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ORIGINAL ARTICLE

Relationship between bone mineral content and bone turnover markers, sex hormones and calciotropic hormones in pre- and early pubertal children

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Abstract

Summary We investigated associations between bone mineral content (BMC) and bone-related biomarkers (BM) in pre-and early pubertal children of both sexes. In this population, we found that bone turnover markers explain a small part of BMC variance.

Introduction It is still debated whether BM including bone turnover markers (BTM), sex hormones and calciotropic (including cortisol) hormones provide information on BMC changes during growth.

Methods Three hundred fifty-seven girls and boys aged 6 to 13 years were included in this study. BM was measured at baseline and BMC twice at 9 months and 4 years using DXA. Relationship between BMs was assessed using principal component analysis (PCA). BM was tested in its ability to explain BMC variation by using structural equation modelling (SEM) on cross-sectional data. Longitudinal data were used to further assess the association between BM and BMC variables.

Results BMC and all BMs, except calciotropic hormones, increased with age. PCA in BM revealed a three-factor solution (BTM, sex hormones and calciotropic hormones). In the SEM, age accounted for 61% and BTM for 1.2% of variance in BMC (cross-sectional). Neither sex nor calciotropic hormones were BMC explanatory variables. In the longitudinal models (with single BM as explanatory variables), BMC, age and sex at baseline accounted for 79–81% and 70–75% in BMC variance at 9 months and 4 years later, respectively. P1NP was consistently associated with BMC.

Conclusion BMC strongly tracks in pre- and early pubertal children. In this study, only a small part of BMC variance was explained by single BTM at the beginning of pubertal growth.

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Keywords Bone mineral content · Bone remodelling · Bone turnover marker · Calciotropic hormones · Gonadal steroid hormones · Puberty

Abbreviations

BM	Biomarkers (including all markers measured in the blood)
BTM	Bone turnover marker
CORT	Cortisol
CTX	C-terminal telopeptide
DHEAS	Dehydroepiandrosterone sulphate
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
FN	Femoral neck
LS	Lumbar spine
OCN	Osteocalcin
25(OH)D	25-Hydroxy vitamin D/calcifediol
PCA	Principal component analysis
P1NP	N-terminal propeptide
PROGE	Progesterone
PTH	Intact parathyroid hormone
SEM	Structural equation modelling
SHBG	Sex hormone-binding globulin
TEST	Testosterone
WB	Whole body

Introduction

Puberty is a period characterized by accelerated bone accrual and major changes in hormone levels in both sexes. Bone mass accumulation during pubertal spurt contributes largely to peak bone mass. This is a determinant of adult bone mineral mass/density, a low level of which can contribute to osteoporosis and fracture risk later in life [1, 2]. Peak bone mass is achieved in most skeletal sites by the end of the second decade of life. Bone mass is primarily determined genetically, genetics accounting for approximately 60 to 80% of the bone mineral mass/structure variance [1]. The remaining variance is explained by lifestyle/environmental factors including nutrition and physical activity [2]. Risks and protective factors in the first two decades of life are thought to have strong influences on the growing skeleton [3]. Thus, the detection of risks for (in)sufficient bone development during growth by means of sensitive biomarkers (BM) would enable early evaluation and preventive measures in optimizing mineral accrual during the critical years of growth.

Bone mineral mass changes are influenced by various hormones, which directly or indirectly regulate bone physiology [4]. These include sex hormones such as estradiol (E2), testosterone (TEST), progesterone (PROGE), parathyroid hormone (PTH), 25-Hydroxyvitamin D [25(OH)D], and cortisol (CORT) that all exert direct or indirect influences on bone growth and on bone health. Their levels may also provide information about bone metabolism [4–6]. In addition, serum or urinary bone turnover markers (BTM) provide information on the rate of bone turnover, which is markedly accelerated during puberty [7–9]. During growth, they may be sensitive markers allowing a dynamic picture of bone turnover processes and may allow an early detection of abnormal bone mass changes [9, 10]. From the studies on the associations between BTM and bone mass, it is still debated whether BTM in children are clinically valuable in informing on the current or later state of bone mineral changes [7, 9–14]. Only few studies have investigated associations of various BMs with bone mineral mass in children [7, 11, 13–18]. Some have suggested a significant association of bone mineral mass with different BMs [11, 13, 15–18], while others did not find evidence for a predictive contribution [14]. These studies have focussed on either BTM, sex hormones or calciotropic hormones separately and have not investigated all BMs jointly [11, 13–18]. They mostly used cross-sectional data [11, 14–18] or applied statistical analyses with drawbacks (e.g. stepwise regression, or analyses without adjustment) [11, 13, 16–18]. More advanced statistical techniques such as principal component analysis (PCA), allowing to explore result patterns [19], or structural equation modelling (SEM), able to model more complex relationships [20], have not been applied yet to bone growth and its determinants in pre- and early pubertal stages.

In this exploratory cross-sectional and longitudinal study conducted in a large sample of pre- and early pubertal healthy girls and boys, we aimed to evaluate whether BMs including BTMs [osteocalcin (OCN), N-terminal propeptide (PINP), and C-terminal telopeptide (CTX)], sex hormones [TEST, E2, PROGE], calciotropic hormones [PTH, 25(OH)D] and CORT are associated with changes in bone mineral content (BMC) as assessed by dual-energy X-ray absorptiometry (DXA). First, we investigated correlations between BMC and BM, and analysed them using PCA. Based on previous results suggesting a relationship between BTM [10, 14], sex hormones [4, 18], and 25(OH)D with PTH [6], and data obtained by PCA, we formed groups of variables (constructs) in a first step. This information was used to build further models (SEM) to investigate whether BM groups (constructs) explained variations in BMC using cross-sectional data. To further investigate results obtained by SEM, we assessed whether baseline BMs explain BMC variance at follow-up (9 months and 4 years) using multiple linear regression on longitudinal data.

Subjects and methods

Setting and sample

This study includes a large cohort of public-school children (n = 357) with samples collected at baseline and follow-up data (at 9 months and 4 years) of the KISS study. The 9-month interval corresponds to the duration of a school year. The KISS study was a cluster-randomized, school-based, physical activity intervention that was conducted between August 2005 (baseline) and July 2006 (post intervention) in 1st and 5th grade students (6- to 13-year-old children). A 4-year follow-up was conducted in 2009. For the present study, a given sample of 357 participants (including intervention and control group participants) was used. Of the 502 children initially participating in the KISS study, 136 participants were excluded due to refusal of blood sampling and/or missing results for BMs. Furthermore, we excluded 9 participants because they were not in a pre- or early pubertal stage at baseline (see Fig. 1 Appendix for further information). The study design and intervention effects on primary and secondary endpoints, including bone mineral density/content, were published previously [21, 22]. Informed consent for all measurements was obtained from children and one of their parents. The protocol has been approved by local ethics committees [21].

Demographics, puberty and anthropometry

Pubertal stage was estimated by a self-assessment questionnaire with a simple explanation summarizing the Tanner stages which we then categorized as pre-pubertal (Tanner 1) and early pubertal (Tanner 2 and 3). Due to the limited reliability and validity of the Tanner stages self-assessment [23], Tanner stage was used only for descriptive purposes and not included in the models to adjust for growth/puberty. Instead, we used age to adjust for growth. Standing height was measured by a wall mounted Harpenden stadiometer (Holtain, Crymych, UK) and body weight was determined using an electronic scale (Seca, Basel, Switzerland; accuracy 0.05 g). Height-for-age and weight-for-age according to World Health Organization references were determined to allow for a comparison of our sample with normal values [24].

Bone mineral content and density

Bone mineral content (BMC, g) and areal density (BMD, g/cm^2) were determined for total body (WB), femoral neck (FN) and L1–L4 vertebrae (LS) in antero-posterior view using DXA (DXA; Hologic QDR-4500, Waltham, MA, USA). The densitometer instrument was located in a truck, allowing for realising the measurements at different schools. The coefficient of variation of repeated measurements for femur, lumbar spine and total body determined in young healthy adults varies between 1–1.6% for BMD, and 0.3–3% for BMC [25]. In addition, BMC and BMD values were z-transformed using age- and sex-specific reference values [26]. In this article, we primarily focused on BMC outcomes.

Biomarkers

Blood was collected between 7:30 and 9:30 a.m. in all children after an overnight fast. Blood was drawn in vacutainers which were immediately put on ice. After the sampling, the blood was transported to the hospital where the blood was centrifuged, divided into 1.0 ml aliquots and stored at − 70 °C until batch analyses. OCN (N-MID-Osteocalcin), P1NP, CTX, PTH, Total TEST, E2, SHBG, PROGE, CORT and dehydroepiandrosterone sulphate (DHEAS) were measured using ECLIA on the automated analyser Elecsys 2010 (Roche Diagnostics, Rotkreuz, Switzerland) [27, 28]. 25(OH)D (25-Hydroxy Vitamin D) was determined using an enzyme-immunoassay (EIA) on the IDS-iSYS (Immunodiagnostic Systems, Frankfurt/Germany) [29]. In order to approximate the free TEST and free E2 serum concentration, the free androgen index [(TEST/SHBG) × 100] and the free estradiol index [(E2/SHBG) × 100] were calculated [30, 31]. All BMs were scaled according to SI units (International System of Units).

Statistics

Sample characteristics are presented as means ± standard deviations for continuous variables and as frequencies (%) for categorical variables. The sex and age stratified distribution of BMs at baseline is shown by boxplots and by 5th, 50th and 95th percentiles. Correlation analyses were used to investigate the distribution and bivariate associations between BMC and BM variables (Pearson and Spearman's rank correlation coefficient). We first analysed cross-sectional and then longitudinal data.

Using cross-sectional baseline data, we applied PCA to reduce the dimensionality of the various BMC and BM variables on a smaller number of components called factors. Those factors still retain most of the variability of the original variables [19]. The number or factors was determined by Scree-Test and parallel analysis. Components were rotated (oblimin) in order to simplify the structure and interpretability. Factor loadings (between − 1 and + 1) show the strength of association of a variable with the respective factor [19].

The components identified in PCA were subsequently analysed in a SEM using latent variable analysis in R [20, 32] in order to model the cross-sectional relationships between BMC and BM. The SEM model was estimated using Maximum Likelihood (ML). To account for the

skewness of the data, we used the Satorra-Bentler scaled statistical test and derived standard errors and p values using non-parametric bootstrapping (replacement sampling, $n = 5000$) [32]. A mean-square error of approximation (RMSEA) below 0.06 was considered good (below 0.10 as acceptable); Standardized Root Mean Square Residual (SRMR) below 0.08, comparative fit index (CFI) above 0.95 and a χ^2/df ratio below 3 were considered as satisfactory fit [20, 33]. Standardized (βstd) and unstandardized (β) coefficients were calculated. Sensitivity analyses for the PCA and the SEM were done by performing analyses for boys and girls separately (to investigate whether they lead to the same conclusion).

Using the longitudinal data, we investigated whether single BTM and sex hormone levels measured at baseline were associated with BMC (FN, LS, WB) at follow-up (9 months and 4 years) by means of robust multiple linear regressions models [34]. These models were adjusted for baseline BMC, age and sex. Additionally, sensitivity analyses were done by adjusting for treatment (treatment vs. control group) and by applying a model including a random intercept (by participant) and a random slope for age. Analyses were performed using R, version 3.5.0 and Stata for Windows version 13.1 (Stata Corp LP, College Station, TX, USA).

Results

Sample characteristics and BMC for girls and boys at baseline and 9 months are shown in Table 1. The majority of participants were in a pre-pubertal (Tanner 1) maturation stage at both time points. Absolute BMC values increased from baseline to follow-up in both genders. Mean z-scores for height, BMI, BMC and BMD showed normal values (in comparison to age-matched healthy individuals [26]).

Figure 1 shows boxplots of all BMs by sex and age groups (6–7 years, 7.1–8 years, 10–11 years and 11.1–13 years). BTM (OCN, P1NP, CTX) increased with age in both sexes (no statistical test applied). This increase was seemingly more pronounced in girls. A similar pattern was found for TEST/SHBG, E2/SHBG and PROGE. PTH, 25(OH)D and CORT showed large variation but remained relatively stable across different age groups. There was a substantial variation for all parameters. Additional information including further parameters (E2, TEST, SHBG, DHEAS) is provided in Appendix Table 4.

Table 2 shows bivariate associations (Pearson) using cross-sectional baseline data of BTM, sex hormones, calciotropic hormones (including CORT), sex, age and height with FN, LS and WB BMC (raw scores, and age- and sex-standardized z-scores). All BMs, except 25(OH)D and CORT were significantly, but weakly associated with BMC raw values. These associations were less pronounced in z-standardized BMC variables. Age and height showed strong and comparable associations with all raw BMC values. Strong and significant relationships were observed among BMC and BMD variables ranging from $r = 0.73$ to $r = 0.97$ ($p < 0.001$, data not shown). Spearman correlations calculated in addition to Pearson to investigate deviations due to non-normal data led to comparable results.

PCA for cross-sectional BMC variables revealed a one-factor solution with variables showing strong loadings between 0.90 and 0.97. These factor solutions were also found for boys and girls when analysed separately (data not shown). PCA in BM variables consistently revealed a three-factor solution (scree and parallel-test) explaining 62% of the total BM variance. Figure 2 shows PCA loadings for BM variables. The first factor explained 26% (Eigenvalue 2.36) of BM variance and was primarily associated with OCN, P1NP and CTX with loadings between 0.77 and 0.89 indicative of a common underlying BTM dimension. The second factor explained 21% of BM variance (Eigenvalue 1.85) on which TEST/SHBG, E2/SHBG and PROGE showed high positive loadings between 0.65 and 0.84 suggesting a common underlying dimension of sex hormones. The third factor was mainly associated with calciotropic hormones (including CORT) explaining 15% of BM variance (Eigenvalue 1.35) showing associations with PTH and 25(OH)D of − 0.55 and 0.75, respectively. This indicates that an increase in factor three was associated with an increase in 25(OH)D and a decrease in PTH. CORT showed the highest loading (0.59) on factor three, but was also associated with factor two (sex hormone factor). Similar results were found by analysing girls and boys separately (data not shown).

Based on previous studies and on information provided by PCA, we determined three predictor constructs and one outcome construct: (a) bone turnover markers (OCN, P1NP, CTX); (b) sex hormones (TEST/SHBG, E2/SHBG, PROGE); (c) calciotropic hormones (25(OH)D, PTH, CORT); and (d) BMC (FN BMC, LS BMC, WB BMC). We first investigated the association between each single predictor construct and the outcome construct. Results for the SEMs on cross-sectional data are shown in Fig. 3 (main models) and Appendix Table 5 (all models).

The calciotropic hormone construct (model a) did not significantly predict BMC. Further, the single indicators (including PTH, 25(OH)D, and CORT) did not sufficiently evaluate the calciotropic hormone construct. The sex

Table 1 Characteristics for pre- and early pubertal girls (n = 182) and boys (n = 175) at baseline and follow-up, puberty and bone mineral content/density (n = 357)

	Girls baseline			Girls follow-up (at 9 months)			Boys baseline			Boys follow-up (at 9 months)		
	n[a]	Mean (SD)	%	n[a]	Mean (SD)	%	n[a]	Mean (SD)	%	n[a]	Mean (SD)	%
Age	182	9.3 (2.1)		176	10.1 (2.1)		175	9.5 (2.1)		167	10.3 (2.1)	
Puberty and anthropometry												
Tanner 1	118		65.2	87		49.7	137		78.7	106		63.1
Tanner 2 and 3	63		34.8	78		44.6	37		21.3	59		35.1
Tanner 4 and 5[b]	0			10		5.7	0			3		1.8
Height cm	180	135.8 (12.7)		176	141.2 (13.4)		175	137.0 (13.4)		167	142.1 (13.8)	
Height-for-age z-score[c]	180	0.16 (0.99)		176	0.25 (1.02)		175	0.27 (0.91)		167	0.30 (0.93)	
Weight kg	180	31.9 (8.5)		176	35.4 (9.7)		175	32.9 (9.7)		167	35.8 (10.7)	
BMI-for-age z-scores[c]	180	0.11 (1.01)		176	0.07 (1.01)		175	0.20 (1.03)		167	0.11 (1.00)	
Bone mineral content (BMC)												
Femoral neck g	173	2.35 (0.65)		129	2.70 (0.70)		167	2.64 (0.81)		125	2.93 (0.92)	
Lumbar spine g	170	23.18 (5.72)		129	26.95 (7.11)		167	23.87 (6.24)		125	26.46 (7.50)	
Total body g	167	760 (200)		129	852 (224)		164	792 (238)		125	851 (266)	
BMC z-scores[d]												
Femoral neck	173	− 0.50 (1.07)		128	− 0.16 (0.99)		167	− 0.26 (1.25)		124	− 0.02 (1.28)	
Lumbar spine	170	− 0.19 (0.94)		128	− 0.07 (1.00)		167	− 0.14 (1.01)		124	− 0.08 (1.08)	
Total body	167	− 0.15 (0.86)		128	− 0.25 (0.94)		164	− 0.06 (0.92)		124	− 0.25 (0.98)	
Bone mineral density (BMD)												
Femoral neck g/cm^2	173	0.64 (0.08)		129	0.68 (0.08)		167	0.68 (0.09)		125	0.72 (0.10)	
Lumbar spine g/cm^2	170	0.61 (0.09)		129	0.65 (0.10)		167	0.60 (0.09)		125	0.62 (0.10)	
Total body g/cm^2	167	0.66 (0.09)		129	0.70 (0.09)		164	0.68 (0.10)		125	0.70 (0.10)	
BMD z-scores[d]												
Femoral neck	173	0.00 (1.07)		128	0.17 (0.94)		167	0.09 (1.00)		124	0.22 (1.03)	
Lumbar spine	170	− 0.12 (0.90)		128	− 0.04 (0.94)		167	0.05 (0.98)		124	0.05 (1.01)	
Total body	167	− 0.32 (0.96)		128	− 0.38 (0.96)		164	− 0.19 (0.95)		124	− 0.37 (0.95)	

[a] Indicates the number of non-missing cases on the respective variable

[b] Individuals with Tanner stage at baseline > 3 were dropped (low case numbers/large heterogeneity)

[c] Z-scores are based on WHO growth reference for school-aged children and adolescents [25]. Weight-for-age z-scores are not shown (only provided by WHO for children ≤ 10 years due to its inability to distinguish between relative height and body mass in older children)

[d] Z-scores based on external reference curves for bone mineral content and density [26]

hormone construct (model b) was a significant predictor of BMC and explained about 34% of its variance and showed a good fit. The BTM (model c) predicted 14% in BMC variance and showed a good fit. To investigate the interaction between sex hormones and BTM in predicting BMC, we included these latent variables in a final model (model d) (Fig. 3a). In this model, sex hormone (βstd. = 0.51, p < 0.001) and BTM (βstd. = 0.22, p < 0.005) were significant predictors explaining about 31% in BMC variance combined and showed an acceptable model fit. Finally, age was added to model d to adjust for growth (model e) (Fig. 3b). Despite a similar fit, the influence of sex hormones became weak and non-significant (βstd. = 0.069, p = 0.32). The BTM construct remained a weak but significant predictor (βstd. = 0.11, p = 0.024) of BMC. Importantly, age was strongly associated with sex hormones (βstd. = 0.62, p < 0.001), BTM (βstd. = 0.33, p < 0.001), and explained 61%, a substantial part of BMC variance (βstd. = 0.78, p < 0.001). Sensitivity analysis for model d/e led to very similar results for girls and boys separately. When weight or height were included as additional covariates to age, the model did not converge.

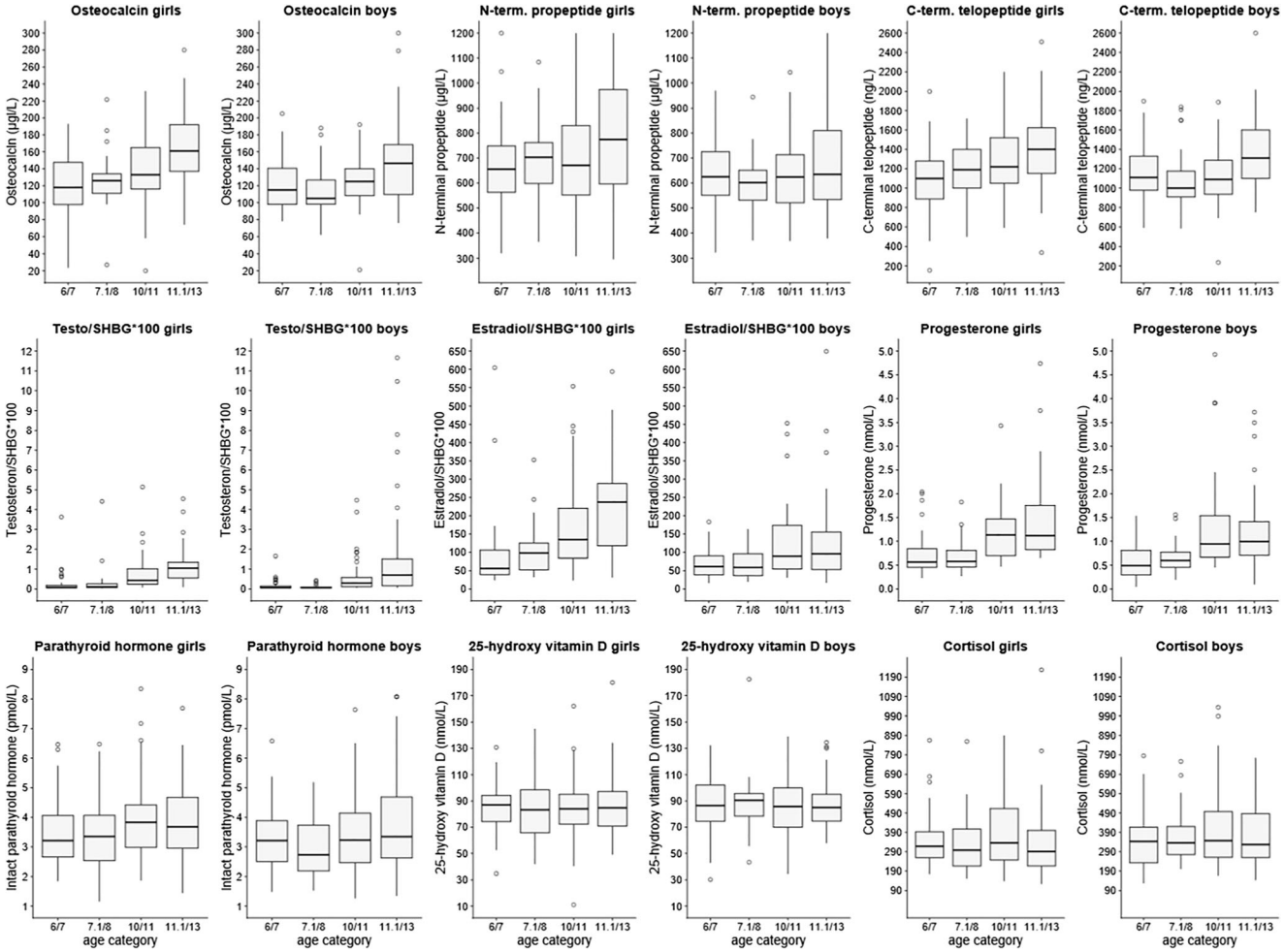

Fig. 1 Boxplot for biomarkers stratified by sex and age groups

Similarly, the relation remained weak when age was replaced by body composition (data not shown). We also included Tanner stages in the model. Only a few subjects were in stage 3 at baseline, most being in stages 1 and 2. BMC was weakly associated with Tanner stages (less of 1% of the variance) (data not shown).

Table 3 shows how baseline values predict BMC values (FN, LS, WB) at follow-up using single BTM, sex hormones, age, BMC and sex at baseline. BMC, age and sex at baseline predicted 79 to 81% of BMC variance at 9-month follow-up. BTM and sex hormones contributed little to the variance (between 1 and 4%). Only P1NP was a consistent and significant but weak predictor of BMC at 9-month follow-up. The association of sex hormones and BMC at follow-up were inconsistent, with large confidence intervals. This appeared not to be related to the intervention. In addition, 70–75% of BMC variance at the 4-year follow-up was explained by BMC at baseline, age and sex (n = 194). BTM and sex hormones showed large confidence intervals, were mostly insignificant, and explained no further variance in addition to baseline BMC, age and sex (data not shown). Further models (random intercept and slope) did not change the conclusion. In addition to BMC, all analyses were performed for BMD that led to the same conclusions (data not shown).

Discussion

The main findings of our cross-sectional model indicate that the major part of variation in BMC (61%) was explained by age. Only a small additional amount was explained by BTM (1.2%), and none by sex hormones using a large representative sample of healthy pre- and early pubertal girls and boys. Likewise, in the longitudinal analyses, at least 70% of variation in single BMC measures (e.g. FN) at follow-up up to 4 years were explained by BMC at baseline, age and sex. Results from the PCA and SEM analyses indicated that single BMC, BTM and sex hormone measures can be represented by

Table 2 Bivariate associations between biomarkers and bone mineral content (raw and z-standardized) measured at different skeletal sites (femoral neck, lumbar spine, whole body) including girls and boys

Predictors	*n*	Femoral neck bone mineral content Rho[a]	Femoral neck bone mineral content z-score Rho[a]	*n*	Lumbar spine bone mineral content Rho[a]	Lumbar spine bone mineral content z-score Rho[a]	*n*	Total body bone mineral content Rho[a]	Total body bone mineral content z-score Rho[a]
Bone turnover markers									
OCN	340	*0.33*	*0.25*	337	*0.38*	*0.12*	331	*0.39*	*0.15*
P1NP	340	*0.17*	*0.13*	337	*0.25*	*0.13*	331	*0.24*	*0.14*
CTX	338	*0.27*	*0.20*	335	*0.31*	*0.11*	329	*0.30*	*0.11*
Sex hormones									
TEST	326	*0.32*	*0.16*	323	*0.35*	0.029	317	*0.35*	− 0.0012
TEST/SHBG	323	*0.34*	*0.19*	320	*0.35*	0.020	314	*0.37*	0.014
E2	336	*0.22*	*0.14*	333	*0.31*	0.056	327	*0.26*	− 0.021
E2/SHBG	333	*0.30*	*0.21*	330	*0.38*	0.085	324	*0.37*	0.074
PROGE	335	*0.35*	*0.21*	332	*0.34*	− 0.021	326	*0.37*	− 0.0063
Calciotropic hormones									
PTH	339	*0.16*	*0.12*	336	*0.16*	0.056	330	*0.16*	0.060
25(OH)D	335	− 0.0038	0.020	332	0.034	0.059	326	0.039	0.093
CORT	329	0.032	− 0.021	326	− 0.03	*− 0.13*	320	− 0.0045	*− 0.13*
Sex	340	*− 0.19*	− 0.10	337	− 0.06	− 0.026	331	− 0.074	− 0.048
Age	340	*0.82*	*0.45*	337	*0.76*	− 0.075	331	*0.84*	− 0.042
Height	340	*0.87*	*0.62*	337	*0.87*	*0.219*	331	*0.93*	*0.294*

Italics denote significant correlations ($p < 0.05$).

OCN osteocalcin, *P1NP* N-terminal propeptide, *CTX* C-terminal telopeptide, *TEST* testosterone, *TEST/SHBG* TEST corrected for SHBG, *E2* estradiol, *E2/SHBG* E2 corrected for SHBG, *PROGE* progesterone, *PTH* intact parathyroid hormone, *25(OH)D* 25-Hydroxy vitamin D, *CORT* cortisol

[a] Pearson correlation

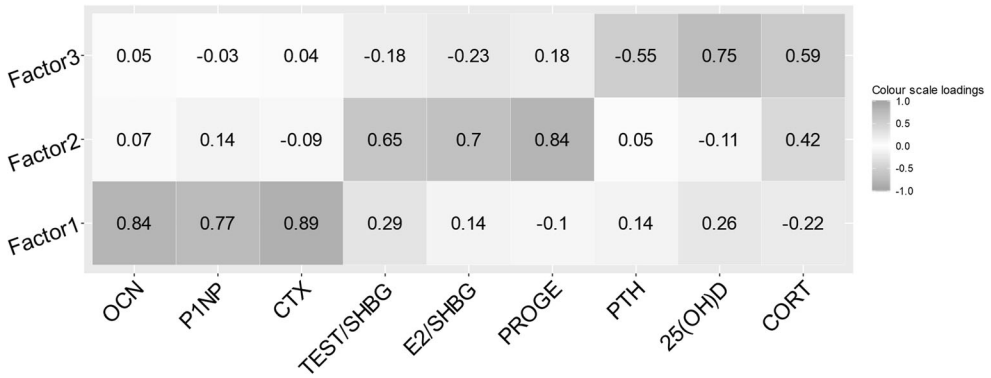

Fig. 2 Principal component analysis (PCA) of biomarkers including girls and boys (n = 309) led to a three-factor solution. Bone turnover markers including N-terminal propeptide (P1NP), osteocalcin (OCN) and C-terminal telopeptide (CTX) showed high positive loadings on factor 1. Sex hormones including estradiol/sex hormone-binding globulin (E2/SHBG), testosterone/sex hormone-binding globulin (TEST/SHBG) and progesterone (PROGE) showed high positive loadings on factor 2. Calciotropic (pre-)hormones including intact parathyroid hormone (PTH) and 25-Hydroxy vitamin D [25(OH)D] were inversely related to factor 3. Cortisol (CORT) showed the highest association with factor 3 as well as a considerable association with factor 2

constructs that allowed us to reduce complex and multidimensional data without a substantial loss of information and a reduction of measurement error. However, neither BTM nor sex hormones explained a substantial part of BMC variance. This does not preclude a possible higher association in pathological conditions instead of in healthy subjects.

Our results indicate that BMC and BMD values [26], height and BMI [24], BTM [7, 10] and hormones [13, 18] in children of this study were comparable to published values of age-matched normal individuals and showed a development pattern similar to that of white healthy subjects [4, 10, 13, 14]. As expected, the increase with age was more pronounced and earlier in girls reflecting their earlier pubertal growth spurt [4, 10, 13, 14].

The one-dimensionality of BMC, BTM and sex hormones shown in the PCA indicates that these variables are probably influenced by common underlying processes. For BMC, this is not surprising since the single variables measure a similar entity, which differs only by measurement site (e.g. femoral neck, lumbar spine). For BTM, this one-dimensionality was expected since BTM of formation and resorption are highly related [10, 13, 14] and reflect processes of bone modelling and remodelling that change in concert [7, 14]. Likewise, sex hormones are already related through their common biosynthesis (e.g. E2 may be synthesised from aromatization of TEST) [4] and were shown to be associated, as suggested in a previous study [18]. Finally, 25(OH)D and PTH inversely loaded on the third factor reflecting their reciprocal regulation, both increasing 25(OH)D levels and suppressing PTH [6].

We then used the components found in the PCA to investigate multiple cross-sectional relationships between BMC with BTM, sex hormones, calciotropic hormones and age using SEM models. The calciotropic hormones showed insufficient construct validity and no evidence for an association with BMC and were thus eliminated from the model. This is not surprizing since these hormones are tightly regulated irrespective of anthropometric or skeletal parameters. The final model d excluding age indicated a relevant association of BTM and hormones with BMC similar to previous research [11, 13, 16–18]. However, most of these associations were not or insufficiently adjusted for growth. Yet, with age adjustment (model e) included, only BTM, but not hormones, explained a small part of BMC variation. In line with these findings, associations between BMC and BTM were substantially weakened by standardization of BMC (z-scores) as shown in the simple descriptive correlation analyses. These findings suggest a major confounding effect of growth (e.g. an increase in BMC and BTM with age). Not surprisingly, previous cross-sectional studies showed that increases in BMC, BTM and sex hormones were strongly related with height, weight, growth velocity or other growth/development-related factors such as age or pubertal stage [4, 7, 13, 17, 35].

In our longitudinal data analyses, the associations with single BTM and sex hormone markers were weak, with large confidence intervals. Only P1NP was consistently associated with BMC values. Interestingly, BMC at 9-month follow-up was largely determined by BMC at baseline which could primarily reflect the tracking of BMC [36, 37]. Similarly, BMC variance at the 4-year follow-up was largely determined by baseline BMC. These results are compatible with a BMC tracking in pre- and early pubertal children, as previously described [36, 38]. Tracking, which results from modelling and remodelling of the growing bone, can be explained by genetics, but

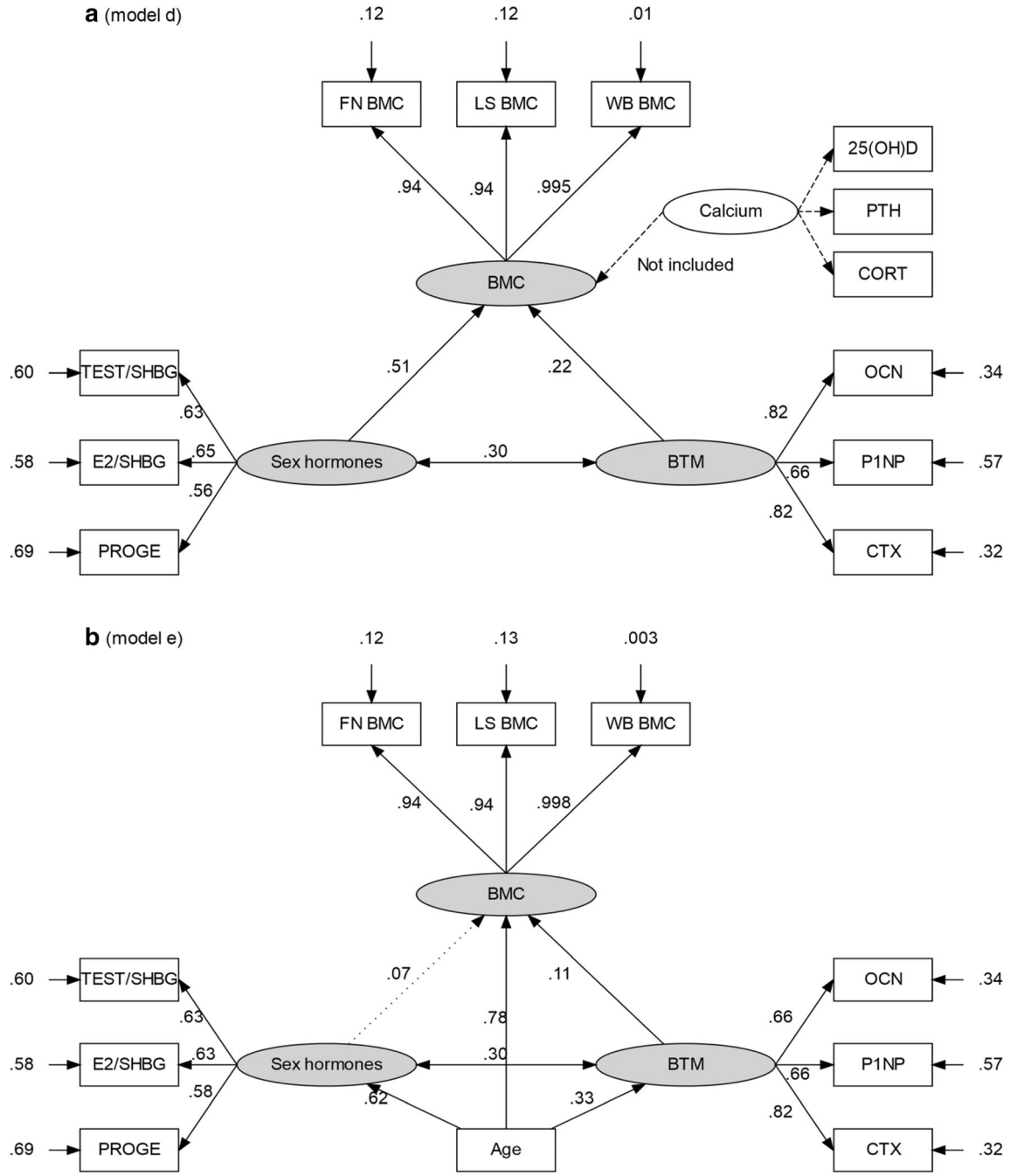

Fig. 3 Structural equation models (n = 309). **a** shows the associations (standardized coefficients) of bone turnover marker (BTM) and sex hormone constructs as predictors of bone mineral content (BMC) without age adjustment (RMSEA 0.087, SRMR 0.054, CFI 0.97, χ^2/df 3.10). BTM explains 5% and sex hormones 26% of variance in BMC. The calciotropic hormone construct was removed due to a lack of construct validity. **b** shows the same model but with age adjustment (RMSEA 0.09, SRMR 0.05, CFI 0.97, χ^2/df 3.18). The strength of association of BTM and sex hormones with BMC is noticeably decreased compared to model **a**. BTM explains 1.2% of variance in BMC, and the association with sex hormones is non-significant. In turn, sex hormones and BTM strongly vary by age. Age determines 61%, a major part of BMC variance. Solid line; $p < .05$, dashed line; construct not included in the model/$p > .05$ (additional information is shown in Appendix Table 5)

possibly also by environmental factors that affect bone like physical activity or nutritional intakes that show some stability over time (e.g. physically active children remain active) [36, 37, 39, 40]. However, changes in these behavioural factors like physical activity and nutrition may modify the tracking [1, 36, 38].

Table 3 Longitudinal (only BMC outcomes were measured repeatedly) model predicting bone mineral content at follow-up (at 9 months later) adjusting for bone mineral content at baseline, age, sex, bone turnover markers and sex hormones (n = 240)

	Femoral neck BMC (g) at follow-up			Lumbar spine BMC (g) at follow-up			Total body BMC (g) at follow-up		
	Beta/R2	CIa	p value	Beta/R2	CIa	p value	Beta/R2	CIa	p value
Basic covariates									
BMC at baseline (T0)	*0.97*	*0.92 to 1.020*	*< 0.0001*	*1.061*	*1.02 to 1.10*	*< 0.0001*	*1.046*	*1.011 to 1.082*	*< 0.0001*
Age	*0.023*	*0.0038 to 0.043*	*0.019*	*0.14*	*0.019 to 0.26*	*0.024*	− 0.29	− 4.021 to 3.45	0.88
Sex	− 0.014	− 0.061 to 0.033	0.56	*0.73*	*0.40 to 1.049*	*< 0.0001*	*9.98*	*1.60 to 18.35*	*0.020*
R2[a]	0.80			0.79			0.81		
Bone turnover markers									
OCN (μg/L)	− 0.00026	− 0.0011 to 0.00063	0.57	0.0047	− 0.0017 to 0.011	0.15	*0.17*	*0.0071 to 0.34*	*0.041*
P1NP (μg/L)	*0.00026*	*0.00011 to 0.00040*	*0.0010*	*0.0019*	*0.00084 to 0.0030*	*< 0.0001*	*0.037*	*0.0097 to 0.065*	*0.008*
CTX (ng/L)	0.0000091	− 0.000082 to 0.00010	0.84	− 0.00037	− 0.0010 to 0.00028	0.26	− 0.016	− 0.033 to 0.00068	0.060
Sex hormones									
TEST/SHBG	0.016	− 0.0031 to 0.035	0.10	*0.33*	*0.19 to 0.46*	*< 0.0001*	*9.17*	*5.60 to 12.75*	*< 0.0001*
E2/SHBG	0.00012	− 0.00012 to 0.00036	0.33	0.0013	− 0.00049 to 0.0030	0.16	*0.064*	*0.019 to 0.11*	*0.0060*
PROGE (nmol/L)	0.0020	− 0.037 to 0.041	0.92	*− 0.31*	*− 0.59 to − 0.036*	*0.027*	*− 9.20*	*− 16.39 to − 2.0034*	*0.012*
R2[b]	0.81			0.82			0.85		

Italics = significant association ($p < 0.05$)

BMC bone mineral content, *OCN* osteocalcin, *P1NP* N-terminal propeptide, *CTX* C-terminal telopeptide, *TEST* testosterone, *TEST/SHBG* TEST corrected for SHBG, *E2* estradiol, *E2/SHBG* E2 corrected for SHBG, *PROGE* progesterone, *PTH* intact parathyroid hormone, *25(OH)D* 25-Hydroxy vitamin D, *CORT* cortisol

[a] R2 (variance explained) for the model including BMC at baseline (T1), age and sex (basic covariates)

[b] R2 (variance explained) for the full model including basic covariates, bone turnover markers and sex hormones

The use of biochemical determinations during bone growth, susceptible to be repeated and reflecting actual bone turnover, may give some insight on the BMC value later during growth at the individual level. Detecting subjects at risk of lower bone mass accumulation could support recommendations for preventive measures such as intense physical activity or nutritional supplements. This could be an alternative to repeated DXA examinations that are a concern for many paediatricians, fearing irradiation. Because of the variations in age and in the beginning of pubertal maturation, of different sexes, of day-to-day biological variation of the biochemical markers of bone turnover, it turned out that a small part of the BMC variance 9 months later could be accounted for by circulating markers levels at baseline. This does not mean that bone remodelling/modelling is not determining BMC. But under our experimental conditions, circulating biochemical markers did not appear to be able to explain a large proportion of BMC variance at a later time during growth in a given individual. However, in a model without baseline BMC and age, bone turnover markers and sex hormones were able to account for more than 30% of BMC variance.

Strengths of this study are the large sample of pre- and early pubertal children and adolescents and standardized measurements of BMC/BMD and BM. Blood samples were collected in fasting individuals during short time intervals to minimize diurnal variations and circumventing the problems associated with urine-derived BMs [7]. The use of PCA and SEM analyses allowed the reduction and simplification of multiple BMC outcomes to one latent variable and flexibly modelling complex relationships between constructs while controlling for measurement error [19, 20]. To our knowledge, these methods have never been used before in the field and might therefore provide an interesting basis for future studies. However, PCA and SEM analyses are usually performed in a two-step procedure in different samples [20]. Therefore, our exploratory analyses by SEM should be interpreted with some caution. Further limitations arise from the high pre- and analytical variability of BM investigated in this study and their complex interactions, which challenge their informative value on BMC development [4, 7, 9, 12, 41, 42]. First, BM show analytical variations and a substantial individual day-to-day variation [9, 12, 41, 42]. The variability may have been amplified by the heterogeneity in the cohort which differed by age, sex, pubertal timing all of which representing important determinants of circulating marker levels. The high variability in bone markers, possibly coupled with the fact that we had only a single measurement of BMs, could have led to a dilution bias [43] resulting in a failure to detect some of the associations. Second, the interplay of biomarkers is complex as they are often linked through multiple biological pathways that are still not fully understood (e.g. it is unknown whether E2 acts directly or via mediators on bone) [4, 7, 44]. Third, specific bone turnover markers reflect different processes including modelling, remodelling and longitudinal growth that may occur concurrently at different skeletal sites in children [7, 9, 10]. Altogether these variabilities in bone markers make it difficult to detect an association between markers and BMC. In consequence, these issues add a substantial amount of complexity to the clinical interpretation in pre- and early pubertal children. Other limitations refer to the DXA measurement in growing children, since assessment of the appropriate locations and size of the regions of interest can be challenging [45].

Conclusion

In this study, the main factors explaining BMC were age in cross-sectional analysis, and age, sex and baseline BMC in longitudinal analyses. Although statistically significant, BTM as a construct and P1NP as single factor explained a small part of BMC variance in cross-sectional and longitudinal analyses covering 4 years. Although it is well known that BTM investigated in this study reflect bone metabolism, their singular use for prognostic or diagnostic purposes on BMC development in youth with different age, sex and maturation may be limited. Neither sex nor calciotropic hormones were BMC explanatory variables. Further longitudinal studies should be performed to reveal whether SEM constructs (or models using combinations of repeated single marker measures) can be used to describe bone health and BM associations over the whole period of puberty and to confirm our exploratory analyses. Further, it would be worthwhile to investigate associations between bone markers, hormones and BMC in diseased children.

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Compliance with ethical standards

Conflicts of interest None.

Appendix

Table 4 Percentile distribution (5th, 50th and 95th percentile) of biomarkers (SI units) by sex and different age groups at baseline (T0)

	Girls				Boys				Girls				Boys			
	n	P5	P50	P95	*n*	P5	P50	P95	*n*	P5	P50	P95	*n*	P5	P50	P95
OCN (μg/L)									P1NP (μg/L)							
6.0 to 7.0 years	48	64.25	118.00	188.75	39	80.00	115.00	184.00	48	428.9	655.5	992.5	39	398.0	625.0	931.0
7.1 to 8.0 years	25	48.30	126.00	210.90	30	64.20	105.00	183.60	25	393.5	703.0	1053.1	30	409.6	602.0	852.1
10.0 to 11.0 years	65	74.30	133.00	212.70	46	87.05	125.00	181.80	65	387.6	671.0	1183.2	46	401.7	624.0	961.2
11.1 to 13.0 years	44	110.50	161.00	246.25	60	86.03	146.50	236.70	44	424.0	774.5	1200.0	60	421.3	634.5	1200.0
CTX (ng/L)									SHBG (nmol/L)							
6.0 to 7.0 years	47	549.00	1100.00	1646.00	39	682.00	1110.00	1780.00	46	32.53	96.80	164.00	39	49.50	97.90	175.00
7.1 to 8.0 years	25	509.60	1190.00	1684.00	30	635.80	1000.00	1823.50	25	43.15	87.20	143.90	30	56.69	107.00	174.15
10.0 to 11.0 years	65	704.40	1220.00	1967.00	46	708.90	1090.00	1696.00	63	35.54	71.10	124.20	45	32.11	77.00	120.70
11.1 to 13.0 years	44	745.50	1400.00	2190.00	59	764.00	1310.00	2020.00	43	34.44	68.30	128.40	59	37.90	75.04	130.00
TEST (nmol/L)									TEST/SHBG × 100[a]							
6.0 to 7.0 years	46	0.07	0.07	0.99	39	0.07	0.07	0.64	46	0.05	0.08	0.99	39	0.04	0.08	0.61
7.1 to 8.0 years	25	0.07	0.07	1.67	30	0.07	0.07	0.42	25	0.05	0.11	3.52	30	0.04	0.07	0.40
10.0 to 11.0 years	61	0.07	0.36	1.17	44	0.07	0.25	1.22	60	0.09	0.44	2.33	44	0.06	0.30	3.41
11.1 to 13.0 years	43	0.12	0.64	4.17	53	0.07	0.49	5.86	42	0.15	1.05	3.73	52	0.07	0.71	8.73
E2 (pmol/L)									E2/SHBG × 100[b]							
6.0 to 7.0 years	46	28.99	54.32	172.55	39	19.08	56.52	121.11	46	28.53	56.17	323.99	39	16.17	61.60	156.29
7.1 to 8.0 years	25	31.27	66.06	182.00	30	29.72	54.50	126.38	25	33.23	97.64	320.11	30	22.73	59.35	149.57
10.0 to 11.0 years	63	44.85	95.05	232.90	45	33.03	62.39	154.32	62	41.89	134.55	428.02	45	32.64	88.97	405.58
11.1 to 13.0 years	43	45.73	132.12	365.46	60	19.22	73.95	169.13	43	53.32	237.30	485.20	58	23.86	95.54	375.59
PROGE (nmol/L)									DHEAS (μmol/L)							
6.0 to 7.0 years	46	0.26	0.57	1.95	39	0.10	0.49	1.44	46	0.17	0.74	2.03	39	0.07	0.64	3.04
7.1 to 8.0 years	25	0.27	0.58	1.68	30	0.21	0.60	1.51	25	0.24	0.98	2.66	30	0.11	1.01	2.93
10.0 to 11.0 years	63	0.51	1.13	2.10	45	0.53	0.94	3.91	63	0.79	2.20	4.59	45	0.80	2.55	6.79
11.1 to 13.0 years	42	0.65	1.12	3.62	60	0.30	0.99	3.18	42	0.97	2.55	5.58	59	0.85	2.82	6.65
PTH (pmol/L)									25(OH)D (nmol/L)							
6.0 to 7.0 years	47	2.10	3.21	6.08	39	1.82	3.21	5.38	46	52.59	87.63	126.81	39	42.75	86.50	123.75
7.1 to 8.0 years	25	1.34	3.35	6.40	30	1.60	2.73	4.94	25	42.65	83.25	144.78	30	49.99	90.50	141.66
10.0 to 11.0 years	65	2.06	3.84	7.00	46	1.90	3.23	6.35	63	60.65	84.00	126.85	46	54.39	85.75	135.70
11.1 to 13.0 years	44	1.66	3.68	6.41	60	1.57	3.34	7.41	42	54.98	84.75	133.24	59	61.25	85.00	130.00
CORT (nmol/L)																
6.0 to 7.0 years	46	180.40	318.00	668.85	39	161.00	343.00	692.00								
7.1 to 8.0 years	25	152.30	298.00	777.40	30	206.05	336.00	716.79								
10.0 to 11.0 years	60	186.65	335.85	844.65	44	172.00	347.00	950.95								
11.1 to 13.0 years	42	133.10	291.05	783.90	58	190.15	327.00	638.10								

Percentile distribution for *OCN* osteocalcin, *P1NP* N-terminal propeptide, *CTX* C-terminal telopeptide, *SHBG* sex hormone-binding globulin, *TEST* testosterone, *TEST/SHBG* TEST corrected for SHBG, *E2* estradiol, *E2/SHBG* E2 corrected for SHBG, *PROGE* progesterone, *DHEAS* dehydroepiandrosterone sulphate, *PTH* intact parathyroid hormone, *25(OH)D* 25-Hydroxy vitamin D, *CORT* cortisol

[a] Free androgen index [(TEST/SHBG) × 100]

[b] Free estradiol index [(E2/SHBG) × 100]

Table 5 Results of different structural equation models using different sets of predictors (models a, b, c, d and e) explaining variability of BMC by calciotropic hormones, sex hormones, bone turnover markers and age (n = 309)

	Model a				Model b				Model c				Model d				Model e			
	β[a]	Std. err	βstd.[b]	p value	β[a]	Std. err	βstd.[b]	p value	β[a]	Std. err	βstd.[b]	p value	β[a]	Std. err	βstd.[b]	p value	β[a]	Std. err	βstd.[b]	p value
Calc.h[c] ← BMC	− 0.12	n.a.	0.12	0.39	n.a.				n.a.				n.a.				n.a.			
Calc.h ← PTH	1.0	n.a.	0.49	n.a.	n.a.				n.a.				n.a.				n.a.			
Calc.h ← 25(OH)D	− 1.05	127.1	− 0.30	0.99	n.a.				n.a.				n.a.				n.a.			
Calc.h ← CORT	− 1.04	1.27	− 0.41	0.41	n.a.				n.a.				n.a.				n.a.			
Sex.h[c] ← BMC	n.a.				*0.15*	*0.027*	*0.58*	*< 0.0001*	n.a.				*0.13*	*0.028*	*0.51*	*< 0.0001*	*0.017*	*0.017*	*0.069*	*0.32*
Sex.h ← TEST/SHBG	n.a.				1.0	n.a.	0.62	n.a.	n.a.				1.0	n.a.	0.63	n.a.	1.00	n.a.	0.63	n.a.
Sex.h ← E2/SHBG	n.a.				*0.089*	*0.012*	*0.65*	*< 0.0001*	n.a.				*0.086*	*0.014*	*0.65*	*< 0.0001*	*0.083*	*0.013*	*0.63*	*< 0.0001*
Sex.h ← PROGE	n.a.				*0.004*	*0.001*	*0.57*	*< 0.0001*	n.a.				*0.004*	*0.001*	*0.56*	*< 0.0001*	*0.004*	*0.001*	*0.58*	*< 0.0001*
BTM[c] ← BMC	n.a.				n.a.				*0.26*	*0.041*	*0.38*	*< 0.0001*	*0.15*	*0.05*	*0.22*	*0.0050*	*0.17*	*0.077*	*0.11*	*0.024*
BTM ← OCN	n.a.				n.a.				1.0	n.a.	0.81	n.a.	1.00	n.a.	0.82	n.a.	1.00	n.a.	0.82	n.a.
BTM ← P1NP	n.a.				n.a.				*0.41*	*0.041*	*0.65*	*< 0.0001*	*0.41*	*0.042*	*0.66*	*< 0.0001*	*0.41*	*0.04*	*0.66*	*< 0.0001*
BTM ← CTX	n.a.				n.a.				*0.94*	*0.102*	*0.83*	*< 0.0001*	*0.93*	*0.10*	*0.82*	*< 0.0001*	*0.93*	*0.1*	*0.82*	*< 0.0001*
Growth																				
Age (years) ← BMC	n.a.				n.a.				n.a.				n.a.				*0.78*	*0.05*	*0.78*	< 0.0001
Fit indices	Model a				Model b				Model c				Model d				Model e			
RMSEA	0.027				0.051				0.070				0.087				0.09			
SRMR	0.028				0.015				0.030				0.054				0.05			
CFI	0.999				0.996				0.993				0.970				0.967			
Chi-square (χ^2/df)	9.73	(1.22)			14.04	(1.76)			19.66	(2.46)			74.32	3.10			95.52	(3.18)		

n.a. parameters not included in this model/no p value available (parameter fixed in the model). Italics denote significant associations ($p < 0.05$)

← Regression (e.g. BMC regressed on BTM)

BMC bone mineral content, *Calc.h* calciotropic hormones, *PTH* intact parathyroid hormone, *25(OH)D* 25-Hydroxy vitamin D, *CORT* cortisol, *Sex.h* sex hormones, *TEST/SHBG* testosterone corrected for SHBG, *E2/SHBG* estradiol corrected for SHBG, *PROGE* progesterone, *BTM* bone turnover markers, *OCN* osteocalcin, *P1NP* N-terminal propeptide, *CTX* C-terminal telopeptide

[a] Unstandardized coefficients

[b] Standardized coefficients (BetaS squared = variance explained)

[c] Regression of BMC (latent construct) on the latent construct predictors including calciotropic hormones, sex hormones and BTM

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