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Fetal and Childhood Growth Patterns Associated with Bone Mass in School-Age Children: The Generation R Study

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ABSTRACT

Low birth weight is associated with lower bone accrual in children and peak bone mass in adults. We assessed how different patterns of longitudinal fetal and early childhood growth influence bone properties at school age. In 5431 children participating in a population-based prospective cohort study, we measured fetal growth by ultrasound at 20 and 30 weeks gestation, and childhood growth at birth, 1, 2, 3, and 4 years of age. We analyzed these growth measurements in relation to total body (less head) BMD measured by DXA at age 6. We used conditional growth modeling; a technique which takes into account correlation between repeatedly measured growth measures. Our results showed that estimated fetal weight gain, femur length growth between 20 and 30 weeks of gestation, femur length growth between 30 weeks and birth, as well as all height and weight growth measurements from birth to 4 years of age were all positively associated with BMC, bone area (BA), and BMD (all $p < 0.01$). Fetal femur length growth between 30 weeks and birth was positively associated with BMC and BA (both $p < 0.001$), but not with BMD. Overall, childhood growth measurements exerted a larger influence on bone measures than fetal growth measures. The strongest effect estimate was observed during the first year of life. Children born small (<10th percentile) for gestational age (SGA) had lower BMC and BA, but not BMD, than children born appropriate for gestational age (AGA), whereas children born large (>90th percentile) for gestational age (LGA) had higher BMC and BA (all $p < 0.001$). These differences were no longer present in children showing subsequent accelerated and decelerated infant growth, respectively. We conclude that both fetal and childhood growth patterns are associated with bone mineral accrual, showing the strongest effect estimates in infancy. Compensatory infant growth counteracts the adverse consequences of fetal growth restriction on bone development. © 2014 American Society for Bone and Mineral Research.

KEY WORDS: FETAL GROWTH; BIRTH WEIGHT; CHILDHOOD GROWTH; BONE MINERAL DENSITY; COHORT STUDY

Introduction

Early life factors influence the development of bone health and osteoporosis during the life-course.[1] Several studies have consistently shown that low birth weight leads to lower bone accrual in children and peak bone mass acquisition in adults.[2,3] However, birth weight is an inappropriate measure of fetal growth, because different adverse fetal growth patterns may still result in the same birth weight.[4] Also, birth weight is strongly correlated with infant growth. A low or high birth weight is frequently compensated for by catch-up growth or catch-down growth during the first 2 years of life.[5] Studies assessing the effects of directly measured fetal growth in different trimesters along with early postnatal growth on bone mineral accrual in later life are scarce. Nevertheless, these studies are important to identify specific early critical periods for bone development. A previous study among 380 children suggested that fetal growth from 19 to 34 weeks of gestation affected childhood bone development at age 4 years.[6] In another study, among the same population, including 628 children, fetal as well as early postnatal growth contributed to bone development at age 4 years.[7] On the other hand, a study among 123 adolescents, found fetal growth and early postnatal growth to be a less crucial determinant of adolescent bone development than

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prepubertal growth.[8] These findings suggest that bone accrual is influenced by different critical periods, though diverse methodological challenges interfere with identifying effects across time in a conclusive manner; when growth measurements are widely separated in time, pinpointing the most influential period of growth is very difficult.[8] Furthermore, the identification of a critical period of growth on subsequent bone development is challenged by the correlation existing between repeatedly collected growth measures,[9] and the unknown influence of growth realignment following an earlier period of growth deviation.

We investigated the independent associations of repeatedly measured fetal and childhood growth characteristics and bone mineral density (BMD) measured by DXA at age 6 years in 5431 children participating in a population-based birth cohort. We applied conditional growth modeling,[10] which enables the simultaneous assessment of correlated growth measures to identify independent critical periods, to further elucidate the independent role of fetal and childhood growth on bone development.

Subjects and Methods

Study design

This study is embedded in the Generation R Study, a population-based prospective cohort study from fetal life onward in Rotterdam, the Netherlands.[11] All mothers who were resident in the study area and had an expected delivery date between April 2002 and January 2006 were eligible. The study aimed toward enrollment in the first trimester but allowed enrollment until delivery of the child. In total, 75% of all mothers enrolled before 18 weeks of gestation. Of all eligible children in the study area, 61% participated at birth in the study. The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam (MEC 198.782/2001/31), and conducted according to the guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants.

In total, 7893 children of mothers who gave consent for follow-up in the preschool phase (0 to 4 years) were eligible for this study. Of the 7696 singleton born children, growth was measured at least once in 7683 children. Of these children, 5602 visited our research center around the age of 6 years. DXA scanning was successfully performed in 5431 children (69% of the eligible population). A flowchart of included participants is shown in Fig. 1.

Fetal and childhood growth characteristics

Fetal ultrasound examinations were performed in each trimester of pregnancy. Medians (interquartile ranges [IQRs]) of these visits were 13.1 (2.4), 20.5 (1.3), and 30.4 (1.1) weeks of gestation for the first, second, and third trimester, respectively. In total, 88% of the examinations took place at either of the two research centers of the study. The remaining examinations were carried out in one of five hospitals in the vicinity under guidance of our research staff. In order to achieve optimal reproducibility all sonographers were experienced and underwent additional training according to guidelines from The Fetal Medicine Foundation.[12] Gestational age was determined at the first fetal ultrasound examination, because 39% of pregnant women had unknown or irregular last menstrual periods and because using last menstrual period for this purpose has several described limitations.[12] In the second and third trimesters, fetal head circumference (HC), abdominal circumference (AC), and femur length (FL) were measured to the nearest millimeter using standardized ultrasound procedures.[13–16] A brief description of the techniques applied is available in the Supporting Information. Fetal weight (EFW) was estimated using HC, AC, and FL in the formula from Hadlock: $\log_{10}\ \mathrm{EFW} = 1.5662 - 0.0108\ (\mathrm{HC}) + 0.0468\ (\mathrm{AC}) + 0.171\ (\mathrm{FL}) + 0.00034\ (\mathrm{HC})^2 - 0.003685\ (\mathrm{AC} * \mathrm{FL})$.[17] In a previous study within the Generation R Study, reference curves were developed based on fetal growth characteristics of the whole study population.[12] In the current study, we used these reference curves to calculate gestational age–adjusted SD scores.

Information about offspring sex, gestational age, and weight at birth was obtained from medical records and hospital registries. Very preterm birth was defined as birth occurring before 32.0 weeks of gestation, and preterm birth as birth between 32.0 and 37.0 weeks of gestation. Small for gestational age (SGA) and large for gestational age (LGA) was defined as sex- and gestational age–adjusted birth weight below the 10th percentile and above the 90th percentile, respectively. Childhood growth was routinely measured at the Community Health Centres at the median ages of 6.2 (IQR 0.4), 11.1 (IQR 0.7), 24.8 (IQR 1.6), 36.7 (IQR 1.4), and 45.8 (IQR 1.3) months following standardized protocols. Sex- and age-adjusted SD scores were calculated using Growth Analyser 3.5 (Dutch Growth Research Foundation, Rotterdam, the Netherlands; http://www.growthanalyser.org).[18,19] In accordance with earlier studies, we defined an increase or decrease in weight greater than 0.67 SD from birth to the age of 24 months as accelerated or decelerated growth, respectively.[20,21] At the age of 6 years, we measured weight in our research center using an electronic personal scale (Seca, Almere, The Netherlands) and height using a Harpenden stadiometer (Holtain Limited, Dyfed, UK) following standardized protocols.

BMD measurements

Total body BMD (g/cm^2), bone mineral content (BMC; g), and bone area (BA; cm^2) were measured at a median age of 6.0 (IQR 0.37) years using a DXA scanner (iDXA; GE Lunar, Madison, WI, USA). As described in detail earlier,[22] well-trained research assistants obtained the DXA scans using the same device and software (enCORE) following standard manufacturer protocols. In our analyses, we used areal total body less head (TBLH) BMD, BMC, and BA as recommended by the International Society for Clinical Densitometry for pediatric evaluations of bone health.[23] All measures were adjusted for skeletal size by using body height or weight as covariate in the models to correct for artifacts arising from periods of rapid growth[24]; this is needed because areal BMD measured on larger bones overestimates true (volumetric) BMD, whereas on smaller bones it can underestimate BMD across individuals.[25,26] In subsequent comparative analyses, we, in addition to the other covariates, corrected BMC for BA to further adjust for size effects.[25]

Covariates

We registered maternal age at enrollment and collected information about maternal education, marital status, parity, and country of birth, and country of birth of the father and grandparents by questionnaire at enrollment in the study. Maternal smoking and alcohol habits were assessed in each trimester. We measured parental height and weight at the research center and obtained information about maternal weight before pregnancy by questionnaire. Because the

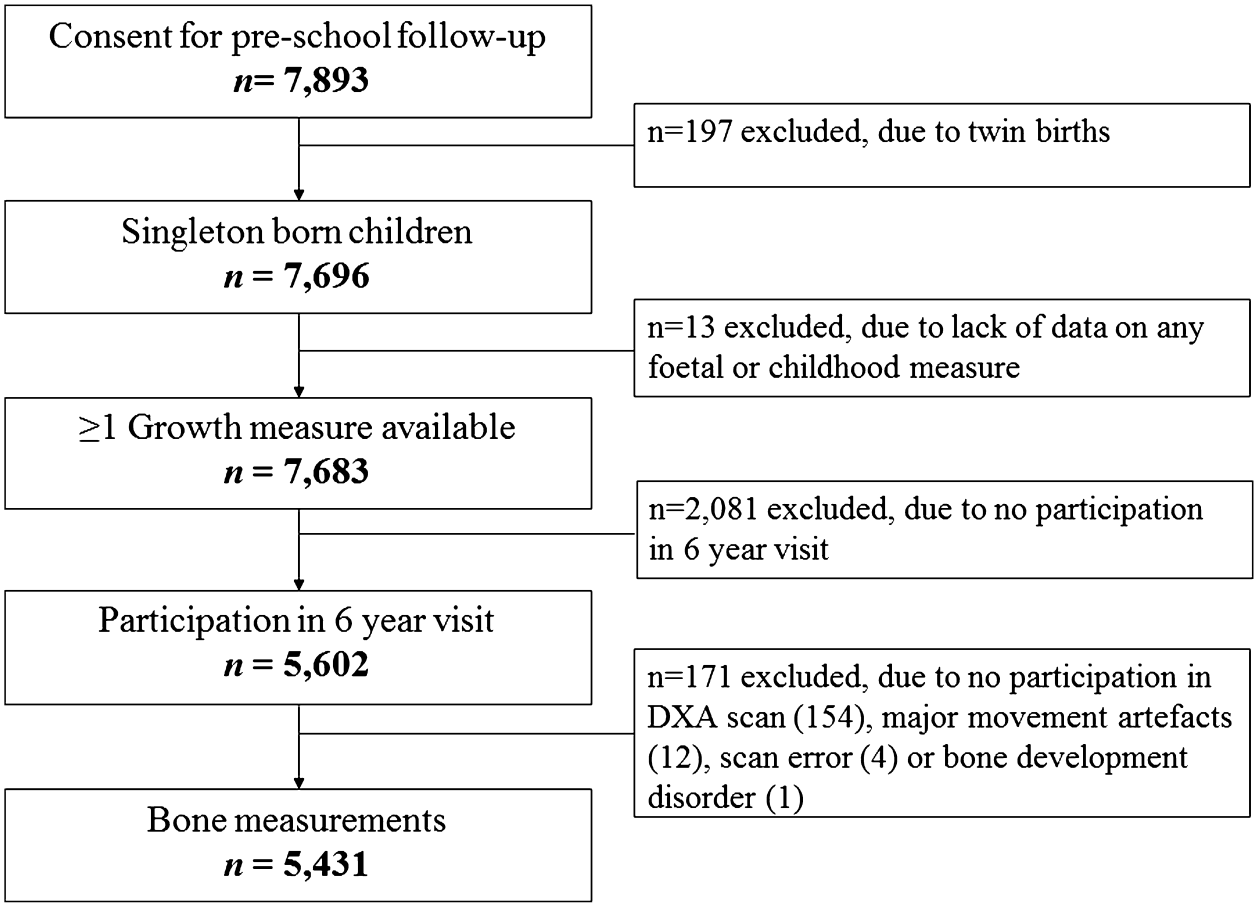

Fig. 1. Flowchart of participants included for analysis, from the Generation R Study, Rotterdam, the Netherlands.

enrollment in our study was during pregnancy, we were not able to measure maternal weight before pregnancy; however, correlation of prepregnancy weight obtained by questionnaire and weight measured at enrollment was high ($\rho = 0.95$; $p < 0.01$). We categorized ethnicity into three main groups: Western (Dutch, Turkish, other European, American, and Oceanic), African (Moroccan, other African, Antillean, Surinamese-Creole, and Cape Verdean), and Asian (Indonesian, other Asian, and Surinamese-Hindu) descent according to the three largest transcontinental ancestral groups. Information about breast feeding[27] and participation in sports was obtained from postnatal questionnaires.

Statistical analysis

We used *t* tests and chi-square tests to compare differences in subject characteristics between boys and girls. We calculated age-adjusted SD scores for all bone measures based on their distribution in the whole study population and analyzed them following four strategies. First, we performed multiple linear regression analyses to assess the individual associations of fetal and childhood growth measures with bone measures at the age of 6 years. Second, we assessed the associations of these growth measures with bone measures using conditional change modeling.[10] In conditional growth modeling, a growth measure at a specific time point is adjusted for growth predicted by prior growth measures. Accordingly, we calculated standardized residuals by regression of the growth measure of interest on prior growth measures,[10] obtaining growth measures independent of prior growth measures and statistically independent of each other across time. This approach enabled a simultaneous analysis of all growth measures with bone measures in order to identify the period of growth most critical to bone development. In an attempt to eliminate potential artifacts caused by bone size and to further distinguish potential effects on bone size from bone mineral accrual, we additionally corrected BMC for BA in a sensitivity analysis. Third, we assessed associations of birth outcomes (gestational age, birth weight, gestational age–adjusted birth weight) with bone measures at the age of 6 years. Fourth, we explored the associations of gestational age–adjusted birth weight with bone measures stratified for the postnatal growth pattern. Based on previous literature, all models were adjusted for maternal age, weight, height, parity, educational level, marital status, alcohol use, smoking, use of folic acid supplements, paternal weight and height, and child's sex, ethnicity, breastfeeding duration, and participation in sports.[28–31] Models concerning weight measures were additionally adjusted for current height, whereas models concerning height were adjusted for current soft tissue weight calculated as "lean + fat mass" (thereby excluding the contribution of bone mass to the child's weight). Because missing values add up in conditional modeling and to prevent bias associated with missing data, we

used multiple imputations (five imputations) to impute missing values in growth measures and covariates. Missing values for growth measures and covariates were imputed based on the correlation of the missing variables with other participant characteristics and other available growth measures, according to the Markov Chain Monte Carlo method.[32] The percentage of missing values for any fetal growth measure was lower than 16%, and for any childhood growth measure it was lower than 38%. Of all children, 6% did not have any data on growth from 1 to 4 years of age. Results from the complete case analyses were similar to results from the imputed analyses. We only present results for the imputed analyses. Comparing infants born SGA and not born SGA, we would be able to detect statistically significant difference in childhood BMD of 0.13 SD (type I error of 5% and a type II error of 20% [power 80%]).[33] Analyses were performed using the SPSS Predictive Analytic Software version 17.0 for Windows (PASW Inc, Chicago, IL, USA).

Results

Characteristics of study population

Subject characteristics for boys and girls separately are shown in Table 1. At 20 and 30 weeks gestation, estimated fetal weight was higher in boys than in girls (Table 2). At 30 weeks gestation femur length was longer in girls than in boys. From birth onward, boys were heavier and taller. At 6 years of age, boys had a higher BMC than girls, whereas no differences in BMD or BA were observed.

Fetal growth, childhood growth, and bone measures

The associations of individually modeled growth measures with bone parameters at age 6 are shown in Supporting Table 1. In short, all fetal length and weight measures were positively associated with BMC and BA (all $p < 0.05$), whereas all childhood growth height and weight measures were positively associated with BMC, BA, and BMD (all $p < 0.01$). The magnitude of the effect estimates increased with advancing age. When applying a conditional model, estimated fetal weight gain between 20 and 30 weeks gestation, 30 weeks and birth, as well as childhood weight gain from birth to 4 years of age were all positively associated with BMC, BA, and BMD (all $p < 0.001$) (Fig. 2). Fetal femur length growth between 20 and 30 weeks gestation and height growth from birth to 4 years were all positively associated with BMC, BA, and BMD (all $p < 0.01$). Fetal femur length growth between 30 weeks gestation and birth was positively associated with BMC and BA (both $p < 0.001$), but not with BMD. Effect estimates (in SD) for childhood height and weight growth measures were larger than for fetal growth. The largest effect estimates were found for the associations of height growth during the first year with BMC and BA, and weight gain during the first year with BMC, BA, and BMD. The size of the effect estimates decreased after the first year of age, except for the association of height growth with BMD, which peaked at 2 to 3 years of age. The corresponding effect estimates are shown in Supporting Table 2.

We further explored whether the associations of height and weight growth during the first year with bone measures were driven by growth during the first 6 months by replacing growth from birth to age 1 year by two separate measures for growth from birth to 6 months and from 6 to 12 months in our models. Height and weight growth during the first 6 months showed stronger associations with bone measures than growth from 6 to 12 months, yet effect estimates were not larger than those

Table 1. Parental, Fetal, and Child Characteristics: the Generation R Study, Rotterdam, the Netherlands

Characteristic	Boys ($n = 2718$)	Girls ($n = 2732$)	p[a]
Maternal characteristics			
Age (years)	30.9 (5.1)	30.8 (5.0)	0.38
Height (cm)	167.9 (7.2)	167.8 (7.5)	0.62
Prepregnancy weight (kg)	66.2 (12.1)	66.8 (12.5)	0.19
Prepregnancy BMI (kg/m^2)	23.4 (4.1)	23.6 (4.2)	0.13
Parity $\geq$1			
No	1466 (54)	1499 (55)	0.39
Yes	1150 (42)	1129 (42)	
Missing	106 (4)	90 (3)	
Single motherhood			
No	2192 (81)	2203 (81)	0.80
Yes	285 (11)	284 (10)	
Missing	245 (9)	231 (9)	
Educational status			
Primary	199 (7)	214 (8)	0.76
Secondary	1021 (38)	1038 (38)	
Higher	1259 (46)	1234 (45)	
Missing	243 (9)	232 (9)	
Smoking during pregnancy			
Never	1766 (65)	1797 (66)	0.08
Until pregnancy was known	199 (7)	233 (9)	
Continued	382 (14)	333 (12)	
Missing	375 (14)	355 (13)	
Alcohol use during pregnancy			
No	1200 (44)	1226 (45)	
Yes	930 (34)	952 (35)	0.23
Missing	592 (22)	1045 (36)	
Start folic acid supplement use			
Preconception	812 (30)	886 (33)	0.18
First 10 weeks	588 (22)	571 (21)	
No	439 (16)	418 (15)	
Missing	883 (32)	843 (31)	
Paternal characteristics			
Age (years)	33.4 (5.4)	33.5 (5.5)	0.44
Height (cm)	182.4 (7.9)	182.4 (7.8)	0.39
Weight (kg)	83.8 (12.8)	84.2 (12.9)	0.95
Body mass index (kg/m^2)	25.2 (3.3)	25.3 (3.4)	0.26
Child characteristics			
Gestational age at birth (weeks)	39.9 (1.7)	39.8 (1.7)	0.17
Ethnicity			
Caucasian	2026 (74)	2022 (74)	0.89
African	413 (15)	403 (15)	
Asian	146 (5)	144 (5)	
Missing	137 (5)	149 (6)	
Breast feeding			
Never	186 (7)	188 (7)	0.64
>0 to 3 months	655 (23)	656 (23)	
>3 months	1021 (38)	1055 (39)	
Missing	819 (30)	777 (29)	

(*Continued*)

Table 1. (*Continued*)

Characteristic	Boys ($n = 2718$)	Girls ($n = 2732$)	p[a]
Participation in sports at age 6 years			
Never	1291 (47)	1226 (45)	<0.001
1/week	739 (27)	976 (36)	
≥2/week	289 (11)	88 (3)	
Missing	403 (15)	428 (16)	

Values reflect the mean (SD) for continuous variables or absolute numbers (%) for categorical variables.

[a]Values of p obtained by Student's t tests for continuous variables and chi-square tests for categorical variables.

observed for growth during the first year as a whole (data not shown).

Size is a major determinant of bone mass. To demonstrate its impact on the conditional growth analysis, Supporting Table 3 shows the results from the analyses unadjusted for size. Size adjustment reduced effect sizes approximately by one third. In a second sensitivity analysis, to further distinguish an increase in bone mineral accrual from bone size, we additionally adjusted BMC models for BA. As a result, fetal femur growth measures were no longer associated with BMC. However, estimated fetal weight measures, and postnatal height and weight growth measures remained positively associated with BMC, although effect estimates were less than half the size of the effect estimates for BMC not adjusted for BA (shown in Supporting Table 4).

Birth outcomes and bone measures

Gestational age at birth showed a weak positive association with BMC (p for trend 0.05) and BA (p for trend 0.02) at 6 years of age, not with BMD (Table 3). Children born preterm had a −0.09 SD lower BMC (95% CI, −0.19, 0.00) and a −0.08 SD lower BA (95% CI, −0.18, 0.01) at school age. Birth weight showed a stronger positive association with both BMC and BA (both p for trend <0.001) and a weak positive association with BMD (p for trend 0.06). When birth weight was adjusted for gestational age at birth, it was still positively associated with BMC and BA, but not with BMD. As compared to children born AGA, children born SGA had a −0.07 SD (95% CI, −0.14, 0.00) lower BMC and a −0.11 SD (95% CI, −0.18, −0.05) lower BA, whereas children born LGA had a 0.12 SD (95% CI, 0.06, 0.18) higher BMC and a 0.16 SD (95% CI, 0.10, 0.23) higher BA.

Birth weight, infant growth, and bone measures

As compared to children born AGA with normal infant growth, children born SGA without growth realignment between 0 and 2 years of age had a −0.30 SD (95% CI, −0.42, −0.18) lower BMC, a −0.35 SD (95% CI, −0.47, −0.24) lower BA, and a −0.21 SD (95% CI, −0.36, −0.06) lower BMD at age 6 years (Fig. 3*A*–*C*). Children born LGA without growth realignment during infancy had a 0.44 SD (95% CI, 0.33, 0.55) higher BMC, a 0.44 SD (95% CI, 0.34, 0.55) higher BA, and a 0.28 SD (95% CI, 0.14, 0.41) higher BMD at age 6 years than children born AGA with normal infant growth. Children born SGA and LGA who did show growth realignment during infancy had a similar BMD, BMC, and BA to that of children born AGA with normal growth. The corresponding effect estimates are shown in Supporting Table 5.

Discussion

Main findings

In this large population-based prospective cohort study of pregnant women and their children in the Netherlands, we found that both fetal and childhood growth, as reflected by height and weight gain, were positively associated with bone accrual at school age. Childhood growth showed larger effect estimates than fetal

Table 2. Fetal Growth, Childhood Growth, and Bone Measures Until Age 6 Years: the Generation R Study, Rotterdam, the Netherlands

Period	(Gestational) Age median (IQR)	Growth characteristic	Boys n	Boys Mean (SD)	Girls n	Girls Mean (SD)	p[a]
Trimester 2	20.5 (1.3) weeks	Femur length (mm)	2292	33.4 (3.5)	2316	33.4 (3.4)	0.72
		Estimated fetal weight (g)	2281	385 (94)	2309	376 (87)	<0.001
Trimester 3	30.4 (1.1) weeks	Femur length (mm)	2368	57.3 (3.0)	2394	57.6 (3.0)	0.007
		Estimated fetal weight (g)	2364	1635 (258)	2383	1619 (260)	0.03
Birth	40.1 (1.9) weeks	Birth length (cm)	1698	50.6 (2.4)	1703	49.9 (2.2)	<0.001
		Birth weight (g)	2719	3503 (562)	2716	3374 (526)	<0.001
1 year	11.1 (0.7) months	Height (cm)	2141	75.1 (2.5)	2116	73.5 (2.5)	<0.001
		Weight (kg)	2143	10.0 (1.1)	2123	9.3 (1.0)	<0.001
2 years	24.8 (1.6) months	Height (cm)	1987	88.9 (3.4)	1984	87.7 (3.4)	<0.001
		Weight (kg)	2023	13.2 (1.5)	2006	12.7 (1.5)	<0.001
3 years	36.7 (1.4) months	Height (cm)	1884	97.9 (3.8)	1911	96.8 (3.8)	<0.001
		Weight (kg)	1908	15.5 (1.8)	1929	15.0 (1.9)	<0.001
4 years	45.8 (1.3) months	Height (cm)	1687	103.7 (4.1)	1666	102.8 (4.2)	<0.001
		Weight (kg)	1697	17.1 (2.2)	1669	16.8 (2.3)	<0.001
6 years	72.2 (4.1) months	Height (cm)	2719	119.5 (5.7)	2713	118.7 (5.7)	<0.001
		Weight (kg)	2719	23.2 (3.8)	2713	22.9 (4.1)	0.008
		BMC (total body less head) (g)[b]	2722	523 (100)	2718	519 (98)	0.01
		BA (total body less head) (cm^2)[b]	2722	942 (115)	2718	941 (109)	0.17
		BMD (total body less head) (g/cm^2)[b]	2722	0.552 (0.051)	2718	0.549 (0.052)	0.66

IQR = interquartile range; BMC = bone mineral content; BA = bone area; BMD = bone mineral density.

[a]Values of p obtained by Student's t tests for continuous variables and chi-square tests for categorical variables.

[b]Bone measures are shown for the total body less head.

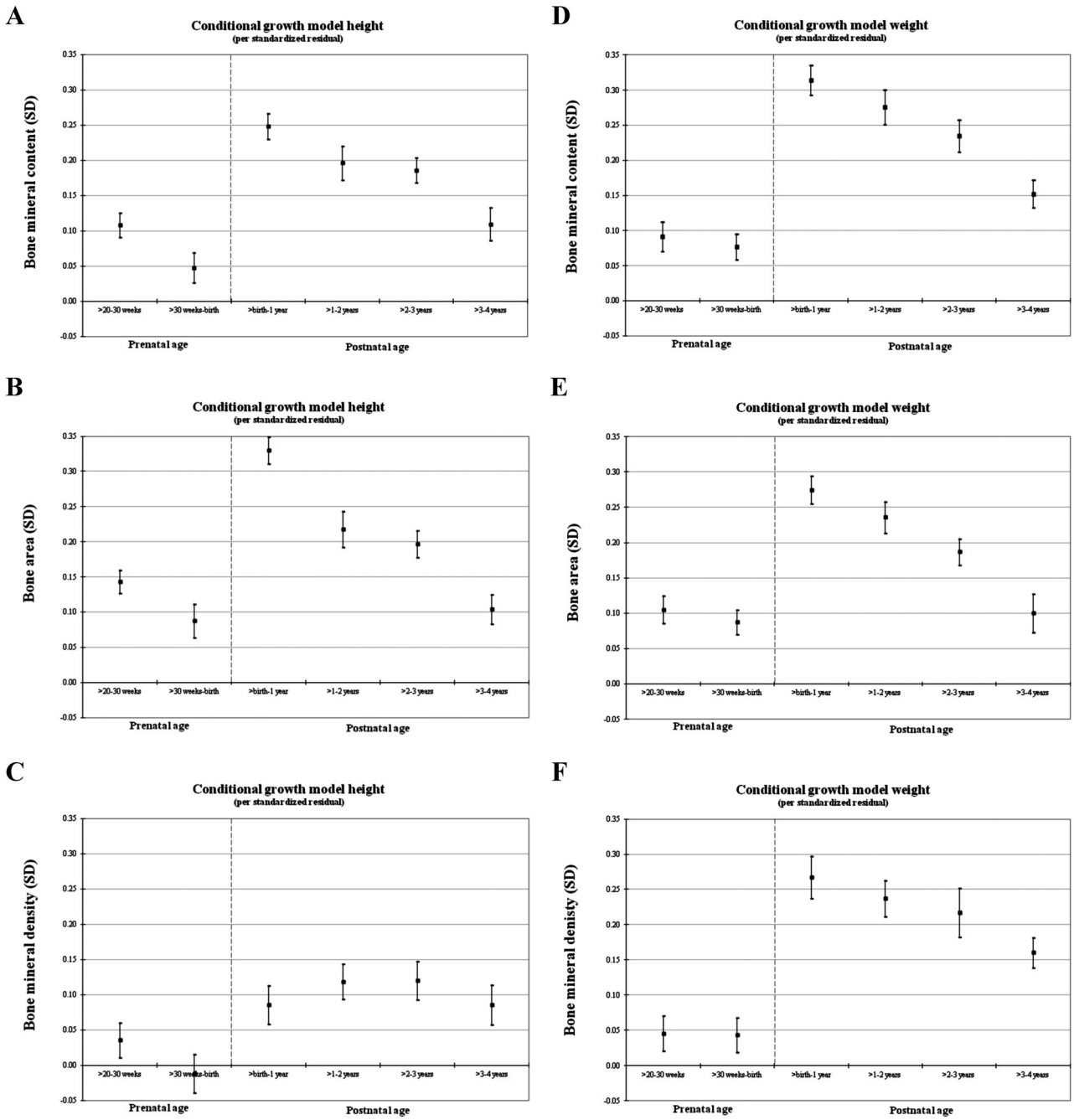

Fig. 2. (*A–F*) Associations of conditional fetal and childhood growth with bone measures at age 6 years, from the Generation R Study Cohort, Rotterdam, The Netherlands. Values are based on multiple linear regression models and reflect the coefficients and 95% CI per standardized residual of conditionally modeled growth. Conditional growth variables are independent of prior growth. Models are adjusted for maternal age, weight, height, parity, educational level, marital status, alcohol use, smoking, daily protein intake, use of folic acid supplements, paternal weight and height, and sex, ethnicity, breastfeeding duration, participation in sports, and for current height (weight models) or weight measured as "lean + fat mass" (height models) of the child and mutually for the other growth measures.

growth, whereas growth occurring in the first year of life showed the strongest positive association with bone mass accrual in later childhood. Gestational duration and birth weight were positively associated with bone parameters at 6 years of age. However, growth realignment between birth and 2 years of age in children born SGA or LGA led to similar bone measures at the age of 6 years to children born AGA who showed normal postnatal growth.

Methodological considerations

One of the major strengths of the study is that it is a large-scale, population-based, prospective cohort study rich in assessments of prenatal and early childhood growth. This unique setting of repeated measures of fetal growth, childhood anthropometrics, and bone measures enabled us to evaluate the independent

Table 3. Associations of Birth Outcomes With Bone Measures at Age 6 Years: the Generation R Study Cohort, Rotterdam, The Netherlands

		Bone mineral content (SD)		Bone area (SD)		Bone mineral density (SD)	
Fetal growth	*n*	β	95% CI	β	95% CI	β	95% CI
Gestational age adjusted for birth weight (weeks)							
<32	28	−0.09	−0.35, 0.17	−0.06	−0.31, 0.19	−0.08	−0.40, 0.23
≥32–37	225	−0.09	−0.19, 0.00	−0.08	−0.18, 0.01	−0.06	−0.18, 0.05
≥37–42 (reference)	4761	0		0		0	
≥42	380	0.01	−0.06, 0.09	0.01	−0.06, 0.08	0.01	−0.08, 0.10
p for trend		0.05		0.03		0.35	
Birth weight (g)							
<2000	64	−0.11	−0.29, 0.07	−0.06	−0.23, 0.11	−0.12	−0.34, 0.09
≥2000–2500	170	−0.10	−0.21, 0.01	−0.15	−0.26, −0.05[a]	−0.01	−0.15, 0.12
≥2500–3000	808	−0.08	−0.14, −0.02[b]	−0.07	−0.13, −0.01[b]	−0.07	−0.15, 0.00[a]
≥3000–3500 (reference)	1912	0		0		0	
≥3500–4000	1755	0.04	−0.01, 0.09	0.10	0.05, 0.14[c]	−0.03	−0.09, 0.02
≥4000–4500	602	0.11	0.04, 0.18[b]	0.15	0.08, 0.21[c]	0.05	−0.04, 0.13
≥4500	124	0.19	0.06, 0.32[b]	0.29	0.16, 0.41[c]	−0.02	−0.18, 0.14
p for trend		<0.001		<0.001		0.06	
Birth weight adjusted for gestational age (SD)							
SGA	501	−0.07	−0.14, 0.00[a]	−0.11	−0.18, −0.05[c]	−0.01	−0.09, 0.07
AGA (reference)	4311	0		0		0	
LGA	576	0.12	0.06, 0.18[c]	0.16	0.10, 0.23[c]	0.02	−0.05, 0.10
p for trend		<0.001		<0.001		0.15	

Values are based on imputed multiple linear regression models and reflect the coefficients and 95% CI for each category. Models are adjusted for maternal age, weight, height, parity, educational level, marital status, alcohol use, smoking, use of folic acid supplements, paternal weight and height, and gender, ethnicity, breastfeeding duration, participation in sports and current height of the child.

SGA = small for gestational age; AGA = appropriate for gestational age; LGA = large for gestational age.

[a] $p < 0.05$.

[b] $p < 0.01$.

[c] $p < 0.001$.

associations of early growth with bone health. We used DXA, a well-validated technique to assess bone mass accrual in children. Nevertheless, our study is not free of limitations. Of the eligible children, 69% participated in the 6-year visit at our research center. Children who did not participate grew slower during fetal life and, accordingly, had lower birth weight and length than those participating in the study. They more often showed accelerated growth in the first 2 years of life, were of non-European descent, and had mothers with a lower educational level (all $p < 0.05$). Further, because in the Netherlands it is not obligatory to attend the routine Community Health Centre visits, only 40% of the children had complete data on growth from 1 to 4 years of age and 6% did not have data from these routine visits at all. Children with incomplete data on average had a higher weight and BMC, and larger BA at the age of 6 years (all $p < 0.05$), but overall similar BMD levels. Although we used multiple imputation, we cannot exclude that missing information may still have led to loss of power or biased estimates. Nevertheless, this will only be the case if effect estimates would have differed systematically between those children included and not included in the analyses. This is unlikely, because loss to follow-up in our cohort is not expected to be related to the studied research question,[34] but cannot be fully excluded.

Establishing gestational age by ultrasound is considered superior to the use of the last menstrual period (LMP)[35] because almost 40% of pregnant women have unknown or irregular last menstrual periods.[12] However, use of first trimester ultrasounds assumes the variation in fetal growth before that ultrasound to be zero, possibly leading to underestimated effect estimates in early pregnancy. We minimized this unwanted side effect by using crown-rump length and biparietal diameter for pregnancy dating,[36,37] but not for assessing fetal growth. Yet as a result of the correlation between fetal growth measures, underestimation of effect estimates may still have occurred. Nonetheless, because we studied relative change in size within time periods by conditional modeling, we do not expect our pregnancy dating strategy to have substantially influenced our results. The validity of ultrasound estimation of fetal weight has often been debated. A systematic review assessed the validity of estimated fetal weight measurements by reviewing measurement errors across 42 studies.[38] In this review, the authors found that all methods used to estimate fetal weight, including the method of Hadlock, have insignificant systematic error. Random error, on the other hand, averaged 10%. Another limitation possibly leading to random error may be the fact that growth measures from age 1 to 4 years were acquired from routine Community Health Centres. Nevertheless, measurements within these clinics were performed using standardized protocols. Even though routinely collected measurements of this type have previously been shown to have good accuracy by lacking systematic error,[39] random error may still have been introduced. Random error may reduce power and lead to underestimation of effects. Furthermore, the 0.67 SD cut-off that we used to define "accelerated growth" has been internationally recognized to represent clinically significant catch-up growth, but does not necessarily represent, and should not be interpreted as a biological phenomenon. Last, although we collected detailed information on many potential confounding variables, residual confounding due to unmeasured sociodemographic and lifestyle factors could still be influencing the results.

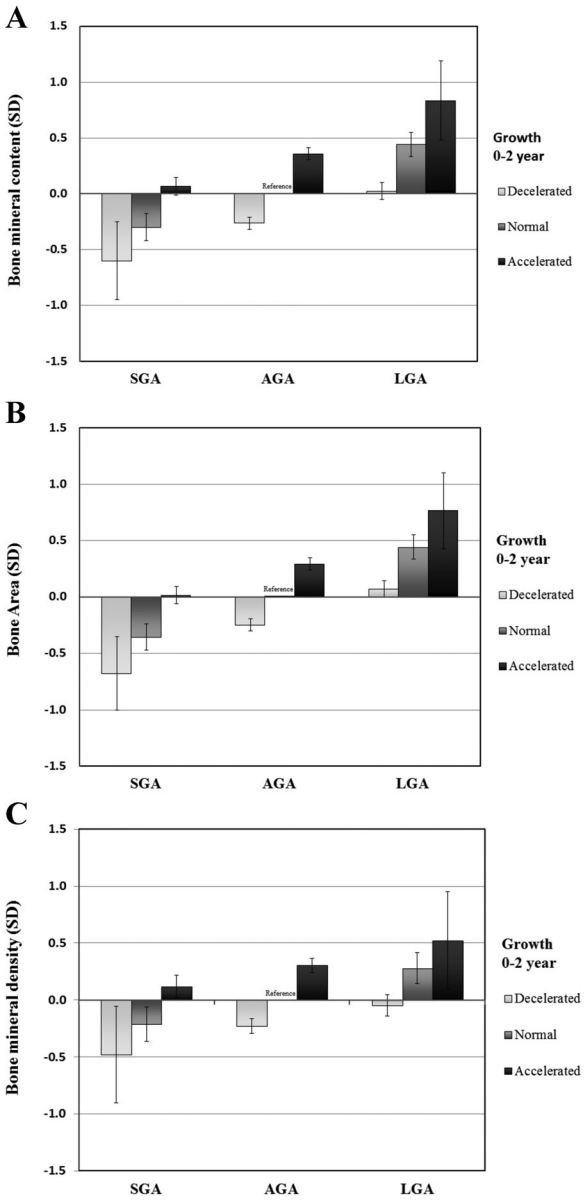

Fig. 3. (*A–C*) Associations of birth weight with bone measures at age 6 years, stratified for postnatal growth patterns, from the Generation R Study Cohort, Rotterdam, The Netherlands. Values are based on multiple linear regression models and the bars and lines reflect the coefficients and 95% CI for each category of birth weight and postnatal growth pattern. Models are adjusted for maternal age, weight, height, parity, educational level, marital status, alcohol use, smoking, use of folic acid supplements, paternal weight and height, and sex, ethnicity, breastfeeding duration, participation in sports, and current height of the child. SGA = small for gestational age; AGA = appropriate for gestational age; LGA = large for gestational age.

Interpretation of main results

Our results confirmed that early growth is associated with both bone size (BA) and bone mineral (BMC) accrual at school age. Overall, effect estimates for models including BMD were consistent in direction, yet smaller in magnitude. This attenuation of the effect on BMD is likely consequence of the influence of changes in bone area on BMD (eg, larger bone areas result in comparatively lower BMD). Further, as DXA is a two-dimensional assessment of a three-dimensional structure, the overestimation of areal BMD in larger bones compared to volumetric BMD may also, although to a lesser extent, reduce the effect estimates. However, the prominent, yet incomplete, attenuation of effect estimates by additional correction of BMC for BA supports the idea that early growth is associated with both increased bone size as well as mineral accrual. The attenuation of effect estimates by size correction seemed to be larger for height than for weight measures. Possibly, height growth, and in particular increase in fetal femur length, are more closely related to the actual skeletal frame size, whereas weight gain is only indicative of the loading effects on the skeleton during postnatal life.

Evidence supporting an influence of early growth on adult peak bone mass acquisition is increasing.[2] Recently, fast weight and height gain during childhood and adolescence were positively associated with bone strength among 1658 adults 60 to 64 years old.[40] Only a few studies have assessed the association of fetal growth with bone development by actually measuring fetal growth instead of using birth weight as a proxy for fetal growth.[6–8,41] In line with our results, Beltrand and colleagues[41] found that fetal growth restriction ($\geq$20 percentile reduction in estimated fetal weight between 22 weeks of gestation and birth) led to lower BMC in 185 newborns, independent of birth weight. Among 380 British children, fetal femur length and abdominal circumference growth during 19 to 34 weeks of gestation were positively associated with BMC and BMD at age 6 years. The effect of fetal abdominal growth was independent of current height, weight, or bone size.[6] Among 119 adolescents living in Denmark, third trimester fetal growth velocity, birth weight, and growth in the first year were positively associated with BMC.[8] However, these associations fully disappeared when adjusted for current height and weight. For that reason, the authors concluded that growth in later life, rather than early growth, may be crucial to bone health in adolescence. However, pinpointing the most influential period of growth is less precise when assessments are so widely separated in time.

Assessment of repeatedly measured growth is challenged by some methodological issues. In fact, "early size" adjusted for "later size" in regression analysis is a measure of change in size between the earlier and later measurement, rather than a measure of absolute growth.[9] To overcome these issues, only one previous study used conditional modeling[10] to study the associations of linear and abdominal growth measured at 11, 19, and 34 weeks of gestation, birth, and 1, 2, 3, and 4 years with bone measures among 628 4-year-olds.[7] The results were consistent with our observations, showing that both fetal and childhood growth are positively associated with bone development at age 4 years, whereas growth in the first 2 postnatal years contributed most strongly. These results are also in line with our results indicating that children born with a low birth weight who showed growth realignment in the first 2 years had similar bone mass to children with a normal birth weight and normal postnatal growth. Our findings highlight the importance of early growth patterns determining bone health later in childhood.

Potential underlying mechanisms

Bone growth during fetal development and postnatal life involves complex regulatory processes mediated by growth factors, cytokines, hormones, mechanical stimuli, and diverse

environmental influences. These processes are largely controlled by genetics, epigenetic regulation, and availability of nutrients and diverse exposures during fetal life, childhood, and adolescence.[42] By adaptation to environmental cues, early growth even from fetal life may already program later bone development.[43] Among others, hormones like leptin, growth hormone (GH), and cortisol have been suggested to play a prominent role in this "programming" of bone mineral accrual.[44,45] However, the exact mechanisms underlying the process remain unclear. Altered leptin levels, resulting from low or high nutrient availability, are proposed to program bone development by stimulating differentiation of mesenchymal stem cells into bone (osteoblasts) precursors, over adipogenic lineages, as well as stimulating cortical bone over trabecular bone formation.[44,46] Further, leptin levels are negatively correlated with fetal growth retardation[47] and positively with postnatal catch-up growth and neonatal BA and BMC.[48,49] Similarly, the GH/IGF-I axis has long been considered a major determinant of bone mass acquisition. The axis is negatively affected by fetal growth restriction[42] and essential to achieve catch-up growth in fetal growth–retarded infants.[50] IGF-I levels in neonates as well as in children are positively correlated with bone mass.[51,52] On the other hand, endogenous cortisol inhibits osteoblast function.[53] Serum cortisol levels are higher in infants born SGA,[54] especially in those who do not achieve catch-up growth.[55] High normal endogenous cortisol levels have been negatively associated with bone mass, predominantly in boys.[56,57] Nutritional aspects such as breastfeeding, calcium and vitamin D intake, and environmental exposures such as sunlight and physical activity may also exert an effect on these associations. Nevertheless, the fact that effect estimates remained essentially unchanged upon correction for a large number of nutritional and environmental factors does not seem to corroborate this contention.

Conclusion

Both fetal and childhood growth predict bone development at 6 years of age. Weight and height growth in the first year of life appeared to have the largest impact on bone mineral accrual. Compensatory growth in the first 2 postnatal years reduced the adverse consequences of slower growth velocity in fetal life on childhood bone mass. Because childhood bone mass tends to track into adulthood, fetal life and infancy may be critical periods to attain optimal bone health and possibly reduce the risk of osteoporosis in later life. The mechanisms underlying these findings are largely unknown and warrant further study.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: DHMH, FR, and VWVJ designed the study; VWVJ, FR, and AH conducted the study; DHMH performed data collection; DHMH performed statistical analyses; DHMH, FR, and VWVJ interpreted the data; DHMH, CMG, JCDJ, EAPS, HR, FR, AH, and VWVJ drafted and revised the manuscript. All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study. All authors read and approved the final manuscript. DHMH, the manuscript's guarantor, takes responsibility for the integrity of the data analysis.

References

1. Cooper C, Westlake S, Harvey N, Javaid K, Dennison E, Hanson M. Review: developmental origins of osteoporotic fracture. Osteoporos Int. 2006;17(3):337–47.
2. Baird J, Kurshid MA, Kim M, Harvey N, Dennison E, Cooper C. Does birthweight predict bone mass in adulthood? A systematic review and meta-analysis. Osteoporos Int. 2011;22(5):1323–34.
3. Martínez-Mesa J, Restrepo-Méndez MC, González DA, et al. Life-course evidence of birth weight effects on bone mass: systematic review and meta-analysis. Osteoporos Int. 2013 Jan;24(1):7–18.
4. Wilcox AJ. Intrauterine growth retardation: beyond birthweight criteria. Early Hum Dev. 1983;8(3–4):189–93.
5. Taal HR, Vd Heijden AJ, Steegers EA, Hofman A, Jaddoe VW. Small and large size for gestational age at birth, infant growth, and childhood overweight. Obesity (Silver Spring). 2013;21(6):1261–8.
6. Harvey N, Mahon P, Robinson S, et al. SWS Study Group. Different indices of fetal growth predict bone size and volumetric density at 4 years old. J Bone Miner Res. 2010 Apr;25(4):920–7.
7. Harvey NC, Mahon PA, Kim M, et al. Intrauterine growth and postnatal skeletal development: findings from the Southampton Women's Survey. Paediatr Perinat Epidemiol. 2012;26(1):34–44.
8. Jensen RB, Vielwerth S, Frystyk J, et al. Fetal growth velocity, size in early life and adolescence, and prediction of bone mass: association to the GH-IGF axis. J Bone Miner Res. 2008;23(3):439–46.
9. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ. 1999;319(7204):245–9.
10. Keijzer-Veen MG, Euser AM, van Montfoort N, Dekker FW, Vandenbroucke JP, Van Houwelingen HC. A regression model with unexplained residuals was preferred in the analysis of the fetal origins of adult diseases hypothesis. J Clin Epidemiol. 2005;58(12):1320–4.
11. Jaddoe VW, van Duijn CM, Franco OH, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739–56.
12. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388–96.
13. Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol. 1975;82(9):702–10.
14. Hadlock FP, Harrist RB, Deter RL, Park SK. Fetal femur length as a predictor of menstrual age: sonographically measured. AJR Am J Roentgenol. 1982;138(5):875–8.
15. Hadlock FP, Deter RL, Harrist RB, Park SK. Fetal abdominal circumference as a predictor of menstrual age. AJR Am J Roentgenol. 1982;139(2):367–70.

16. Shepard M, Filly RA. A standardized plane for biparietal diameter measurement. J Ultrasound Med. 1982;1(4):145–50.
17. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol. 1985;151(3):333–7.
18. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). Acta Paediatr Scand. 1991;80(8–9):756–62.
19. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955–1997. Pediatr Res. 2000;47(3):316–23.
20. Heppe DH, Kiefte-de Jong JC, Durmus B, et al. Parental, fetal, and infant risk factors for preschool overweight: the Generation R Study. Pediatr Res. 2013;73(1):120–7.
21. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967–71.
22. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr. 2013;98(1):224–32.
23. Lewiecki EM, Gordon CM, Baim S, et al. Special report on the 2007 adult and pediatric Position Development Conferences of the International Society for Clinical Densitometry. Osteoporos Int. 2008;19(10):1369–78.
24. Heaney RP. Bone mineral content, not bone mineral density, is the correct bone measure for growth studies. Am J Clin Nutr. 2003; 78(2):350–1; author reply 351–2.
25. Prentice A, Parsons TJ, Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. Am J Clin Nutr. 1994; 60(6):837–42.
26. Warner JT, Cowan FJ, Dunstan FD, Evans WD, Webb DK, Gregory JW. Measured and predicted bone mineral content in healthy boys and girls aged 6–18 years: adjustment for body size and puberty. Acta Paediatr. 1998;87(3):244–9.
27. Durmus B, van Rossem L, Duijts L, et al. Breast-feeding and growth in children until the age of 3 years: the Generation R Study. Br J Nutr. 2011;105(11):1704–11.
28. Cooper C, Harvey N, Cole Z, Hanson M, Dennison E. Developmental origins of osteoporosis: the role of maternal nutrition. Adv Exp Med Biol. 2009;646:31–9.
29. Cooper C, Cawley M, Bhalla A, et al. Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res. 1995; 10(6):940–7.
30. Rudang R, Mellstrom D, Clark E, Ohlsson C, Lorentzon M. Advancing maternal age is associated with lower bone mineral density in young adult male offspring. Osteoporos Int. 2012;23(2):475–82.
31. Harvey NC, Javaid MK, Poole JR, et al. Paternal skeletal size predicts intrauterine bone mineral accrual. J Clin Endocrinol Metab. 2008; 93(5):1676–81.
32. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological, clinical research: potential, pitfalls. BMJ. 2009;338:b2393.
33. Lenth RV. Java Applets for Power and Sample Size [Computer software]. Retrieved 2014 May 15. Available from: http://www.stat.uiowa.edu/~rlenth/Power.
34. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? Epidemiology. 2006;17(4):413–8.
35. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol. 1996; 8(3):178–85.
36. Altman DG, Chitty LS. New charts for ultrasound dating of pregnancy. Ultrasound Obstet Gynecol. 1997;10(3):174–91.
37. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol. 1979;86(7):525–8.
38. Dudley NJ. A systematic review of the ultrasound estimation of fetal weight. Ultrasound Obstet Gynecol. 2005;25(1):80–9.
39. Howe LD, Tilling K, Lawlor DA. Accuracy of height and weight data from child health records. Arch Dis Child. 2009;94(12):950–4.
40. Kuh D, Wills AK, Shah I, et al. Growth from birth to adulthood and bone phenotype in early old age: a British birth cohort study. J Bone Miner Res. 2014;29(1):123–33.
41. Beltrand J, Alison M, Nicolescu R, et al. Bone mineral content at birth is determined both by birth weight and fetal growth pattern. Pediatr Res. 2008;64(1):86–90.
42. Setia S, Sridhar MG. Changes in GH/IGF-1 axis in intrauterine growth retardation: consequences of fetal programming? Horm Metab Res. 2009;41(11):791–8.
43. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61–73.
44. Devlin MJ, Bouxsein ML. Influence of pre- and peri-natal nutrition on skeletal acquisition and maintenance. Bone. 2012;50(2):444–51.
45. Goodfellow LR, Cooper C, Harvey NC. Regulation of placental calcium transport and offspring bone health. Front Endocrinol (Lausanne). 2011;2:3.
46. Wong IP, Nguyen AD, Khor EC, et al. Neuropeptide Y is a critical modulator of leptin's regulation of cortical bone. J Bone Miner Res. 2013 Apr;28(4):886–98.
47. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab. 1998;83(4):1243–6.
48. Javaid MK, Godfrey KM, Taylor P, et al. Umbilical cord leptin predicts neonatal bone mass. Calcif Tissue Int. 2005;76(5):341–7.
49. Jaquet D, Leger J, Tabone MD, Czernichow P, Levy-Marchal C. High serum leptin concentrations during catch-up growth of children born with intrauterine growth retardation. J Clin Endocrinol Metab. 1999;84(6):1949–53.
50. Cianfarani S, Ladaki C, Geremia C. Hormonal regulation of postnatal growth in children born small for gestational age. Hormone Res. 2006;65(Suppl 3):70–4.
51. Javaid MK, Godfrey KM, Taylor P, et al. Umbilical venous IGF-1 concentration, neonatal bone mass, and body composition. J Bone Miner Res. 2004;19(1):56–63.
52. van Coeverden SC, Netelenbos JC, de Ridder CM, Roos JC, Popp-Snijders C, Delemarre-van de Waal HA. Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin Endocrinol (Oxf). 2002;57(1):107–16.
53. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr. 2007;97(6):1064–73.
54. Verkauskiene R, Jaquet D, Deghmoun S, Chevenne D, Czernichow P, Levy-Marchal C. Smallness for gestational age is associated with persistent change in insulin-like growth factor I (IGF-I) and the ratio of IGF-I/IGF-binding protein-3 in adulthood. J Clin Endocrinol Metab. 2005;90(10):5672–6.
55. Cianfarani S, Geremia C, Scott CD, Germani D. Growth, IGF system, and cortisol in children with intrauterine growth retardation: is catch-up growth affected by reprogramming of the hypothalamic-pituitary-adrenal axis? Pediatr Res. 2002;51(1):94–9.
56. Remer T, Boye KR, Hartmann MF, et al. Adrenal steroid hormones and metaphyseal bone in children. Hormone Res. 2004;62(5):221–6.
57. Remer T, Boye KR, Hartmann M, et al. Adrenarche and bone modeling and remodeling at the proximal radius: weak androgens make stronger cortical bone in healthy children. J Bone Miner Res. 2003;18(8):1539–46.