BMC l.s. – f.n. – total hip + BMD f.n. ○ BMD l.s. – total hip Regression analysis + BMC l.s. – total hip
Schlüssel et al. (2010)	n = 496 23 yrs. Brazil	DXA: BMC – BMD – BA - Lumbar spine - Proximal femur - Femoral neck	Linear regression (birth tertiles) + BMC/BA l.s. - p.f. – f.n. (males) + BMD spine (males) + BMC/BMD spine (females) + BA l.s. – p.f. – f.n. (highest birth weight tertile) (females) Multiple regression (with adj.) + BA f.n. – p.f. ○ BA spine + BMC spine – f.n. – p.f. ○ BMD spine – f.n. – p.f.
Wang et al. (2010)	n = 236 16 – 20 yrs. All females Finland	DXA: BA – BMC - Total body - Femoral neck - Lumbar spine pQCT: bone strength index (BSI) – CSA - Radius - Tibia	DXA + BA t.b. – l.s. – f.n. + BMC t.b. – l.s. – f.n. pQCT + BSI tibia ○ BSI radius + CSA radius – tibia

+ : the results show a significant and positive relationship; - : the results show a significant and inverse relationship; ○ : no relationship was found. L.s. lumbar spine; t.b. total body; f.n. femoral neck; p.f. proximal femur; r.s. radial shaft; d.t. distal tibia; d.r. distal radius.

Other factors influencing bone health in children

In the studies examining the relationship between birth weight and bone health, many other variables were investigated as well, since their influence on one of both dependent variables. These variables can be split in characteristics of the child and parental characteristics.

1. Characteristics of the children

Most studies have focused on growth related factors. Nine studies investigated the influence of current height and weight (at the time of the bone measurements) on bone health [18-22, 24, 28, 42, 43].

Second, weight gain or catch-up growth in weight in the postnatal period was investigated by six researchers. Nevertheless, results are difficult to compare by differences in the period of measured weight gain [19, 25, 28, 29, 33, 43]. Yet, not only weight gain but also height gain can be considered: the influence of delta height on lumbar spine BMD and total body BMD was recently studied [29]. Apart from birth weight, also birth length or crown-heel length at birth has been investigated [18, 29, 41, 44]. Moreover, the influence of weight and length, at different ages, on bone density has been frequently studied. Weight and length at one till six months was measured and tested for associations with bone health [19, 25, 41]. Most often, the age of one year was taken to measure the weight and length [23, 31, 32]. Finally, some less obviously growth variables were studied such as skeletal age [43], fetal growth [25, 31], growth hormone [31] and gestational age [18, 20, 25, 26, 44]. Apart from growth, also fat mass, BMI, ponderal index [21, 24, 25, 29, 42, 43], season of birth [26] and physical activity [21, 24, 28] have been examined on their effect on bone density.

Other studies confirm the influence of other factors on bone health in children: the nutrition of the child (e.g. calcium intake), genetic factors, hormonal influences and lifestyle factors such as tobacco smoking and weight-bearing exercise [7, 45-47].

2. Parental characteristics

This part enumerates behavioral, environmental and socio-economic factors of parents that can contribute to the bone health in offspring.

- Nutritional status or diet such as calcium intake during pregnancy [18, 21, 22].
- Duration and exclusivity of breastfeeding [18, 21, 25, 32].
- Parity, the number of times a mother has given birth [18, 20, 24].
- Body characteristics of the parents such as maternal and paternal height, weight, BMI and bone density [18, 20, 22, 25, 41].
- Background ultraviolet B exposure during pregnancy and of the child [22].
- Maternal smoking during pregnancy [21, 24].
- Alcohol consumption during pregnancy [21, 24].
- The influence of housing density [21, 24].

Additional analyses and results

Covariate analysis

Through combining potential covariates found in (e.g.) the literature and the existing covariates available in our study, thorough univariate analyses were performed. The covariates were divided into two main groups: on the one hand covariates with influence on the dependent continuous variable birth weight and on the other covariates with influence on the dependent continuous variables BUA, SOS and SI. In relation to a dichotomous variable, an independent T-test was used in normally distributed variables and a Mann-Whitney U test in non-normally distributed variables. The analysis between categorical variables and the dependent variables were performed using an F-test (ANOVA) in normally distributed variables and a Kruskal-Wallis test in non-normally distributed variables. Finally correlation analysis (Pearson) was used to explore the relation between two continuous variables, a covariate and one of the four dependent variables. The influence of the covariates on the dependent variables was calculated for the total group as for boys and girls separately. P-values ≤ 0.2 were considered as potential influential variables and included in further analyses. Appendix 1 contains the results of the univariate analyses whereby the influence of the covariates on (1) birth weight and (2) BUA, SOS and SI is presented separately.

For the dependent variable **birth weight**, the covariates were divided into three groups: factors related to the pregnancy (e.g. alcohol or tobacco use), shared genetic factors (e.g. height of the mother and father, income category) and factors related to the child (e.g. gender of the child, season of birth). Appendix 1 (1. Birth weight) shows the results of the analyses between birth weight and twenty-one covariates. Eighteen variables showed an influence on birth weight ($p \leq 0.2$). Several factors related to the pregnancy such as smoking of the mother, delivery by caesarian section, mother's gained weight during pregnancy and the age of the mother at time of delivered influenced birth weight for total group and boys and girls separately. All shared genetic factors showed an influence on birth weight except the BMI of the father (both as a continuous or categorical variable). All factors related to the child, the number of weeks born too early, the gender and season of birth, revealed significant influences and were considered influential covariates.

As shown in appendix 1 (2.1 Broadband Ultrasound Attenuation) thirty-one variables were investigated and categorized into three groups: factors related to the birth (e.g. birth weight, birth length, breastfeeding), other variables (current age of the child, hours of sleep, physical activity) and factors related to anthropometry or body composition (e.g. height, skinfold, fat-free mass of the child). Twenty-four variables showed an influence on the bone health parameter **BUA**. Three factors related to the birth (gender of the child, birth weight and birth length) did significantly influence BUA. Of the 'other variables' the age and the number of hours of sleep were influential covariates. Finally all anthropometric variables, such as current height, weight, waist, BMI, skinfolds and (%) fat-free mass, were positively correlated with BUA.

Mainly inverse associations were found between the tested covariates and **SOS** due to the nature of this dependent variable. Appendix 1 (2.2 Speed of Sound) shows the same thirty-one variables as in BUA and were tested for the dependent variable SOS. In accordance to BUA, the covariates birth weight, birth length, current age of the child, the number of hours of sleep and all factors related to anthropometry and body composition variables (e.g. height, weight and skinfolds) were significantly associated with SOS. In contrast to the results of BUA, gender ($t = 0.094$; $p = 0.925$ for total group) and % fat-free mass ($r = 0.041$; $p = 0.316$ for total group) did not influence SOS in univariate analyses.

The same thirty-one variables, as used in the analyses with the other bone health parameters SOS and BUA, were analyzed to assess the influence on the third bone health parameter **SI**. In appendix 1 (2.3 Stiffness Index) the results of the univariate analyses between the covariates and SI are presented. In general fewer variables showed associations with the calculated parameter SI. Similar associations were found for gender and the current age of the child in the first two categories compared to SOS and BUA. Within the category on factors related to anthropometry or body composition, only a few variables showed significant results: current height, current weight, log-transformed tricipital skinfold thickness, fat-free mass and % fat-free mass.

Stepwise multiple regression

The tested covariates in univariate analyses with an influence on a dependent variable (birth weight, BUA, SOS or SI) were listed and used in further analyses. First, several different models with the influential covariates were tested in multiple regression analyses. Second, more specific pre-final models were tested for each dependent variable whereby the results are explained below. Finally, all these previous analyses resulted in final models tested in stepwise multiple regression analyses whose results are discussed in the scientific article. In all analyses adjustments for age and gender were made. Table 1 shows five models that were tested for the relationship between birth weight and **BUA**. The covariates FFM, %FFM, weight z-score and height z-score were added separately or together, depending on the model, with the ENTER-method. In general the adjusted R square of all models was relatively high (between 0.251 and 0.352). Model 1 and model 3 showed problems with multicollinearity whereby a tolerance measure of ≥0.4 was accepted. A relationship was found between birth weight and BUA with age as a significant predictor in model 4. Models 2, 4 and 5 showed an influence of age in contrast with the absence of influence of the variable gender. The anthropometric variables %FFM, height z-score and weight z-score gave, depending on the model, significant results. Summarized, only models 2, 3 and 5 could be interpreted whereby the influence of age, %FFM, weight z-score and height z-score are important. This knowledge was used to design five final and concrete models to use in the scientific article. The influence of birth weight on **SOS** was analyzed with two different models in multiple regression analysis and the results are shown in table 2. Besides age and gender, weight z-score and height z-score were added separately or together depending on the model. The adjusted R square was 0.359 in model 1 and 0.366 in model 2. No problem with multicollinearity was observed. Both weight z-score and height z-score proved to be significant predictors of SOS. With this result, four final models were used in the scientific article. Five similar models as within the parameter BUA were analyzed for the last bone health parameter **SI**. The covariates FFM, %FFM, weight z-score and height z-score were used separately or together, with the ENTER-method. Overall the adjusted R square was low (between 0.028 and 0.033). Model 1 and 3 could not be interpreted due to the problem of multicollinearity. Apart from age, only %FFM was a significant predictor of SI. This resulted in three final models used in the scientific article.

Table 1. Results of multiple regression analyses using calcaneal BUA (n = 596) as a dependent variable and various other variables as independent variables

Confounders (ENTER)				
Model 1 - Birth weight - Age - Gender - FFM - Weight z-score	Model 2 - Birth weight - Age - Gender - % FFM - Weight z-score	Model 3 - Birth weight - Age - Gender - % FFM - Weight z-score - Height z-score	Model 4 - Birth weight - Age - Gender - % FFM	Model 5 - Birth weight - Age - Gender - Weight z-score - Height z-score
Model summary				
Durbin-watson 1.927 Adj R square 0.344 ANOVA F = 63.400 p < 0.001	Durbin-watson 1.924 Adj R square 0.351 ANOVA F = 65.338 p < 0.001	Durbin-watson 1.929 Adj R square 0.352 ANOVA F = 54.795 p < 0.001	Durbin-watson 1.912 Adj R square 0.251 ANOVA F = 50.801 p < 0.001	Durbin-watson 1.939 Adj R square 0.346 ANOVA F = 64.048 p < 0.001
Predictors				
Birth weight - t = 0.951 - p = 0.342 Age - t = 4.329 - **p < 0.001** Gender - t = -0.131 - p = 0.896 FFM - t = 2.629 - **p = 0.009** Weight z-score - t = 3.716 - **p < 0.001**	Birth weight - t = 0.957 - p = 0.339 Age - t = 14.682 - **p < 0.001** Gender - t = -0.007 - p = 0.994 %FFM - t = 3.645 - **p < 0.001** Weight z-score - t = 9.597 - **p < 0.001**	Birth weight - t = 0.876 - p = 0.382 Age - t = 14.708 - **p < 0.001** Gender - t = -0.164 - p = 0.870 %FFM - t = 2.517 - **p = 0.012** Weight z-score - t = 4.529 - **p < 0.001** Height z-score - t = 1.303 - p = 0.193	Birth weight - t = 3.282 - **p = 0.001** Age - t = 13.590 - **p < 0.001** Gender - t = -0.754 - p = 0.451 %FFM - t = 0.351 - p = 0.726	Birth weight - t = 0.944 - p = 0.346 Age - t = 14.934 - **p < 0.001** Gender - t = -1.123 - p = 0.262 Weight z-score - t = 3.760 - **p < 0.001** Height z-score - t = 2.936 - **p = 0.003**
Collinearity statistics (tolerance measure ≥0.4 = acceptable)				
Age 0.197 FFM 0.149 Weight z-score 0.393	All acceptable	Weight z-score 0.280 Height z-score 0.316	All acceptable	All acceptable

BUA: Broadband Ultrasound Attenuation; FFM: fat-free mass (lean mass); %FFM: percentage fat-free mass (lean mass); P-values < 0.05 are indicated in bold

Table 2. Results of multiple regression analyses using calcaneal SOS (n = 600) as a dependent variable and various other variables as independent variables

Confounders (ENTER)	
Model 1 - Birth weight - Age - Gender - Weight z-score	Model 2 - Birth weight - Age - Gender - Weight z-score - Height z-score
Model summary	
Durbin-watson 2.102 Adj R square 0.359 ANOVA F = 84.845 p < 0.001	Durbin-watson 2.095 Adj R square 0.366 ANOVA F = 70.054 p < 0.001
Predictors	
Birth weight - t = -1.794 - p = 0.073 Age - t = -14.225 - **p < 0.001** Gender - t = -0.727 - p = 0.468 Weight z-score - t = -11.241 - **p < 0.001**	Birth weight - t = -1.568 - p = 0.117 Age - t = -14.204 - **p < 0.001** Gender - t = -0.933 - p = 0.351 Weight z-score - t = -5.579 - **p < 0.001** Height z-score - t = -2.701 - **p = 0.007**
Collinearity statistics (tolerance measure ≥0.4 = acceptable)	
All acceptable	All acceptable

SOS: Speed of Sound; P-values < 0.05 are indicated in bold

Table 3. Results of multiple regression analyses using calcaneal SI (n = 827) as a dependent variable and various other variables as independent variables

Confounders (ENTER)				
Model 1 - Birth weight - Age - Gender - FFM - Weight z-score	Model 2 - Birth weight - Age - Gender - % FFM - Weight z-score	Model 3 - Birth weight - Age - Gender - % FFM - Weight z-score - Height z-score	Model 4 - Birth weight - Age - Gender - % FFM	Model 5 - Birth weight - Age - Gender - Z-score weight - Height z-score
Model summary				
Durbin-watson 1.330 Adj R square 0.030 ANOVA F = 6.112 p < 0.001	Durbin-watson 1.323 Adj R square 0.033 ANOVA F = 6.641 p < 0.001	Durbin-watson 1.322 Adj R square 0.033 ANOVA F = 5.619 p < 0.001	Durbin-watson 1.310 Adj R square 0.032 ANOVA F = 7.864 p < 0.001	Durbin-watson 1.333 Adj R square 0.028 ANOVA F = 5.696 p < 0.001
Predictors				
Birth weight - t = -1.140 - p = 0.254 Age - t = 0.843 - p = 0.400 Gender - t = -0.788 - p = 0.431 FFM - t = 1.502 - p = 0.134 Weight z-score - t = -0.824 - p = 0.410	Birth weight - t = -1.187 - p = 0.235 Age - t = 4.737 - **p < 0.001** Gender - t = -0.657 - p = 0.511 %FFM - t = 2.193 - **p = 0.029** Weight z-score - t = 1.311 - p = 0.190	Birth weight - t = -1.137 - p = 0.256 Age - t = 4.686 - **p < 0.001** Gender - t = -0.507 - p = 0.612 %FFM - t = 2.241 - **p = 0.025** Weight z-score - t = 1.353 - p = 0.177 Height z-score - t = -0.729 - p = 0.466	Birth weight - t = -0.858 - p = 0.391 Age - t = 4.761 - **p < 0.001** Gender - t = -0.801 - p = 0.423 %FFM - t = 1.835 - p = 0.067	Birth weight - t = -1.089 - p = 0.276 Age - t = 4.916 - **p < 0.001** Gender - t = -1.624 - p = 0.105 Weight z-score - t = -0.054 - p = 0.957 Height z-score - t = 0.536 - p = 0.592
Collinearity statistics (tolerance measure ≥0.4 = acceptable)				
Age - 0.195 FFM - 0.143 Weight z-score - 0.374	All acceptable	Weight z-score - 0.267 Height z-score - 0.309	All acceptable	All acceptable

SI: Stiffness Index; FFM: fat-free mass (lean mass); %FFM: percentage fat-free mass (lean mass); P-values < 0.05 are indicated in bold

Additional points of discussion

A thorough and extensive discussion of this research can be found in the scientific article. The following paragraphs discuss (1) a brief summary of the main results of this additional report and (2) additional discussion points including some (3) additional strengths and limitations.

This study investigated the relationship between birth weight and bone strength assessed as calcaneal BUA, SOS and SI-values measured by QUS in 827 healthy children between 3 and 11 years old. The main findings of the analyses within this additional report are the existence of many variables that influence birth weight on the one hand and bone health parameters on the other. Those covariates were taken into account when analyzing the influence of birth weight on bone health. Gender and age were defined as confounders and included in all analyses. Pre-final multiple regression analysis showed that age, %FFM, weight z-score and height z-score act as predictors of bone health assessed by QUS. The final models are discussed in the scientific article.

Three out of the seven studies investigating the influence of birth weight on bone health in primary school children adjusted for potential covariates in multiple regression analysis. Steer & Tobias used three different models. First, adjusting for gestational age, gender and current age of the child gave similar significant results as this research. After additionally adjusting for parental height and weight, the relationship persisted, what is in contrast to this study. The researchers additionally adjusted for current height and weight in a third model whereby the relationship decreased but was still significant with exception of BMC and bone area [22]. Jones & Dwyer used three models with different covariates. The researchers started immediately with the adjustment for gender and growth variables together in a first model whereby a significant influence was found for BMD but not for BMAD. Similar results as in the first model were obtained after additionally correcting for environmental factors (e.g. breastfeeding and maternal smoking). In a third model maternal BMD was added, but no associations remained significant [19]. This is in line with the findings of this study whereby the association decline or disappear when adding several covariates. The third study that took covariates into account used two models to analyze a large number of variables at once [23]. Vidulich et al. adjusted in a first model for the variables bone age, gender, race, SES, current height and weight. No

significant associations between birth weight and bone health were found. This finding is in line with the third model of Jones & Dwyer and the results of this research. After additionally correcting BMC for bone area (BA), a significant relation between birth weight and BMC femoral neck in 8 year olds was shown.

Summarized, our research using the radiation-free QUS gave similar results as literature using bone health parameters assessed by DXA: an association between birth weight and bone health parameters after correcting for age and sex, but less or no association after correcting for additional confounders (mainly anthropometric characteristics of the child).

Strengths and limitations

The strength of this study is the availability of a large representative population-based sample of both Belgian pre-school and primary school boys and girls. The possibility of testing a large battery of covariates lies in the fact that data on early life factors, environmental and family factors, QUS measurements and anthropometric measurements were complete for all 827 children. The parental questionnaire consisted of many variables from information on breastfeeding till the height, weight and BMI of the parents. Hence the possibility to analyze many models in multivariate regression analyses.

First important limitation that should be mentioned here again, is the absence of an exact gestational age per child as this could be an important potential confounder [18, 20, 25, 26]. Besides the drawbacks mentioned in the research article, some minor limitations can be added. First, exact data that shows whether the participant is part of a twin or triplet, if they did not all participated in the study, is missing. Known twins or triplets were excluded, but there is a small chance that other twins or triplets stayed unnoticed. Second, birth weight was obtained through a parental questionnaire and the accuracy of this information can be questioned. Overall, these minor flaws will not have much influence on the results of this study.

Conclusion of this additional report

The overall conclusion of this research is written in the scientific article. The conclusion of this additional report is formulated in the next section. Osteoporosis is a silent epidemic whereby at the one hand many factors determine the gain of bone early in life linked with the foundation of the disease and at the other the bone loss later in life linked with the manifestation of the disease. The present findings support the importance of attaining maximal peak bone mass in healthy children and adolescents to prevent osteoporosis later in life. In general very few studies investigated the influence of birth weight on bone health, as a determinant of osteoporosis later in life, in pre-school and primary school children. This study points towards the importance of a normal birth weight even in healthy children (including preschool children). Many variables proved to be of influence on bone health and were used in analyses to purify the relation between birth weight and bone strength assessed by QUS. Public health strategies should insist also on the importance of an optimal birth weight, through the mothers' health and environmental factors, which could positively influence chronic diseases (e.g. osteoporosis) later in life. Further research could investigate if the findings of this study are consistent in large study samples when using the QUS method and purifying for potential covariates in statistical analyses.

Choice of journal

Since this master's thesis is written as a combination of a scientific article and an additional report, a scientific journal for submission of the article had to be chosen. Possible journals with their impact factor and scope are presented in appendix 2. Firstly, the journals related to bone metabolism are listed since that is the main focus variable of this study. Three studies had a relative high impact factor and a few studies had a strong clinical focus. The journals *Calcified Tissue International* and *Metabolism – clinical and experimental* were preferred. Second, journals related to pediatrics are listed. One journal has a high impact factor and a few articles have a less relevant scope. The journals *European journal of pediatrics* and *Child: care, health and development* were preferred. Thirdly, a category 'other' is presented with one article related to epidemiological research. In agreement with the promotor of this master's thesis, dr. Isabelle Sioen, it was decided to submit the scientific article to *Calcified Tissue International* for its focus on bone in many facets. The confirmation of the submission is attached to the scientific article.

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Appendix

Appendix 1: Analyses of covariates on birth weight, BUA, SOS and SI.

Appendix 2: List of possible scientific journals.

Appendix 1: Univariate analyses of covariates on birth weight, BUA, SOS and SI[1].

1. On birth weight

Covariates	Total group	Boys	Girls	Test
1.1. Pregnancy				
Smoking mother →dichotomous (0 and 1) Dicho_tobacco_pregnancy	t = 2.858 p = 0.004	t = 1.515 p =0.131	t = 2.975 p =0.003	Indep. T-test
	n = 743	n = 387	n = 356	
Smoking mother →gradations (0 to 3) Grad_tobacco_pregnancy	F = 3.118 p = 0.026	F = 1.533 p = 0.217	F = 3.287 p = 0.021	F-test (ANOVA)
	n = 743	n = 387	n = 356	
Drinking mother →dichotomous (0 and 1) Dicho_alcohol_pregnancy	Levene = sign. p = 0.11 T-test t = -1.226 p = 0.221	Levene = sign. p = 0.021 T-test t = -0.981 p = 0.327	t = -0.673 p = 0.501	Indep. T-test
	n = 742	n = 382	n = 360	
Non-Param test (Mann-Whitney U Test) Dichot. alcohol mother	p = 0.397	p = 0.423	p = 0.829	NP test
	n = 742	n = 382	n = 360	
Drinking mother →gradations (0 to 3) Grad_alcohol_pregnancy	Levene = sign p = 0.008	Levene = sign p = 0.035	F = 1.008 p = 0.366	F-test (ANOVA)
	n = 742	n = 382	n = 360	
→**Non-Param test** (Kruskal-Wallis Test) Drinking mother gradations	p = 0.200	p = 0.634	p = 0.267	NP test
	n = 742	n = 382	n = 360	
C-section Pregnancy – C-section done c_section (1 and 2)	Levene = sign. p = 0.001 T-test t = -2.854	Levene = sign. p = 0.001 T-test t = -2.386	t = -1.556 p = 0.120	Indep. T-test

[1] marked when $p \leq 0.200$

	p = 0.005	p = 0.019		
	n = 818	n = 421	n = 397	
→Non-Param test (Mann-Whitney U Test) C-section	p = 0.007	p = 0.044	p = 0.088	NP test
	n = 818	n = 421	n = 397	
Mother's gained weight Preg_w_up	r = 0.138 p < 0.001	r = 0.123 p = 0.014	r = 0.166 p = 0.001	Correlate
	n = 776	n = 399	n = 377	
Age mother at time of delivery age_birth	r = 0.100 p =0.005	r = 0.114 p = 0.021	r = 0.076 p = 0.136	Correlate
	n = 801	n = 414	n = 387	
1.2. Shared genetic factors				
Height mother Height_m	r = 0.155 p < 0.001	r = 0.117 p = 0.017	r = 0.208 p < 0.001	Correlate
	n = 801	n = 418	n = 383	
Height father Height_f	r = 0.117 p = 0.002	r = 0.125 p = 0.016	r = 0.119 p = 0.031	Correlate
	n = 698	n = 368	n = 330	
Weight mother Weight_m	r = 0.161 p < 0.001	r = 0.166 p = 0.001	r = 0.155 p = 0.002	Correlate
	n = 789	n = 409	n = 380	
Weight father Weight_f	r = 0.072 p = 0.060	r = 0.102 p = 0.051	r = 0.016 p = 0.774	Correlate
	n = 686	n = 363	n = 323	
BMI mother Continuous	r = 0.098 p = 0.006	r = 0.120 p = 0.016	r = 0.065 p = 0.208	Correlate
	n = 789	n = 409	n = 380	
BMI mother (In 4 groups) cat_BMI_mother	F = 2.608 p = 0.051	F = 1.912 p = 0.127	F = 1.764 p = 0.154	F-test (ANOVA)
	n = 788	n = 408	n = 380	
BMI father Continuous	r = 0.006 p = 0.883	r = 0.024 p = 0.649	r = -0.045 p = 0.417	Correlate
	n = 680	n = 358	n = 322	
BMI father (In 4 groups) cat_BMI_father	F = 0.330 p = 0.804	F = 0.259 p = 0.855	F = 0.074 p = 0.974	F-test (ANOVA)

	n = 680	n = 357	n = 321	
ISCED parents Isced_max	F = 2.747 p = 0.018	F = 3.785 p = 0.005	F = 1.414 p = 0.218	F-test (ANOVA)
	n = 782	n = 400	n = 382	
Income category Income_cat	F = 3.054 p = 0.016	F = 3.914 p = 0.004	F = 1.018 p = 0.398	F-test (ANOVA)
	n = 674	n = 349	n = 325	
Non-Param test Kruskal_Wallis test Income_cat	p = 0.017	p = 0.006	p = 0.269	NP test
	n = 674	n = 349	n = 325	
1.3. Child				
Weeks born too early Weeksearly	r = -0.560 p < 0.001	r = -0.572 p < 0.001	r = -0.548 p < 0.001	Correlate
	n = 328	n = 176	n = 152	
Weeks born too early (with missing = 0) All_weeks_early	r = -0.470 p < 0.001	r = -0.476 p < 0.001	r = -0.482 p < 0.001	Correlate
	n = 827	n = 427	n = 400	
Gender Sex_child	t = 5.218 p < 0.001	/	/	Indep. T-test
	n = 827	/	/	
Season_birth	F = 2.945 p = 0.032	F = 1.995 p = 0.114	F = 1.659 p = 0.175	F-test (ANOVA)
	n = 827	n = 427	n = 400	

2. On QUS parameters

2.1. Broadband Ultrasound Attenuation (dB/MHz)

Covariates	Total group	Boys	Girls	Test
2.1.1. Birth				
Gender Sex_child	t = 1.845 p = 0.066	/	/	Indep. T-test
	n = 596	/	/	
Birth weight Birth_w	r = 0.128 p = 0.002	r = 0.129 p = 0.026	r = 0.102 p = 0.077	Correlate
	n = 596	n = 297	n = 299	
Birth length Birth_h	r = 0.156 p < 0.001	r = 0.173 p = 0.003	r = 0.121 p = 0.037	Correlate
	n = 593	n = 295	n = 298	
Birth season Season_birth	F = 0.073 p = 0.975	F = 0.255 p = 0.858	F = 0.289 p = 0.833	F-test (ANOVA)
	n = 596	n = 297	n = 299	
Weeks born too early Weeksearly	r = -0.005 p = 0.941	r = 0.027 p = 0.770	r = -0.045 p = 0.633	Correlate
	n = 237	n = 121	n = 116	
Weeks born too early (with missing values = 0) All_weeks_early	r = -0.022 p = 0.598	r = -0.030 p = 0.608	r = -0.011 p = 0.853	Correlate
	n = 596	n = 297	n = 299	
Breastfeeding Months_exclusive_breastfeeding	r = -0.021 p = 0.718	r = 0.009 p = 0.916	r = -0.057 p = 0.488	Correlate
	n = 290	n = 142	n = 148	
2.1.2. Other variables				
Age children Age	r = 0.489 p < 0.001	r = 0.449 p < 0.001	r = 0.533 p < 0.001	Correlate
	n = 596	n = 297	n = 299	
Physical activity (Average count per minute) Avg_cpm	r = -0.050 p = 0.367	r = -0.072 p = 0.357	r = -0.059 p = 0.460	Correlate
	n = 329	n = 168	n = 161	
Hours of sleep Sleep_hour	r = -0.212 p < 0.001	r = -0.184 p = 0.013	r = -0.233 p = 0.003	Correlate

	n = 350	n = 184	n = 166	
Calcium CA_U	r = 0.049 p = 0.375	r = -0.001 p = 0.993	r = 0.088 p = 0.258	Correlate
	n = 336	n = 169	n = 167	
Phosphate NANP	r = 0.058 p = 0.287	r = 0.037 p = 0.629	r = 0.060 p = 0.440	Correlate
	n = 336	n = 169	n = 167	
2.1.3. Anthropometry – body composition				
Current height (Cole) Height_score_cole	r = 0.303 p < 0.001	r = 0.320 p < 0.001	r = 0.279 p < 0.001	Correlate
	n = 596	n = 297	n = 299	
Current height height	r = 0.574 p < 0.001	r = 0.540 p < 0.001	r = 0.609 p < 0.001	Correlate
	n = 596	n = 297	n = 299	
Current height (categories) Height_cat	Levene = sign. p = 0.037	Levene = sign. p = 0.003	F = 1.753 p = 0.175	F-test (ANOVA)
	n = 596	n = 297	n = 299	
→**Non-Param Test** (Kruskal-Wallis test) Height_cat	p = 0.003	p = 0.041	p = 0.098	NP test
	n = 596	n = 297	n = 299	
Current weight (Cole) Weight_score_cole	r = 0.293 p < 0.001	r = 0.316 p < 0.001	r = 0.278 p < 0.001	Correlate
	n = 596	n = 297	n = 299	
Current weight weight	r = 0.527 p < 0.001	r = 0.173 p = 0.003	r = 0.558 p < 0.001	Correlate
	n = 596	n = 295	n = 299	
Waist (categories) Waist_cat_CDC	F = 3.151 p = 0.005	F = 1.059 p = 0.387	F = 3.157 p = 0.005	F-test (ANOVA)
	n = 595	n = 296	n = 299	
→**Non-Param Test** (Kruskal-Wallis test) Waist_cat_CDC	p = 0.008	p = 0.202	p = 0.015	NP test
	n = 595	n = 296	n = 299	
Waist (Cole) Waist_score_cole	r = 0.236 p < 0.001	r = 0.238 p < 0.001	r = 0.248 p < 0.001	Correlate
	n = 595	n = 296	n = 299	
BMI	F = 1.636	F = 0.763	F = 1.565	F-test

IOTF grade	p = 0.148	p = 0.550	p = 0.170	(ANOVA)
	n = 596	n = 297	n = 299	
BMI (z-score) Bmi_score_cole	r = 0.163 p < 0.001	r = 0.177 p = 0.002	r = 0.161 p = 0.005	Correlate
	n = 596	n = 297	n = 299	
Triceps Fat_tric	r = 0.135 p = 0.001	r = 0.095 p = 0.105	r = 0.283 p < 0.001	Correlate
	n = 591	n = 296	n = 295	
Subscapular Fat_scap	r = 0.135 p = 0.001	r = 0.163 p = 0.005	r = 0.176 p = 0.002	Correlate
	n = 593	n = 296	n = 297	
Sum Triceps & Subscapular SUMfat_tric_scap	r = 0.145 p < 0.001	r = 0.137 p = 0.018	r = 0.223 p < 0.001	Correlate
	n = 590	n = 295	n = 295	
Waist/hip ratio Waist_to_hip	r = -0.170 p < 0.001	r = -0.261 p < 0.001	r = -0.112 p = 0.053	Correlate
	n = 595	n = 296	n = 299	
Triceps log Triceps_log	r = 0.117 p = 0.005	r = 0.082 p = 0.158	r = 0.228 p < 0.001	Correlate
	n = 591	n = 296	n = 295	
Subscapular log Scapular_log	r = 0.146 p < 0.001	r = 0.187 p = 0.001	r = 0.177 p = 0.002	Correlate
	n = 593	n = 296	n = 297	
Sum Triceps & Subscapular log SUMfat_tric_scap_log	r = 0.139 p = 0.001	r = 0.142 p = 0.015	r = 0.217 p < 0.001	Correlate
	n = 590	n = 295	n = 295	
Waist/hip ratio log Waist_to_hip_log	r = -0.171 p < 0.001	r = -0.260 p < 0.001	r = -0.115 p = 0.048	Correlate
	n = 595	n = 296	n = 299	
Fat-free mass (lean mass) FFM	r = 0.570 p < 0.001	r = 0.560 p < 0.001	r = 0.580 p < 0.001	Correlate
	n = 595	n = 297	n = 298	
% Fat-free mass (lean mass) FFM_P	r = 0.083 p = 0.043	r = 0.118 p = 0.042	r = 0.038 p = 0.510	Correlate
	n = 595	n = 297	n = 298	

2.2. Speed of Sound (m/sec)

Covariates	Total group	Boys	Girls	Test
2.2.1. Birth				
Gender Sex_child	t = -0.094 p = 0.925	/	/	Indep. T-test
	n = 600	/	/	
Birth weight Birth_w	r = -0.157 p < 0.001	r = -0.160 p = 0.005	r = -0.158 p = 0.006	Correlate
	n = 600	n = 301	n = 299	
Birth length Birth_h	r = -0.151 p < 0.001	r = -0.159 p = 0.006	r = -0.151 p = 0.009	Correlate
	n = 597	n = 299	n = 298	
Birth season Season_birth	Levene = sign. p = 0.013	F = 0.727 p = 0.537	Levene = sign. p = 0.047	F-test (ANOVA)
	n = 600	n = 301	n = 299	
Weeks born too early Weeksearly	r = -0.028 p = 0.669	r = -0.052 p = 0.563	r = -0.007 p = 0.945	Correlate
	n = 240	n = 124	n = 116	
Weeks born too early (with missing values = 0) All_weeks_early	r = 0.046 p = 0.256	r = 0.013 p = 0.828	r = 0.079 p = 0.171	Correlate
	n = 600	n = 301	n = 299	
Breastfeeding Months_exclusive_ breastfeeding	r = -0.027 p = 0.645	r = -0.126 p = 0.134	r = 0.074 p = 0.372	Correlate
	n = 292	n = 144	n = 148	
2.2.2. Other variables				
Age children Age	r = -0.451 p < 0.001	r = -0.476 p < 0.001	r = -0.430 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Physical activity (Average count per minute) Avg_cpm	r = -0.002 p = 0.965	r = 0.003 p = 0.964	r = -0.026 p = 0.744	Correlate
	n = 332	n = 171	n = 161	
Hours of sleep Sleep_hour	r = 0.164 p = 0.002	r = 0.210 p = 0.004	r = 0.110 p = 0.160	Correlate

	n = 354	n = 188	n = 166	
Calcium CA_U	r = -0.007 p = 0.891	r = -0.021 p = 0.788	r = 0.005 p = 0.953	Correlate
	n = 338	n = 171	n = 167	
Phosphate NANP	r = -0.056 p = 0.308	r = -0.052 p = 0.501	r = -0.061 p = 0.433	Correlate
	n = 338	n =171	n = 167	
2.2.3. Anthropometry – body composition				
Current height (Cole) Height_score_cole	r = -0.356 p < 0.001	r = -0.421 p < 0.001	r = -0.299 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Current height height	r = -0.571 p < 0.001	r = -0.615 p < 0.001	r = -0.533 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Current height (categories) Height_cat	Levene sign. (p < 0.001)	Levene sign. (p < 0.001)	F = 1.406 p = 0.034	F-test (ANOVA)
	n = 600	n = 301	n = 299	
→**Non-Param Test** (Kruskal-Wallis test) Height_cat	p < 0.001	p < 0.001	p = 0.012	NP test
	n = 600	n = 301	n = 299	
Current weight (Cole) Weight_score_cole	r = -0.377 p < 0.001	r = -0.462 p < 0.001	r = -0.310 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Current weight weight	r = -0.574 p < 0.001	r = -0.630 p < 0.001	r = -0.525 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Waist (categories) Waist_cat_CDC	F = 5.241 p < 0.001	F = 4.748 p < 0.001	F = 1.663 p = 0.130	F-test (ANOVA)
	n = 599	n = 300	n = 299	
→**Non-Param Test** (Kruskal-Wallis test) Waist_cat_CDC	p < 0.001	p < 0.001	p = 0.021	NP test
	n = 599	n = 300	n = 299	
Waist (Cole) Waist_score_cole	r = -0.343 p < 0.001	r = -0.458 p < 0.001	r = -0.267 p < 0.001	Correlate
	n = 599	n = 300	n = 299	
BMI	F = 5.929	F = 4.253	F = 2.894	F-test

IOTF grade	p < 0.001	p = 0.002	p = 0.014	(ANOVA)
	n = 600	n = 301	n = 299	
BMI (z-score) Bmi_score_cole	r = -0.257 p < 0.001	r = -0.328 p < 0.001	r = -0.207 p < 0.001	Correlate
	n = 600	n = 301	n = 299	
Triceps Fat_tric	r = -0.259 p < 0.001	r = -0.307 p < 0.001	r = -0.266 p < 0.001	Correlate
	n = 595	n = 300	n = 297	
Subscapular Fat_scap	r = -0.216 p < 0.001	r = -0.332 p <0.001	r = -0.188 p = 0.001	Correlate
	n = 597	n = 300	n = 297	
Sum Triceps & Subscapular SUMfat_tric_scap	r = -0.257 p < 0.001	r = -0.351 p < 0.001	r = -0.246 p < 0.001	Correlate
	n = 594	n = 299	n = 295	
Waist/hip ratio Waist_to_hip	r = 0.161 p < 0.001	r = 0.284 p < 0.001	r = 0.077 p = 0.186	Correlate
	n = 599	n = 300	n = 299	
Triceps log Triceps_log	r = -0.254 p < 0.001	r = -0.301 p < 0.001	r = -0.256 p < 0.001	Correlate
	n = 595	n = 300	n = 295	
Subscapular log Scapular_log	r = -0.257 p < 0.001	r = -0.373 p < 0.001	r = -0.209 p < 0.001	Correlate
	n = 597	n = 300	n = 297	
Sum Triceps & Subscapular log SUMfat_tric_scap_log	r = -0.273 p < 0.001	r = -0.367 p < 0.001	r = -0.250 p < 0.001	Correlate
	n = 594	n = 299	n = 295	
Waist/hip ratio log Waist_to_hip_log	r = 0.163 p < 0.001	r = 0.286 p < 0.001	r = 0.079 p = 0.173	Correlate
	n = 599	n = 300	n = 299	
Fat-free mass (lean mass) FFM	r = -0.552 p < 0.001	r = -0.633 p < 0.001	r = -0.502 p < 0.001	Correlate
	n = 599	n = 301	n = 298	
% Fat-free mass (lean mass) FFM_P	r = 0.041 p = 0.316	r = 0.073 p = 0.208	r = 0.038 p = 0.517	Correlate
	n = 599	n = 301	n = 298	

2.3. Stiffness Index

Covariates	Total group	Boys	Girls	Test
2.3.1. Birth				
Gender Sex_child	t = 1.661 p = 0.097	/	/	Indep. T-test
	n = 827	/	/	
Birth weight Birth_w	r = -0.024 p = 0.496	r = -0.017 p = 0.729	r = -0.055 p = 0.271	Correlate
	n = 827	n = 427	n = 400	
Birth length Birth_h	r = 0.003 p = 0.926	r = 0.025 p = 0.610	r = -0.040 p = 0.422	Correlate
	n = 823	n = 425	n = 398	
Birth season Season_birth	F = 1.982 p = 0.115	F = 1.171 p = 0.320	F = 2.528 p = 0.057	F-test (ANOVA)
	n = 827	n = 427	n = 400	
Weeks born too early Weeksearly	r = -0.021 p = 0.703	r = -0.15 p = 0.847	r = -0.032 p = 0.699	Correlate
	n = 328	n = 176	n = 152	
Weeks born too early (with missing values = 0) All_weeks_early	r = 0.016 p = 0.649	r = -0.25 p = 0.604	r = 0.061 p = 0.226	Correlate
	n = 827	n = 427	n = 400	
Breastfeeding Months_exclusive_breastfeeding	r = 0.029 p = 0.560	r = -0.003 p = 0.968	r = 0.074 p = 0.297	Correlate
	n = 395	n = 194	n = 201	
2.3.2. Other variables				
Age children Age	r = 0.171 p < 0.001	r = 0.187 p < 0.001	r = 0.151 p = 0.003	Correlate
	n = 827	n = 427	n = 400	
Physical activity (average count/min) Avg_cpm	r = 0.014 p = 0.749	r = 0.015 p = 0.808	r = -0.009 p = 0.889	Correlate
	n = 490	n = 257	n = 233	
Hours of sleep Sleep_hour	r = -0.122 p = 0.017	r = -0.117 p = 0.094	r = -0.126 p = 0.097	Correlate
	n = 382	n = 207	n = 175	
Calcium	r = 0.035	r = -0.007	r = 0.074	Correlate

CA_U	p = 0.435	p = 0.914	p = 0.251	
	n = 506	n = 265	n = 241	
Phosphate NANP	r = -0.010 p = 0.820	r = -0.038 p = 0.541	r = 0.003 p = 0.962	Correlate
	n = 506	n = 265	n = 241	
2.3.3. Anthropometry – body composition				
Current height (Cole) Height_score_cole	r = 0.016 p = 0.649	r = 0.015 p = 0.753	r = 0.011 p = 0.822	Correlate
	n = 827	n = 427	n = 400	
Current height height	r = 0.153 p < 0.001	r = 0.165 p = 0.001	r = 0.134 p = 0.007	Correlate
	n = 827	n = 427	n = 400	
Current height (categories) Height_cat	Levene = sign.	F = 0.349 p = 0.705	F = 0.698 p = 0.498	F-test (ANOVA)
	n = 827	n = 427	n = 400	
→**Non-Param Test** (Kruskal-Wallis test) Height_cat	p = 0.577	p = 0.719	p = 0.429	NP test
	n = 827	n = 427	n = 400	
Current weight (Cole) Weight_score_cole	r = 0.005 p = 0.879	r = 0.012 p = 0.808	r = -0.002 p = 0.971	Correlate
	n = 827	n = 427	n = 400	
Current weight weight	r = 0.111 p = 0.001	r = 0.129 p = 0.008	r = 0.092 p = 0.067	Correlate
	n = 827	n = 427	n = 400	
Waist (categories) Waist_cat_CDC	F = 0.632 p = 0.705	F = 0.523 p = 0.791	F = 0.822 p = 0.554	F-test (ANOVA)
	n = 824	n = 425	n = 399	
→**Non-Param Test** (Kruskal-Wallis test) Waist_cat_CDC	p = 0.529	p = 0.527	p = 0.724	NP test
	n = 824	n = 425	n = 399	
Waist (Cole) Waist_score_cole	r = 0.005 p = 0.879	r = -0.002 p = 0.962	r = 0.014 p = 0.782	Correlate
	n = 824	n = 425	n = 399	
BMI IOTF grade	F = 0.290 p = 0.919	F = 0.714 p = 0.582	F = 0.400 p = 0.849	F-test (ANOVA)
	n = 827	n = 427	n = 400	

BMI (z-score) Bmi_score_cole	r = -0.014 p = 0.686	r = -0.007 p = 0.892	r = -0.019 p = 0.702	Correlate
	n = 827	n = 427	n = 400	
Triceps Fat_tric	r = -0.042 p = 0.234	r = -0.049 p = 0.311	r = -0.006 p = 0.905	Correlate
	n = 820	n = 426	n = 394	
Subscapular Fat_scap	r = -0.006 p = 0.863	r = 0.002 p = 0.960	r = 0.015 p = 0.764	Correlate
	n = 819	n = 426	n = 393	
Sum Triceps & Subscapular SUMfat_tric_scap	r = -0.027 p = 0.450	r = -0.028 p = 0.570	r = 0.005 p = 0.920	Correlate
	n = 815	n = 425	n = 390	
Waist/hip ratio Waist_to_hip	r = -0.014 p = 0.694	r = -0.012 p = 0.810	r = -0.024 p = 0.630	Correlate
	n = 824	n = 425	n = 399	
Triceps log Triceps_log	r = -0.060 p = 0.084	r = -0.073 p = 0.131	r = -0.015 p = 0.764	Correlate
	n = 820	n = 426	n = 394	
Subscapular log Scapular_log	r = -0.030 p = 0.396	r = -0.015 p = 0.765	r = -0.010 p = 0.837	Correlate
	n = 819	n = 426	n = 393	
Sum Triceps & Subscapular log SUMfat_tric_scap_log	r = -0.048 p = 0.169	r = -0.048 p = 0.323	r = -0.013 p = 0.799	Correlate
	n = 815	n = 425	n = 390	
Waist/hip ratio log Waist_to_hip_log	r = -0.010 p = 0.777	r = -0.004 p = 0.930	r = -0.024 p = 0.632	Correlate
	n = 824	n = 425	n = 417	
Fat-free mass (lean mass) FFM	r = 0.164 p < 0.001	r = 0.181 p < 0.001	r = 0.128 p = 0.010	Correlate
	n = 824	n = 427	n = 397	
% Fat-free mass (lean mass) FFM_P	r = 0.094 p = 0.007	r = 0.129 p = 0.008	r = 0.057 p = 0.261	Correlate
	n = 824	n = 427	n = 397	

Appendix 2: List of possible scientific journals.

Name	Impact-factor	Scope
Bone		
Journal of bone and mineral research	7.059 → **too high**	The biology and physiology of bone, hormones that regulate bone and mineral metabolism, and the pathophysiology and treatment of disorders of bone and mineral metabolism.
Osteoporosis international	4.859 → **too high**	- The diagnosis, **prevention**, treatment and management of osteoporosis and other metabolic bone diseases. - While focusing on **clinical** research, the Journal will also accept submissions on more basic aspects of research, where they are considered by the editors to be **relevant** to the human disease spectrum.
Bone	4.601 → **too high**	- Cell and molecular biology of bone cells - Bone and bone disease; trauma and stress - Therapeutic agents; physical studies of calcium and bone - Radiology of bone; metabolism of bone matrix and mineral ; mechanisms of normal and disordered calcification C**linical studies/pathology**
Calcified Tissue International	2.759	- **Structure and function of bone** - Connective tissues and cells, ion transport, and metabolism of hormones, nutrition, mineralized tissue ultrastructure, molecular biology, and research on humans which reveal important **facets of the skeleton** or bear upon bone and mineral metabolism
Journal of bone and mineral metabolism	2.238	- Relevant issues in bone and mineral research. - The journal is aimed at researchers and clinicians dedicated to improvements in **research, development,** and **patient-care** in the fields of bone and mineral metabolism. **Clinical focus**
Metabolism – clinical and experimental	2.538	Studies in humans, animal and cellular models. Work with strong translational potential is prioritized. incl.: - Energy Expenditure and Obesity - Metabolic Syndrome and Diabetes - Nutrition, Exercise, and the Environment - Genetics, Proteomics, and Metabolomics - Carbohydrate, Lipid, and Protein Metabolism - Endocrinology and Hypertension - Mineral and Bone Metabolism - Cardiovascular Diseases and Malignancies
Journal of osteoporisis	/	Open-access
Clinical endocrinology →A number of articles from the literature review were published in this journal.	3.323	- Papers and reviews which focus on the **clinical aspects** of endocrinology, incl. the clinical application of molecular endocrinology. - Essential reading not only for those engaged in endocrinological research but also for those involved primarily in clinical practice **Apparently not only clinical studies.**

Child		
Pediatrics	5.391 → **too high**	- Original Research Articles, Clinical and Laboratory Observations (case reports), reviews of Medical Progress in pediatrics and related fields, Grand Rounds (clinicopathologic conferences [CPC] or didactic discussions), Invited Commentaries, Special Articles, Association of Medical School Pediatric Department Chairs, Inc. (AMSPDC) commentaries, Insights, Letters to the Editor, and Supplements. - **Related fields** such as nutrition, surgery, dentistry, public health, child health services, human genetics, basic sciences, psychology, psychiatry, education, sociology, and nursing.
Academic pediatrics	2.597	- To strengthen the **research and educational base** of academic general pediatrics. - Leadership in pediatric education, **research**, patient care and advocacy. Content areas include pediatric education, emergency medicine, injury, abuse, behavioral pediatrics, holistic medicine, child health services and health policy and the **environment**. - Research relating to the quality of child health care, health care policy and the organization of child health services. **Less relevant**
European journal of pediatrics	1.644	- Covering the field of **pediatrics in all its aspects** - Original papers, reviews, research letters, and letters - Subject areas: cardiology, dermatology, endocrinology, gastroenterology, **growth and development**, hematology and oncology, immunology and allergology, infectious diseases, intensive care medicine, medical genetics and metabolic diseases, neonatology, nephrology and urology, neuropediatrics, nutrition, pneumology, preventive pediatrics and epidemiology, and psychology. **Relevant**
Child: care, health and development	1.308	- **All aspects of the health and development of children** and young people (quantitative and qualitative research) - **From all disciplines** working in child health and child development. - A forum for discussion of **global child health issues** and are happy to publish both primary research and systematic reviews. We welcome submissions concerned with: - The health of children and young people - **Physical**, psychomotor, emotional and social **development** - **Social and environmental factors, including the family, affecting health and development** - Evaluation of specific interventions for children - Government policies and the organization of services **Relevant**
Journal of pediatrics and child health	1.221	- All aspects of hospital/**community paediatrics** and neonatology - Emphasis: clear, concise presentation of information of direct **clinical relevanc**e to both **hospital and**

		community-based paediatricians. - Focused primarily on the Asia Pacific region, the Journal attracts readers and contributors from more than 30 countries.
Clinical pediatrics	0.896	A peer-reviewed monthly journal, is a must read for the busy pediatrician. CLP contains state-of-the-art, accurate, concise and down-to earth information on **practical, everyday child care** topics whether they are clinical, scientific, behavioral, educational, or ethical. **Less relevant**
Pediatrics international	0.755	- Japan Pediatric Society, - Improvement of **child health delivery** for the benefit of children everywhere through facilitation of the sharing ideas, experiences and achievements. **No clear description**
Journal of Child Health Care	0.673	- Journal of Child Health Care is a professionally focused, peer reviewed journal which addresses child health issues from a **multi-disciplinary** perspective. - Critical understanding of the neonate, child and adolescent in **health and illness**. - Children = 'as a part of the community in which we live and considers health issues arising from it.'
Other		
Paediatric and Perinatal Epidemiology →An article from the literature review was published in this journal.	1.928	Crosses the boundaries between the epidemiologist and the paediatrician, obstetrician or specialist in child health, ensuring that important paediatric and perinatal studies reach those clinicians for whom the results are especially relevant. **Epidemiological aspect**