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Early childhood as a sensitive period for the effect of growth on childhood bone mass: Evidence from Generation XXI birth cohort

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ABSTRACT

Background: To identify sensitive periods for the effect of early life growth on childhood bone mass we compared the associations between weight and length/height velocities from birth to age six and bone mineral content (BMC) and areal density (aBMD) at 7 years of age.

Methods: We analyzed data from 1853 participants from the Generation XXI birth cohort scanned with a whole body dual-energy X-ray absorptiometry system. Velocities of growth in weight and length/height were obtained through linear spline multilevel models on the basis of data collected during routine health examinations. Using linear regression we computed associations of birth weight, birth length, five weight velocities ("early neonatal": 0–10 days, "early infancy": 10 days–3 months, "late infancy": 3–12 months, "early childhood": 1–3 years, and "later childhood": 3–6 years) and four length/height velocities ("early infancy": 0–3 months, "late infancy": 3–12 months, "early childhood": 1–3 years, and "later childhood": 3–6 years) with outcomes BMC, aBMD, height and height-adjusted BMC at age seven. Confounding by maternal and child characteristics was addressed and effects of growth velocities were adjusted to preceding growth.

Results: Weight and length/height velocities up to the age of six were associated with increased bone mass, areal density and height at 7 years with the strongest associations observed for growth in early childhood. In this age period, after concurrent height and confounder adjustment, one standard deviation (SD) increase in weight velocity was associated with higher BMC z-scores: 0.27 (95%CI: 0.22, 0.32) in girls and 0.24 (95%CI: 0.19, 0.29) in boys. Height velocity was also associated with greater height-adjusted BMC z-score: 0.12 (95%CI: 0.07, 0.17) per SD in girls and 0.11 (95%CI: 0.06, 0.16) in boys. The pattern of associations was similar, albeit attenuated, after adjusting for preceding growth.

Conclusion: Growth in second and third years of life may represent a sensitive period for the effect of growth on childhood bone mass, partly through their effect on concurrent body size.

1. Introduction

Body size, as measured by height and weight, is amongst the strongest determinants of bone mineral properties throughout life, largely reflecting skeletal adaptation to loading, as well as the close constitutional relation between anthropometrics and body composition [1,2]. Beyond its short-term mechanical effect, there is growing evidence that the velocity of growth in height and weight during infancy and childhood is a probable determinant of later bone mass and density [3–5]. In addition, our previous work has shown that the overall trajectory of weight gain from birth is likely to contribute to cumulative bone mass accrued before puberty, since children in above-average weight gain trajectories had higher bone mineral content and areal density at age seven than those who gained less weight, with the strongest associations for children with persistent weight gain since birth [6]. Nevertheless, from an etiologic perspective, there is an increasing interest in the identification of sensitive periods, during which programmed phenotypes such as bone mass may be particularly responsive to growth velocity [7,8]. This may provide insights into the timing in childhood when targeted interventions to promote bone health are most promising.

Previous studies of two interval cohorts that examined the effect of

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specific growth periods on childhood bone mass have found that growth in the first three years of life has the strongest associations with bone properties, with the magnitude of associations decreasing thereafter [9–12]. Despite adding valuable evidence, interval cohorts are by definition examined as part of periodic follow-up assessments whose timing is defined a priori by researchers and whose frequency is necessarily lower than in the context of health care provision. Height and weight records obtained as part of routine health care are a valuable source of information collected at short time intervals that may be used to complement interval cohort data in order to refine the identification of meaningful periods of growth [13]. Specifically, we believe that examining growth velocity during time periods defined empirically on the basis of a large amount of observed data may improve precision in the identification of sensitive periods for the effect of height and weight changes on bone mass measured using dual-energy X-ray absorptiometry (DXA).

Therefore, our objective was to compare, across different age periods defined on the basis of empirical growth data, the associations of height and weight velocities with DXA-derived bone measures at 7 years of age in a large birth cohort.

2. Materials and methods

2.1. The Generation XXI cohort

All pregnant women delivering live-born infants between April 2005 and August 2006 at each of the five level III maternity units covering the metropolitan area of Porto were invited to participate. Overall, 8647 infants with gestational age above 23 weeks and their mothers were enrolled (91.4% participation). These children were followed up at ages 4, 7 and 10 years. The 13 years evaluation is ongoing as of May 2019. At ages four and seven, 7459 (86.3%) and 6889 (79.7%) children, respectively, had follow-up data. The present study is based on a subsample of 1853 of those children, whose selection criteria and comparison with the remaining cohort participants are described below. The Ethics Committee of Hospital de São João approved the study protocol. The study complies with the Ethical Principles expressed in the Helsinki Declaration and with the national legislation and is registered with the Portuguese Authority for Data Protection. In all evaluations, participants were informed about the potential discomfort caused by participation and only children providing oral assent and whose legal guardians provided written informed consent were considered participants. Detailed descriptions of the Generation XXI cohort have been published elsewhere [14,15].

2.2. Growth measures

Birth weight and length were obtained from clinical records held at the maternity units and subsequent weight and length/height measurements were abstracted from the National Health Service official children's health books. At the 4 years follow-up evaluation, legal guardians were asked to provide, whenever possible, all measures of the child's weight and length/height from books, comprising data from birth up to 70 months of age, which allowed to model growth up to the age of 6 years. Average weight and length/height growth trajectories were then estimated with linear spline multilevel models. Full details on the modelling of growth trajectories have been described elsewhere [13]. Briefly, the best-fitting splines for length/height and weight, in both girls and boys, had knots at 3 months, 12 months and 3 years, with an extra knot at 10 days for weight. These models were the ones that best fitted observed data and provided interpretable summaries of the pattern of growth throughout childhood. Thus, length/height growth was modelled as four linear splines while weight growth was modelled as five linear splines, characterized by different linear growth rates that are initially higher and then decrease with age (Table 1, from reference [13]). From these models, we obtained individual estimates of weight velocity for five age periods ("early neonatal": 0 to 10 days, "early infancy": 10 days to 3 months, "late infancy": 3 to 12 months, "early childhood": 1 to 3 years, and "later childhood": 3 to 6 years) and length/height velocity for four age periods ("early infancy": 0 to 3 months, "late infancy": 3 to 12 months, "early childhood": 1 to 3 years, and "later childhood": 3 to 6 years).

Table 1
Estimated birth weight, length and growth velocities from birth to 6 years predicted by linear spline multilevel models for 5282 children in Generation XXI (Howe LD, et al. (2016) Stat Methods Med Res 25: 1854–1874 [13]).

	Girls	Boys	Girls	Boys
	Weight (kg)		Length (cm)	
Birth	3.11 (0.40)	3.22 (0.43)	48.33 (1.87)	48.97 (1.94)
	Weight velocities (kg/month)		Length/height velocities (cm/month)	
Early neonatal	0.42 (0.64)	0.52 (0.70)	3.79 (0.22)	4.12 (0.22)
Early infancy	0.87 (0.16)	0.99 (0.17)		
Late infancy	0.40 (0.08)	0.41 (0.09)	1.70 (0.17)	1.70 (0.18)
Early childhood	0.22 (0.05)	0.22 (0.05)	0.90 (0.07)	0.88 (0.07)
Later childhood	0.19 (0.05)	0.18 (0.05)	0.53 (0.02)	0.51 (0.02)

Values represent mean (standard deviation) of birth weight and length, weight velocities and length/height velocities.
Girls (n = 2611); Boys (n = 2671).
Weight velocities for early neonatal (0–10 days), early infancy (10 days–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years) periods. Length/height velocities for early infancy (0–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years) periods.

At 7 years of age, height and weight were updated by trained examiners according to standard procedures. Height was measured with a wall stadiometer to the nearest 0.1 cm and weight was measured with a digital scale to the nearest 0.1 kg, while the child was barefoot and in light clothing.

2.3. DXA-derived bone measures

At the 7 years follow-up, a subsample of children consecutively attending the evaluation (43.8% of the participants) were invited to undergo a whole body DXA. Selection was chronologic and solely related with equipment availability. Scans were performed using a Hologic Discovery QDR® 4500 W device (Hologic Inc., Bedford, Massachusetts, USA), software version 13.3.0.1 according to standard manufacturer protocols while children were barefoot in light indoor clothing and without metal accessories. We obtained total body less head bone mineral content (g) (BMC) and areal bone mineral density (g/cm^2) (aBMD) [16]. Two trained operators checked all scans independently and excluded those showing unacceptable artefacts.

2.4. Potential confounders

Maternal and child factors which have been previously associated with early growth and skeletal development through genetic or environmental effects (maternal pre-pregnancy body mass index (BMI), smoking status during pregnancy, age and educational level at childbirth, and child gestational age) were reported in the baseline questionnaire completed within 72 h after delivery, and complemented with information retrieved from birth records.

2.5. Statistical analysis

Analyses presented in this paper were restricted to the 1853 (48.0% girls) participants included in the growth analysis, who had a valid DXA scan and with complete data on all potential confounders. Specifically, of the 7459 participants who attended the 4-year-old follow-up evaluation, 2077 did not provide growth data from health records and 100

were excluded because they were twins, whose birth weight and subsequent growth rates differ considerably from those of singletons. We also excluded 732 participants who did not attend the 7 years evaluation, 2259 who were not invited for bone densitometry on the basis of equipment availability, 377 who refused to perform the DXA scan, 11 whose images had unacceptable technical quality and 50 who did not have complete data for all potential confounders.

Birth weight and birth length, as well as the five weight velocities and the four length/height velocities predicted by the linear spline model, were standardized (z-scored) by sex. Age- and sex-specific z-scores for BMC and height based on the means and standard deviations (SD) derived from the study sample (n = 1853) were computed. Race was not collected or taken into account in the calculation of any of the z-scores since the Portuguese Constitution explicitly forbids the collection of statistical data on race, ethnicity or skin color. In terms of potential impact, immigration is comparatively infrequent in the region of Porto compared to other Western European settings or the USA: only 4.5% of Generation XXI mothers are first-generation migrants, of whom 3.5% from non-European countries [17].

DXA-derived measures of bone mass are strongly influenced by body size, which in turn is a result of overall growth trajectories up to the point of measurement. To address this, we selected the following four outcomes, all measured at age seven: DXA-derived BMC and aBMD, height, and height-adjusted BMC (through standard multivariate regression adjustment), the latter being an approach to estimate of skeletal growth relative to height up to that point, more closely related to cumulative bone growth than to the physical properties of bone tissue.

Linear regression models were used to quantify associations between each of the exposures (birth weight, birth length, early neonatal weight velocity, early infancy weight/length velocity, late infancy weight/length velocity, early childhood weight/height velocity and later childhood weight/height velocity) and each of the outcomes measured at age 7: BMC, aBMD, height, and height-adjusted BMC. Regression coefficients represent the change in each standardized outcome per one standard deviation increase in birth weight/length or in weight or length/height velocity in each age period.

We examined the effects of growth on bone outcomes with 4 models. Model 1 represents the crude analysis. Model 2 was adjusted for potential confounders (maternal age, educational level, pre-pregnancy body mass index, smoking during pregnancy and child gestational age). Model 3 was similar to model 2 with further adjustment for earlier growth, i.e. each weight or height velocity was adjusted for birth weight or height, respectively, plus all of the preceding velocities. For weight growth, a model 4 with additional adjustment for birth length, preceding length/height velocities, and length/height velocity over the same age period was fitted to obtain estimates for body weight after accounting for statural growth. Due to the well-documented sex differences in growth and bone accrual since early stages [18,19], all analyses were stratified by sex. This was an a priori decision, even though when we formally tested for interaction by including cross-product terms with each weight and length/height velocity and sex in the regression models, we found no evidence of statistical interaction.

Sensitivity analyses were conducted to test the robustness of our findings. Owing to possible differences in growth profile, analyses excluding preterm children and children born to women who gained excessive weight during pregnancy, one group at a time, were carried out.

Statistical analyses were carried out with the use of Stata software, version 11.2 for Windows (Stata Corp. LP, College Station, Texas, USA).

3. Results

Characteristics of the 1853 children and their mothers included in our study are summarized in Table 2. Compared with the remaining cohort participants, children included in our study were similar with regard to maternal prepregnancy BMI, gestational age, birth weight and birth length. Those included were more likely to have older mothers (mean 30.2 years vs 28.6 years) with higher education (32% vs 22%) and employed (79% vs 69%) and less likely to report a monthly household income lower than 1000€ (31% vs 38%). As shown in Table 2, using as reference all Generation XXI participants eligible for growth analysis, mean z-scores for length and weight velocities in the sample included are very close to zero, suggesting that the sample was on average very similar to the remaining participants at each age period. There were no differences between girls and boys in maternal characteristics, gestational age at birth and weight at 7 years. However, when compared to boys, girls were slightly lighter and shorter at birth,

Table 2
Maternal and child characteristics: Generation XXI, Porto, Portugal.

	Girls (n = 889)	Boys (n = 964)	p[a]
Maternal characteristics			
Age at childbirth (years), mean (SD)	30.4 (5.1)	30.1 (5.0)	0.135
Educational level (years), n (%)			
≤9	364 (40.9)	371 (38.5)	0.425
10–12	239 (26.9)	283 (29.4)	
> 12	286 (32.2)	310 (32.2)	
Prepregnancy BMI (kg/m^2), mean (SD)	24.6 (4.7)	24.3 (4.1)	0.186
Ever smoked during pregnancy, n (%)	186 (20.9)	188 (19.5)	0.447
Child characteristics			
Gestational age at birth (weeks), mean (SD)	38.8 (1.6)	38.7 (1.6)	0.232
Preterm birth, n (%)	51 (5.7)	67 (7.0)	0.285
Birth weight, mean (SD)			
Crude (g)	3146.6 (471.6)	3242.4 (480.9)	< 0.001
Z-score, WHO reference[b]	−0.24 (1.09)	−0.27 (1.07)	0.560
Birth length, mean (SD)			
Crude (cm)	48.4 (2.0)	49.1 (2.3)	< 0.001
Z-score, WHO reference[b]	−0.38 (1.09)	−0.40 (1.22)	0.647
Weight velocity (Z-score[c]), mean (SD)			
Early neonatal	0.04 (0.98)	0.00 (1.05)	0.437
Early infancy	−0.01 (0.98)	0.01 (0.98)	0.745
Late infancy	0.03 (0.96)	−0.02 (0.97)	0.228
Early childhood	−0.01 (1.03)	−0.06 (0.92)	0.327
Later childhood	−0.03 (1.03)	−0.07 (0.92)	0.385
Length/height velocity (Z-score[c]), mean (SD)			
Early infancy	0.03 (0.98)	0.07 (1.02)	0.441
Late infancy	−0.04 (1.02)	−0.00 (0.99)	0.411
Early childhood	−0.06 (1.03)	−0.01 (0.97)	0.219
Later childhood	0.03 (1.07)	0.02 (1.07)	0.831
Weight at 7 years, mean (SD)			
Crude (kg)	27.3 (5.9)	27.0 (5.1)	0.272
Z-score, WHO reference[b]	0.76 (1.13)	0.70 (1.16)	0.220
Height at 7 years, mean (SD)			
Crude (cm)	124.1 (5.5)	125.2 (5.6)	< 0.001
Z-score, WHO reference[b]	0.18 (0.91)	0.20 (0.98)	0.634
DXA-derived BMC at 7 years (g), mean (SD)	591.6 (85.4)	600.8 (86.4)	0.021
DXA-derived aBMD at 7 years (g/cm^2), mean (SD)	0.612 (0.057)	0.624 (0.053)	< 0.001

Age intervals in weight growth: early neonatal (0–10 days), early infancy (10 days–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years). Age intervals in length/height growth: early infancy (0–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years).
SD, standard deviation; BMI, body mass index; BMC, bone mineral content; aBMD, areal bone mineral density; DXA, dual-energy X-ray absorptiometry.
[a] Maternal and child characteristics were compared between girls and boys with two-sample Student's *t* or chi-squared tests.
[b] Age and sex-specific Z-scores computed using WHO reference data [18].
[c] Sex-specific Z-scores of length/height and weight velocities predicted by the linear spline multilevel models computed based on the means and standard deviations derived from 5282 children from Generation XXI [13].

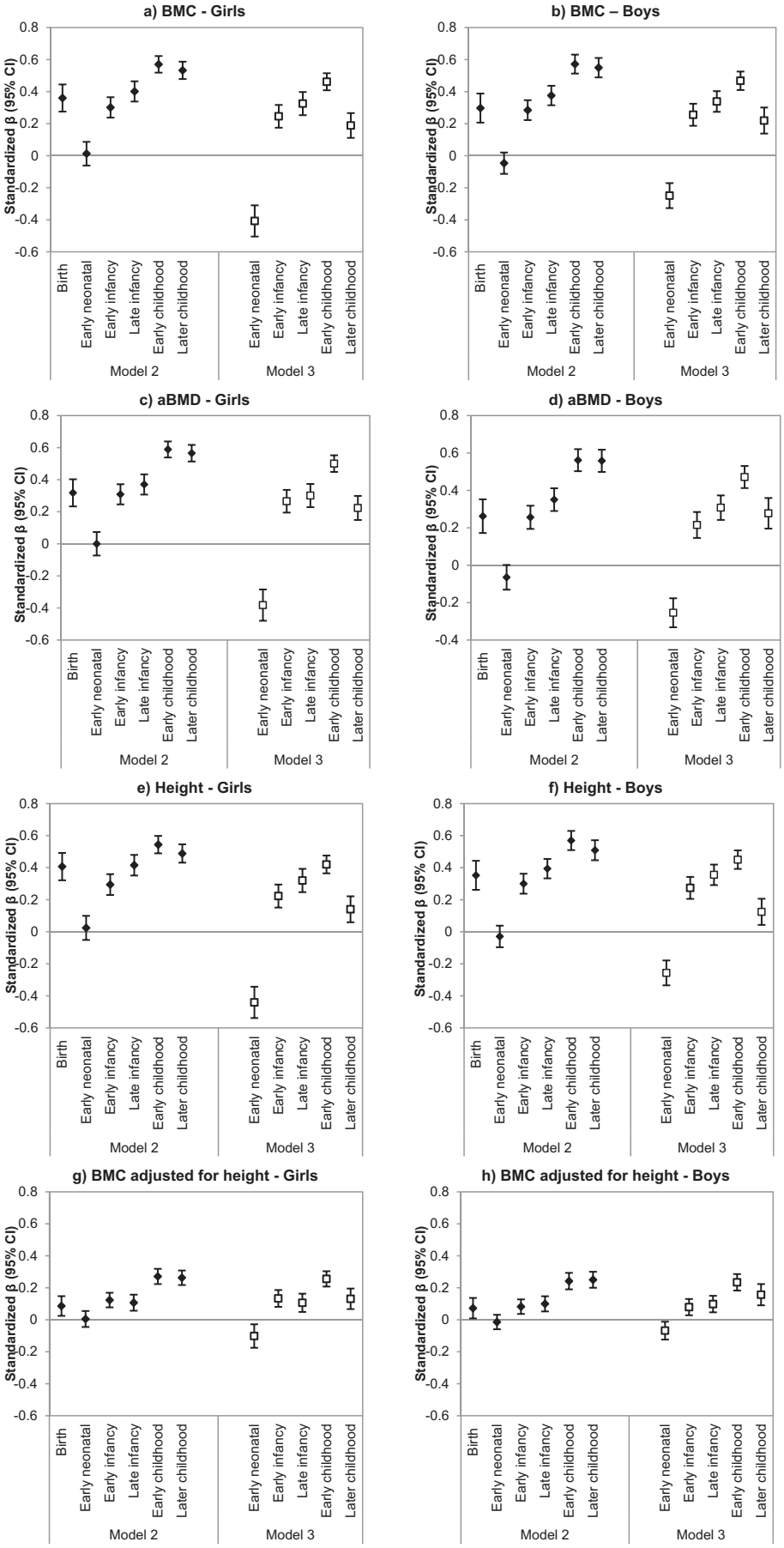

Fig. 1. Adjusted linear regression coefficients (95% CIs) for the associations between weight velocities and bone content, bone areal density and height at 7 years of age.

Legend: Regression coefficients (95% CIs) that represent the change in standardized BMC (a and b), aBMD (c and b), height (e and f) and BMC adjusted for height (g and h) per 1 standard deviation increase in birth weight or in weight velocity in each age period, in girls (left) and boys (right).

Diamond: estimates adjusted for maternal age, educational level, pre-pregnancy body mass index, smoking during pregnancy and child gestational age (model 2). Square: estimates additionally adjusted for birth weight and all of the preceding weight velocities (model 3).

Age intervals: early neonatal (0–10 days), early infancy (10 days–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years).

BMC, bone mineral content; aBMD, areal bone mineral density.

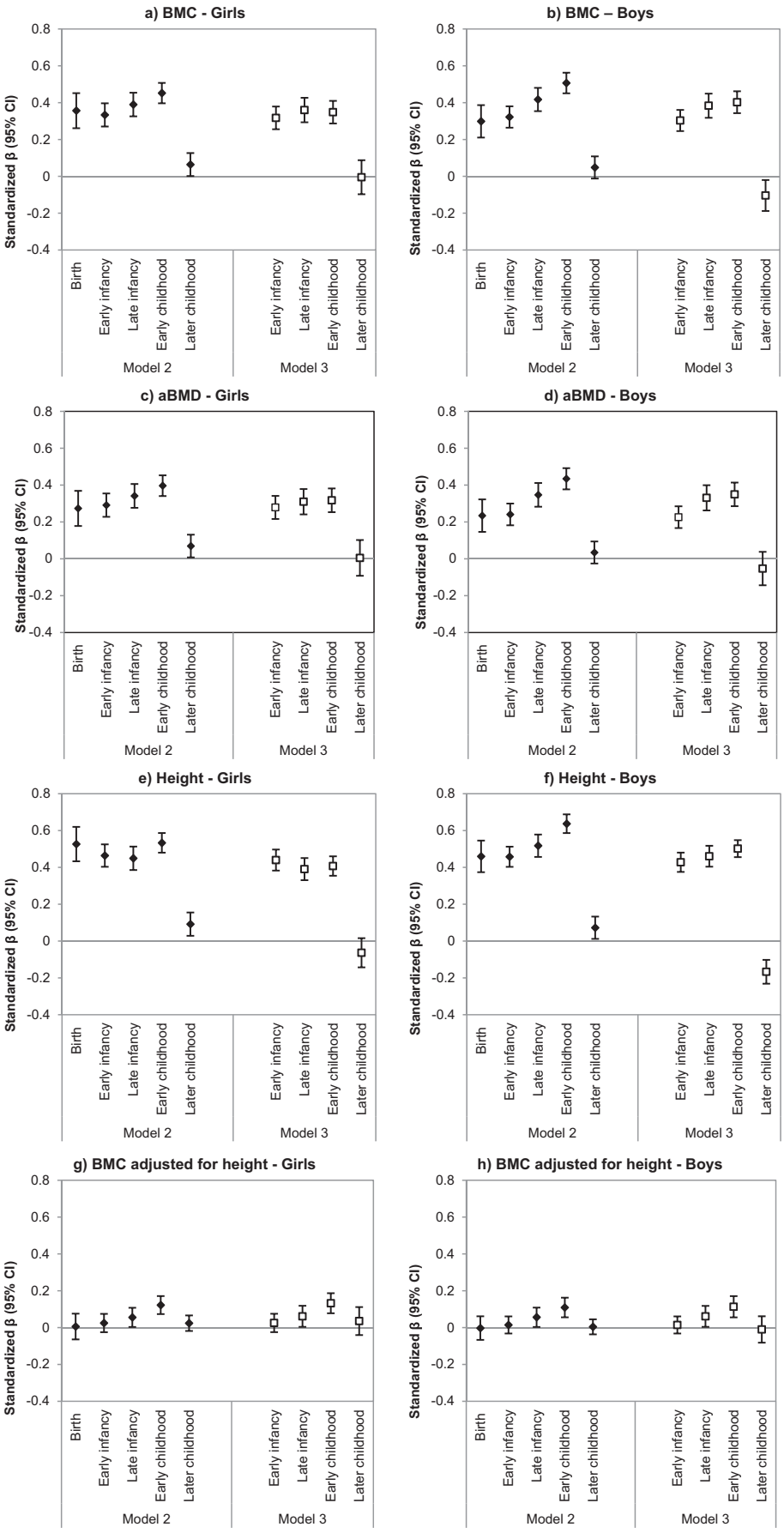

Fig. 2. Adjusted linear regression coefficients (95% CIs) for the associations between length/height velocities and bone content, bone areal density and height at 7 years of age.

Legend: Regression coefficients (95% CIs) that represent the change in standardized BMC (a and b), aBMD (c and d), height (e and f) and BMC adjusted for height (g and h) per 1 standard deviation increase in birth length or in length/height velocity in each age period, in girls (left) and boys (right).

Diamond: estimates adjusted for maternal age, educational level, pre-pregnancy body mass index, smoking during pregnancy and child gestational age (model 2). Square: estimates additionally adjusted for birth length and all of the preceding length/height velocities (model 3).

Age intervals: early infancy (0–3 months), late infancy (3–12 months), early childhood (1–3 years) and late childhood (3–6 years).

BMC, bone mineral content; aBMD, areal bone mineral density.

shorter at 7 years of age and more likely to have lower DXA-derived bone mass and areal density.

Figs. 1 and 2 show estimates of the associations between weight and length/height velocities and BMC, aBMD, height and BMC adjusted for height at 7 years of age, after adjustment for confounders only (model 2) and after additional adjustment for previous growth (model 3). Numerical values of all regression coefficients for Models 1 through 4 are presented in Supplemental Tables 1 and 2.

3.1. Birth anthropometry

Birth weight was positively associated with bone content, areal density and height in crude and adjusted analysis (Fig. 1, models 2 and 4 and Supplemental Table 1, model 1). Birth length was positively associated with BMC, aBMD and height (Fig. 2, model 2 and Supplemental Table 2, model 1). Adjustment for height at 7 years largely attenuated associations between birth anthropometry and BMC (Figs. 1 and 2, panels e) and f) and Supplemental Tables 1 and 2).

3.2. Weight velocity

Crude and confounder-adjusted estimates revealed a lack of association between early neonatal weight velocity and childhood bone mass (Fig. 1, model 2 and Supplemental Table 1, model 1). Additional adjustment for birth weight and length originated negative associations between early neonatal weight velocity and 7-years-old BMC, aBMD, height and BMC adjusted for height. In contrast, weight velocity in the subsequent four age periods predicted childhood bone content, areal density and height significantly and positively. We found the strongest associations for early childhood weight velocity: after confounder adjustment, 1 SD increase in weight velocity during the second and third years of life was associated with average increases in BMC z-score of 0.57 (95%CI: 0.52, 0.62) in girls and 0.57 (95%CI: 0.51, 0.63) in boys, in aBMD z-score of 0.54 (95%CI: 0.49, 0.60) in girls and 0.56 (95%CI: 0.50, 0.62) in boys and in height z-score of 0.54 (95%CI: 0.49, 0.60) and 0.57 (95%CI: 0.51, 0.63) (Fig. 1, model 2). Adjustment for height measured concurrently to BMC clearly attenuated all associations from early infancy up to later childhood towards the null, even though associations of early childhood growth with BMC persisted: 1 SD increase in early childhood weight velocity was associated with average increases in height-adjusted BMC z-score of 0.27 (95%CI: 0.22, 0.32) in girls and 0.24 (95%CI: 0.19, 0.29) in boys. All estimates were also attenuated upon adjustment for birth weight and preceding weight velocities, but the same overall pattern remained with positive associations for all periods after 10 days of age and greater magnitudes for weight velocity during early childhood (Fig. 1, model 3). Additional adjustment for preceding and concurrent velocities of growth in length/height was responsible for further attenuation of most estimates, with the exception of those between later childhood weight velocity and bone and height measures (Supplemental Table 1, model 4). Nevertheless, associations remained strongest for weight velocity during early childhood.

3.3. Height velocity

Length/height velocities in early infancy, late infancy and early childhood were positively associated with BMC and height at seven, while later childhood height velocity was only associated with height, with the largest effect estimates being observed during early childhood. In girls and boys, respectively, after confounder adjustment, 1 SD increase in early childhood height velocity was associated with average increases in BMC z-score of 0.45 (95%CI: 0.40, 0.51) and 0.51 (95%CI: 0.45, 0.56), in aBMD z-score of 0.40 (95%CI: 0.34, 0.45) and 0.43 (95%CI: 0.38, 0.49) and in height z-score of 0.53 (95%CI: 0.48, 0.59) and 0.64 (95%CI: 0.59, 0.69) (Fig. 2, model 2). As observed for weight, adjustment for concurrent height resulted in an attenuation of the estimates but stronger associations for height velocity in early childhood remained: 1 SD increase in height velocity during the second and third years of life was associated with average increases in adjusted for height BMC z-score of 0.12 (95%CI: 0.07, 0.17) in girls and 0.11 (95%CI: 0.06, 0.16) in boys. Also, birth length and preceding length/height velocities adjustment resulted in an attenuation of the estimates (Fig. 2, model 3). We found little evidence of association between height velocity in later childhood and bone content and areal density (Fig. 2, models 2 and 3 and Supplemental Table 2, model 1).

3.4. Weight vs height velocities

We found that, in earlier periods, specifically during infancy, length/height velocity was more strongly associated with bone mass at 7 years than weight velocity, while, in early and later childhood, weight velocity stronger correlate with bone mass (Supplemental Table 3). Our results for height were remarkably consistent with those obtained for BMC, which suggest that the relations between growth and bone mass in children are to a large extent attributable to body size. The direction of the associations was also similar for males and females, with only minor differences in the magnitude of the estimates that were not consistently stronger for one gender.

Results from sensitivity analyses after excluding children born preterm, or children born to women who gained excessive weight during pregnancy, showed a similar pattern of associations to those observed for the whole sample.

4. Discussion

In this population-based birth cohort, height and weight velocity in the first 6 years of life were associated with bone mass at age seven. In comparison to the remaining periods, growth between 1 and 3 years presented the strongest associations with childhood DXA-derived bone measures. Despite a strong statistical dependence on body size, this finding held for both height and weight, and in both genders. Our study suggests that the toddler years might be a particularly sensitive period for the effect of growth on bone development. If a causal interpretation is to be extracted, our results point to the importance of identifying disturbances to normal growth in early childhood that may impact bone health before peak bone mass. From a practical point of view, this study also highlights the informative potential of complementing data from interval cohorts with children's routine health information, with the objective of improving the detection of sensitive periods in life course research.

The generalized assumption that prepubertal bone mass tracks up until peak bone mass and that both determine bone mass into older ages deserves discussion. Studies in several contexts have generally suggested that both body size in the first year of life, as well as growth in height in the first 2 years, are predictive of adolescent and adult bone mass [5,20–24]. However, evidence of bone mass tracking in longitudinal studies is relatively short-term and its implications are contingent on the assumption of the peak bone mass model [25,26]. While it is undeniable that childhood is an important period for the attainment of bone density because of the great amount of bone mineral acquired during this stage, the relevance of early life bone mass acquisition for adult bone mass has been questioned [27,28]. If bone mass is governed by a homeostatic system acting in the short-term with any perturbation tending to be corrected over time (as per the mechanostat theory), bone mass may depend primarily on recent conditions rather than on the distant past [29]. This work was restricted to estimating an effect of early life growth on later bone mass in prepubertal children and the extent to which such an influence could persist up to peak bone mass and beyond to decrease the risk of fragility fracture is unclear.

DXA-derived bone measurements are strongly dependent on growth and partially reflect bone size rather than the intrinsic properties of the mineralized tissue. In order to clarify the size dependency of bone mass

we used as outcomes not only BMC and aBMD but also height at age 7, as a measure of linear growth, and BMC adjusted to height, as a measure of bone mass relative to body size. The latter outcome aimed to provide an estimate of skeletal growth relative to height up to that point, more closely related to cumulative bone growth than to the physical properties of bone tissue. The fact that associations of growth velocities with BMC were strongly attenuated once height was taken into account was expected given the close constitutional relation between bone mass and height. Nevertheless, we acknowledge that the inclusion of current height measurements in the models may represent an adjustment for an intermediate step and could have introduced some degree of collider bias [30].

A number of practical limitations should be noted. First, our study population is composed of children born in Portugal, whose mothers are comparatively homogeneous in terms of geographical origin. In addition, of the overall cohort, 21.4% of children had information on growth and bone variables to be included in the analyses of this study. This proportion is the result of both design choices and attrition or missing data. Children who did not participate were more likely to have lower socioeconomic position than those included in the study, but overall the magnitude of the differences was minor. Also, participants and nonparticipants were similar in terms of the main exposures under study: birth weight, birth length, and growth velocities at each age period. Therefore, we do not expect nonparticipation in Generation XXI follow-up assessments to be strongly related to the study's research question [31]. Another limitation is the fact that growth measures obtained from routine child health records are typically performed by different health professionals with different equipment. Although we were unable to test the validity of routinely-collected length/height measurements in Portugal, this source of information has previously shown little systematic error [32]. Random error is more likely, which would probably reduce the statistical power, although we do not expect this to have had a major impact on our results [33]. Moreover, although we collected detailed information on potential confounding variables, residual confounding due to unmeasured sociodemographic and lifestyle factors could still influence the results.

Despite those potential limitations, our results were consistent with four previous studies that have used regression with measures of conditional growth, residual growth model or two-stage and joint multilevel linear spline models to analyze associations between growth and bone content and density in children [9,10,12]. In a sample of 628 children from the Southampton Women's Survey (SWS), positive associations between growth in height in 1-year time intervals and bone measures at 4 years of age were reported, which were stronger for growth up to 1 year (for BMC) and between 1 and 2 years (for aBMD), than for subsequent growth intervals [9]. Also in SWS, the first and second years of life were identified as the most important age periods for the effect of conditional growth in height on bone mass at 6 years of age [10]. More recently in SWS participants, stronger associations with 6-year BMC was shown for growth in height between 1 and 3 years (through residual growth models), between birth and 6 months (through two-stage multilevel linear spline models) or between 1 and 3 years (through joint multilevel linear spline models) [11]. Only one study assessed the effect of both height and weight velocities on childhood bone mass and suggested different sensitive periods for their effects on aBMD at 6 years of age. In this study from Generation R, weight velocity in the first year of life had the strongest positive association with aBMD, whereas height velocity during the second and third years of life contributed most strongly to aBMD. For BMC, the strongest positive associations were found during the first year of life, for both height and weight velocities [12]. A general observation from previous studies that we also replicate here is a lack of gender heterogeneity in sensitive periods in this life stage.

Our results are also coherent with studies in populations of adolescents and adults that point to the first two years of life, in comparison to subsequent periods, as the most important for the effect of height velocity on BMC/aBMD from 15 to 20 years, on BMC/aBMD in the third decade of life and on BMC at 60 years of age [5,23,24]. Our results contradict, however, previous research on the effect of weight velocity on later adolescent and early old age bone mass, where growth closer to the age of bone mass assessment was more strongly associated to DXA-derived bone measures [3,5]. In another study, weight velocity during childhood was irrelevant as a determinant of bone mass at 60 years of age [24]. Direct comparison with previous research is hampered by differences in the periods of growth identified, which were defined almost always as relative changes in height and weight in interval cohorts, whose timing of measurement is planned in a single chronological age that does not necessarily correspond to the biological age and its intraindividual variation [34].

Positive associations between faster growth in weight or height and childhood bone mass are expected, and likely to be explained by the accretion of both adipose and muscle tissue, which promote osteogenesis both through increased mechanical loading and via the effects of secretory products such as growth hormone (GH), leptin, and cortisol [35]. Therefore, variations in the magnitude of effect estimates between different age periods should be largely attributable to changes in these underlying mechanisms. Our results suggest that the strong association with bone mass of growth in early childhood, in comparison to other early life periods, is related to biological changes specific to the second and third years of life. A documented underlying change in this life stage includes a change from predominantly GH-independent to GH-dependent growth [36]. In addition to this long-term process, however, we believe that the most likely explanation for the singularity of that period in relation to the remaining stages is the relatively fast acquisition of the vertical posture and the attainment of independent walking at around 12 to 18 months of age, with corresponding changes in gravitational and muscular stimuli to the skeleton [37,38]. The onset of locomotion seems to be a key macrostructural factor in defining the amount of physical strain imposed on spinopelvic and lower limb bone tissue, as already suggested in our previous work with posture that showed a clear association between sagittal morphotype and bone mass [39]. Changes in this balance of forces are so great from 1 to 3 years that growth in later periods may become comparatively less relevant. From the statistical point of view, this is compatible with later growth being less predictive of bone mass and observable as a decrease in the magnitude of the associations towards later childhood.

As a starting point, we estimated the effects of birth length and weight as markers of prenatal growth. As in previous studies, we found that body size at birth was positively associated with later bone mass, but less strongly than postnatal growth [9,10,12,40]. After birth size adjustment, we found an inverse association between early neonatal weight velocity and childhood BMC and aBMD. This is in agreement with our observation that early neonatal weight velocity was inversely related to weight velocities in subsequent age periods (Supplemental Fig. 1) which may be due to compensatory growth. Due to national policy, early neonatal measurements are particularly frequent in Portugal, which provides high data density in our sample but scarce opportunities for comparison with other settings.

An additional relevant finding was on the balance between height and weight velocities in terms of their associations with BMC and aBMD across periods. Length/height velocity was more strongly associated with bone mass than weight velocity in the earlier periods, whereas the relative contribution of weight velocity grew stronger later on in childhood. Indeed, height velocity in later childhood showed weak or negligible (and in one case even inverse) associations with BMC and aBMD. This pattern of associations probably reflects linear growth as the main driver of bone accrual in the earlier stages, later surpassed by an increasing importance of changes to bone diameter imposed by weight gain through gravitational and muscle forces [12].

In addition to generally corroborating the body of evidence of the first three years of life as a sensitive period for childhood bone mass, the present study adds important evidence. Specifically, it suggests the

strongest role for growth during the toddler years, regarding both height and weight velocities and possibly a major contribution of the upright posture. We believe our analytical approach makes the present results particularly robust since we studied velocities of growth defined from an empirical approach. By taking advantage of a large amount of data, we were able to identify growth periods from statistical knot points that indicate changes in average observed velocities that are likely to have relevant biological meaning. The advantages of linear spline multilevel models to summarize childhood growth have been described in detail [13,41]. Briefly, this approach models growth data efficiently as it allows using all available data, taking into account different timing and number of measurements between individuals, and not restricting the analysis to those with complete data at all times. In this way, this approach identifies age intervals that differ in velocity of growth, rather than using arbitrary intervals limited by data availability, and deals efficiently with collinearity between repeated measures and bias due to missing data [13,34]. In Generation XXI, linear spline multilevel models have shown good fit to the observed data. Also, the knot points identified were similar to those in other settings where the same analytical approach was used, which supports the validity of this model to identify periods of growth that are biologically, as well as statistically, relevant [13]. We opted to use DXA, the preferred method to assess bone status in children due to its good accuracy, short analysis time, safety, low cost, and wide availability [42]. Importantly, BMC measured by DXA has been shown to be an accurate predictor of fracture risk, which is ultimately a key clinical validation [43]. We also used measurements of total body less head which is the recommended option for obtaining DXA-derived bone measures in pediatric research, due to precision and reproducibility [16]. In addition, our effect estimates were robust to several sensitivity analyses, as well as to adjustments to maternal and child factors, representing potential confounding by genetic and environmental characteristics, and to adjustments to previous growth. They were also concordant between different DXA-derived measures.

In this population-based prospective cohort, growth in the first six years of life predicted bone mass at 7 years of age, partly through concurrent body size. Possibly due to the adoption of the upright position and the development of walking abilities, our findings identify the second and third years of life as a sensitive period of growth for skeletal development.

CRediT authorship contribution statement

Teresa Monjardino: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Funding acquisition, Writing - original draft. **Joana Amaro:** Validation, Writing - review & editing. **Maria João Fonseca:** Validation, Writing - review & editing. **Teresa Rodrigues:** Validation, Writing - review & editing, Supervision. **Ana Cristina Santos:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Project administration, Funding acquisition, Writing - review & editing, Supervision. **Raquel Lucas:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Funding acquisition, Writing - review & editing, Supervision.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.07.002.

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