Published in final edited form as:

J Pediatr. 2008 October ; 153(4): 484–490. doi:10.1016/j.jpeds.2008.04.028.

Interpretation of Biomarkers of Bone Metabolism in Children: Impact of Growth Velocity and Body Size in Healthy Children and Chronic Disease

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Abstract

Objectives—To determine the effects of growth, maturation, and whole body bone mineral content (WB-BMC) accrual on biomarkers of bone formation [bone specific alkaline phosphatase (BSAP)] and resorption [urine deoxypyridinoline/creatinine (DPD)] in healthy children and children with Crohn's disease.

Study design—BSAP and DPD were measured at baseline, and growth and dual energy x-ray absorptiometry (DXA) WB-BMC were measured at baseline and six months in 202 controls and 110 subjects with Crohn's disease, ages 5 to 21 years. Multivariable linear regression identified determinants of biomarkers in controls and subjects with Crohn's disease.

Results—In controls, BSAP and DPD were significantly and independently associated with sex, Tanner stage, WB-BMC, height velocity, and WB-BMC accrual rates; these covariates explained 77–80% of the variability in bone biomarkers. Subjects with Crohn's disease had lower height-for-age ($p < 0.001$) and WB-BMC-for-height ($p < 0.05$), compared with controls. Crohn's disease was associated with lower BSAP ($p < 0.001$) and greater DPD ($p < 0.001$), independent of growth, maturation, baseline WB-BMC, and WB-BMC accrual, compared with controls.

Conclusions—These data illustrate the potential confounding effects of growth and WB-BMC on bone metabolism biomarkers in children. After adjustment for these effects, Crohn's disease was associated with lower biomarkers of bone formation and greater bone resorption.

Keywords

Crohn's disease; height velocity; bone-specific alkaline phosphatase; urine deoxypyridinoline; dual energy x-ray absorptiometry; bone mineral content

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Edited by TW and WFB

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The authors declare no conflicts of interest.

Biochemical markers are released into the circulation during the process of bone formation and resorption, providing information about the dynamic process of bone metabolism. During bone *remodeling*, bone formation by osteoblasts and bone resorption by osteoclasts are tightly coupled in time and space within the bone multicellular unit.(1) Drugs that promote bone formation (e.g. parathyroid hormone) increase biomarkers of formation and resorption, and drugs that inhibit resorption (e.g. bisphosphonates) decrease biomarkers of formation and resorption.(2) Biomarkers of bone turnover predict fractures and changes in bone mineral density in adults, and can be used to monitor the effectiveness of therapy.(3,4)

Skeletal modeling during growth and development results in new bone formation at a different location than the site of resorption, with alteration in bone shape and net bone accrual. In children, biomarkers of bone metabolism represent the aggregate effects of endochondral bone formation, longitudinal growth, and increases in bone circumference and thickness, as well as bone remodeling.(5,6) The pubertal growth spurt is associated with dramatic elevations in biomarkers of bone metabolism paralleling growth velocity.(6,7)

Biomarkers of bone metabolism in children and adolescents have traditionally been interpreted in the context of sex and age or pubertal stage. (8) However, overall skeletal mass [e.g. whole body bone mineral content (WB-BMC)], height velocity, and rates of bone accrual may also be important determinants. The impact of these factors on biomarkers has not been established and may have important implications for clinical and research applications.

Serum levels of biomarkers of bone formation were significantly reduced in children with newly diagnosed Crohn's disease, and biomarkers of bone resorption were variably reduced in comparison with controls; thus, bone remodeling may be suppressed in incident Crohn's disease due to a primary impairment in bone formation. (9) The accompanying editorial suggested that these findings support the use of anabolic agents as opposed to anti-resorptive agents in childhood Crohn's disease.(10) However, low overall bone mass (i.e. reduced numbers of osteoblasts and osteoclasts) and reduced bone modeling secondary to poor growth and delayed pubertal maturation in children with Crohn's disease may contribute to low biomarker levels, as opposed to reduced bone remodeling alone.

The first objective of this study was to identify independent determinants of biomarkers of bone metabolism in healthy children and adolescents. The second objective was to determine if Crohn's disease was associated with reductions in biomarkers of bone formation and resorption, independent of the poor growth and low bone mass that frequently complicates this chronic disease.(11,12). Of note, the objective of this study was not to identify Crohn's disease specific factors (such as site of disease or therapies) that impact biomarkers of bone metabolism. Rather, the objective was to determine if low biomarker levels in a disease associated with poor growth, delayed maturation, and low bone mass persisted after adjustment for these potential confounding factors.

Methods

Study Subjects

A total of 800 healthy control subjects, ages 5 to 21 years, were recruited from urban and suburban general pediatric practices in the greater Philadelphia area, and through newspaper advertisements. Controls were ineligible if they had a height or body mass index (BMI) below the 3rd percentile for age and sex, or chronic illnesses or medications that may affect growth, nutritional status, dietary intake, pubertal development or bone mineral accrual. Among these, a random subset of 300 subjects were enrolled in a longitudinal study of bone health with repeat anthropometry and dual energy x-ray absorptiometry (DXA) scans after six and twelve months.

Children and adolescents with new-onset or previously diagnosed Crohn's disease, between the ages of 5 and 21 years, were recruited from the Children's Hospital of Pennsylvania (CHOP) and regional gastroenterology practices. The diagnosis of Crohn's disease was confirmed histologically. Subjects were ineligible if they had other medical conditions unrelated to Crohn's disease, such as chronic kidney disease or diabetes, that could potentially affect growth or bone mineral accrual. Given the changes in growth and nutritional status following initiation of therapy among children with newly diagnosed Crohn's disease (13), the data included in this analysis were limited to the 110 children and adolescents with baseline measures obtained at least six months after Crohn's disease diagnosis, and repeat DXA scans and anthropometric measures obtained six months after the baseline measures.

The study protocol was approved by the Institutional Review Board at the Children's Hospital of Philadelphia. Informed consent was obtained directly from study subjects older than 18 years, and assent along with parental consent from subjects less than 18 years of age.

Laboratory tests

Biomarkers of bone metabolism were collected at the baseline visit only. Serum bone specific alkaline phosphatase (BSAP, $\mu\text{g/L}$) was measured as a marker of bone formation in all subjects with Crohn's disease, and control subjects who agreed to undergo phlebotomy. BSAP was performed at Quest Diagnostics Laboratories (San Juan Capistrano, CA) using a two-site immunoradiometric assay with an inter-assay coefficient of variation (CV) of 8.5%. BSAP was chosen as the biomarker of bone formation because of its high bone specificity, lack of circadian variability, and ease of comparability with prior studies.(4,6) The ratio of deoxypyridinoline to creatinine (DPD, nmol/mmol creatinine), as measured on a second morning void, was used as a marker of bone resorption. Urine DPD was assayed using high performance liquid chromatography at Quest Diagnostics Laboratories (San Juan Capistrano, California), with an inter-assay CV between 6.2% and 7.8%. DPD was chosen as the biomarker of bone resorption because of its low extraosseous concentrations, long-term stability in urine, and osteoclast specificity.(4,6)

Among the 202 controls with baseline and 6 month measures of growth and bone mass, 101 assented to phlebotomy for serum BSAP at the baseline visit. Of note, there were no significant differences in sex or racial distributions, growth or bone measures according to availability of BSAP values. However, the subjects that declined phlebotomy were significantly younger (11.4 vs. 12.9 yrs, $p < 0.001$). A total of 172 (85%) of the control subjects provided urine for DPD measurement.

DXA

Whole body DXA scans were obtained at the baseline and six-month visit using a Hologic Delphi densitometer (Hologic, Inc. Bedford, MA; software version 12.4). The measurements were obtained with standard positioning techniques and were analyzed to generate estimates of WB-BMC (g) and whole body lean mass (WB-LM, kg). The DXA instrument was calibrated using a hydroxyapatite spine phantom daily and with a whole body phantom three times per week. The in vitro CV at our institution is less than 0.6%; the in vivo CV is less than 1%.

Anthropometric and Pubertal Development

At each study visit, weight and height were measured using a digital scale to the nearest 0.1 kg (Scaltronix, White Plains, NY) and a stadiometer to the nearest 0.1 cm (Holtain Ltd., Crosswell, Crymch, United Kingdom), respectively. Pubertal development stage was assessed via a validated self-assessment questionnaire,(14,15) and classified according to the method of Tanner.(16)

Statistical Analysis

Statistical analyses were performed using STATA 9.0 (Stata Corporation, College Station, Texas). Descriptive analyses included means, standard deviations, medians, and ranges of continuous variables, and distributions of categorical variables. Differences of means were assessed using Student's t-test if the variable was normally distributed. The Wilcoxon rank-sum test was used if these data were not normally distributed. Group differences in categorical variables were assessed using the Chi-square test. Two-sided tests of hypotheses were used, and a p value < 0.05 was considered statistically significant.

Height and body mass index (BMI) were converted to age- and sex-specific standard deviation scores (z-scores) using the National Center for Health Statistics 2000 Centers for Disease Control reference data with the LMS method.(17) In order to assess WB-BMC relative to height, the LMS method was used to generate race (black vs. non-black) and sex-specific z-scores for WB-BMC relative to height based on the cross-sectional data in the entire sample of 800 healthy controls.(18,19)

Potential determinants of BSAP and DPD in the control cohort included age, sex, race, baseline height, baseline Tanner stage, baseline WB-BMC, height velocity (cm/yr), and WB-BMC accrual velocity (g/yr). BSAP, DPD, WB-BMC, WB-LM, height velocity, and bone accrual velocity were non-normally distributed. These variables were transformed to achieve normal distributions using the *lnskew0* function (natural log transformation) in Stata 9.0. Multivariable linear regression models were used to determine the independent effects of each of these potential covariates. Polynomial terms were used as indicated to improve the fit of the models if graphical displays of the data suggested non-linear relationship between BSAP or DPD and the covariate of interest. Furthermore, since urine DPD is normalized to urine creatinine, WB-LM was included in the regression analysis for DPD to account for the potential effects of muscle mass on urinary creatinine excretion. Multiplicative interaction terms were used to identify potential interactions between sex and Tanner stage given sex differences in growth and maturation. Finally, in order to determine the independent effect of Crohn's disease on bone biomarker levels, sequential, multivariable linear regression using Crohn's disease status and significant covariates from the control regression analysis was performed.

RESULTS

Subject Characteristics

Baseline subject characteristics are summarized in Table I. There were proportionally fewer black subjects among the cohort with Crohn's disease, consistent with the racial distribution of Crohn's disease and the demographics of the control catchment area. A smaller proportion of subjects with Crohn's disease were pubertal. Adjusted for age, subjects with Crohn's disease were at earlier Tanner stages, compared with controls (p<0.05), consistent with delayed pubertal maturation. Subjects with Crohn's disease had significantly lower height z-scores (p < 0.001), BMI z-scores (p < 0.01) and WB-BMC-for-height z-scores (p < 0.05), compared with healthy controls. Height velocity over the 6 month interval was significantly lower in children with Crohn's disease compared with controls, adjusted for sex and Tanner stage: Subjects with Crohn's disease grew, on average, 0.65 cm less per year [95% confidence interval: -1.23, -0.06, p < 0.05] than the healthy controls. Subjects with Crohn's disease had a median disease duration of 7.5 months (range 6 to 26 months) at the time of biomarker measurement. Thirteen (12%) of the subjects with Crohn's disease were receiving or had received Infliximab therapy and 66 (60%) had a history of previous or current systemic corticosteroid therapy at the time of bone biomarker measurement. Pediatric Crohn's Disease Activity Index (PCDAI) scores were available in 94 (85%) of the subjects with Crohn's disease: 54 had inactive disease

(PCDAI ≤ 10), 31 had mild (11–30), 5 had moderate (31–40), and 4 had severe disease activity (>40).

Biomarkers of Bone Metabolism

Healthy Controls—BSAP and DPD levels were examined according to Tanner stage within males and females. The results are summarized in Figure 1 and Figure 2 (available at www.jpeds.com). In males, both BSAP and DPD were comparable across Tanner stages 1 to 4, and significantly lower in Tanner stage 5 ($p < 0.001$). In females, BSAP and DPD levels were comparable across Tanner stages 1 to 3, significantly lower in Tanner stage 4 compared with earlier Tanner stages ($p < 0.001$), and even lower in Tanner stage 5 ($p < 0.001$). Therefore, for the purposes of biomarker regression analysis, Tanner stage was categorized as stages 1 to 3, stage 4, and stage 5. The decrement in BSAP levels in Tanner stage 4 in the females (but not males), is consistent with the observation that bone mineral accrual and growth velocity decline after menarche in females (Tanner stage 3 – 4) and after Tanner stage 4 in males.(20, 21)

Univariate analyses revealed that BSAP and DPD were significantly and positively correlated with height velocity (BSAP: $r=0.83$, DPD: $r=0.76$, both $p<0.001$) and WB-BMC accrual velocity (BSAP: $r=0.65$, DPD: $r=0.44$, both $p<0.001$); but negatively correlated with baseline age (BSAP: $r=-0.73$, DPD: $r=-0.75$), height (BSAP: $r=-0.54$, DPD: $r=-0.63$), and WBBMC (BSAP: $r=-0.63$, DPD: $r=-0.71$)(all $p<0.0001$). These negative correlations were consistent with the lower or absent height velocity in the oldest, tallest subjects who had the lowest biomarker levels. The strong associations between height velocity and the biochemical markers of bone metabolism are illustrated in Figures 3 and Figure 4 (available at www.jpeds.com).

The results of the multivariable linear regression model for independent determinants of BSAP levels in healthy controls are presented in Table II. Advanced Tanner stage (4 and 5), height velocity, and WB-BMC accrual rates were significant and independent determinants of BSAP levels. The significant negative interaction between sex and Tanner stages 4 and 5 illustrates the greater decrement in BSAP in Tanner stage 4 and 5 females, compared with males. Race was not significant in the full model and is not included. Sex, Tanner stage, and their interaction explained 62% of the variability in BSAP levels among controls ($R^2s = 0.62$). Inclusion of measures of growth velocity, bone mass, and WB-BMC accrual rates increased the R^2 to 0.80. That is, the combined variables summarized in Table II explain 80% of the variability in BSAP levels.

As shown in Table III (available at www.jpeds.com), a similar linear regression model was developed for DPD; confirming that advanced Tanner stage, baseline WB-BMC, height velocity and WB-BMC accrual rates were independent and significant determinants of DPD levels in healthy controls. Race was not significant in the full model and is not included. Sex, advanced Tanner stage, and their interaction explained 47% of the variability in DPD levels ($R^2 = 0.47$). Adjustment for height velocity, bone mass and bone accrual rates increased the R^2 to 0.77. Finally, in order to determine if differences in lean mass impacted urine DPD levels, WB-LM was included in the model, and was not significant (data not shown).

Crohn's Disease—A series of sequential multivariate analyses were used to examine differences in biomarkers of bone metabolism in subjects with Crohn's disease and controls, adjusted for differences in growth, maturation, and bone accrual rates (Table IV and Table 5; available at www.jpeds.com). Each row in these tables represents a separate model examining the independent effect of Crohn's disease status on the biomarker levels, adjusted for the covariates listed in the left column. In the base model, BSAP was adjusted for Tanner stage, sex, and their interaction. Crohn's disease was associated with significantly lower BSAP levels compared with controls ($p < 0.001$). In order to determine if Crohn's disease status was

associated with lower BSAP levels, independent of poor growth and low bone mass, the models were sequentially expanded to incorporate WB-BMC, growth velocity, and WB-BMC accrual velocity. The overall magnitude of the effect of Crohn's disease on BSAP (indicated by the β -coefficient) in the regression analysis remained largely unchanged with the sequential addition of each covariate. The finding of lower BSAP in Crohn's disease remained significant in the fully adjusted model. To illustrate the magnitude of the differences, a typical Tanner IV control female has a BSAP of 45 $\mu\text{g/L}$ and a Tanner IV female with Crohn's disease has a BSAP of 33.7 $\mu\text{g/L}$, adjusted for average values of the other covariates.

In contrast to BSAP, urine DPD levels were significantly greater in subjects with Crohn's disease compared with controls ($p < 0.01$). The β -coefficients in Table V represent the difference in log transformed DPD levels between

Subjects with Crohn's disease and controls, adjusted for Tanner stage, sex, and their interaction. In order to determine if lower growth rates or bone mass in subjects with Crohn's disease resulted in an underestimate of the elevation in bone resorption, the models were sequentially expanded to include measures of WB-BMC, height velocity, and WB-BMC accrual rates. In the sequentially adjusted models, the finding of a higher DPD in Crohn's disease remained significant. Finally, in order to determine if lower muscle mass, and attendant reductions in urinary creatinine excretion in children with Crohn's disease contributed to the elevated DPD to creatinine ratio, the model was adjusted for WB-LM. This did not attenuate the elevation in DPD in Crohn's disease (data not shown). To illustrate the differences, a typical Tanner IV control female has a DPD value of 49.4 nmol/mmol creatinine and a similar Tanner IV female subject with Crohn's disease has a value of 61.3 nmol/mmol creatinine, adjusted for the average values of the other covariates.

Additional multivariate linear regression models were constructed to examine the independent associations of disease activity or corticosteroid exposure on BSAP and DPD levels within the subjects with Crohn's disease, adjusted for height velocity, Tanner stage, baseline WB-BMC, and changes in WB-BMC. These analyses revealed that the PCDAI score was negatively and independently associated with BSAP levels (β -coefficient of -0.11 , 95% CI: -0.19 , -0.04 , $p < 0.01$); that is, greater disease activity was associated with lower BSAP levels. However, PCDAI was not significantly associated with DPD levels. Current or prior corticosteroid exposure was not significantly associated with BSAP or DPD levels in the multivariate models.

DISCUSSION

It is well-recognized that biomarkers of bone metabolism vary significantly with age, sex, and Tanner stage, with a peak during the pubertal growth spurt following by a rapid decline to adult levels.(8,22–25) Although prior studies have demonstrated that biomarkers of bone metabolism were positively correlated with growth velocity, (7,24) these studies were limited to correlations between biomarkers and growth velocity without consideration of the independent effects of pubertal maturation, bone mass, and bone accrual on biomarker levels. Our data in healthy controls demonstrates that Tanner stage, sex, WB-BMC, height velocity, and WB-BMC accrual rates were all significant and independent determinants of bone biomarker levels during childhood and adolescence. That is, among subjects of the same sex and Tanner stage, with comparable growth velocity; those with greater bone mass and bone mineral acquisition rates had greater biomarker levels. Similar results were seen for biomarkers of both bone formation and resorption, reflecting the synchronized modeling activities that result in linear growth and bone accrual.

Biomarkers of bone metabolism are frequently used to assess metabolic bone disorders and bone remodeling in chronic diseases. Childhood chronic diseases are frequently associated

with poor growth, delayed maturation, low bone mass and poor bone accrual. We hypothesized that reports of low bone biomarker levels for age and sex in childhood chronic disease reflected the aggregate effects of low bone mass (and hence fewer bone cells) for age and sex, reduced modeling activity, and reduced endochondral growth - rather than suppressed bone remodeling within the multicellular unit. Therefore, we used childhood Crohn's disease as a model of a disorder that is associated with poor growth, delayed maturation, low bone mass, and poor bone accrual in order to determine if these factors explained the low bone biomarker levels reported in this disease.

The data presented here demonstrate that Crohn's disease was associated with lower BSAP levels and greater DPD levels, adjusted for sex and Tanner stage, compared with controls. We anticipated that adjustment for low growth velocity, low bone mass, and impaired WB-BMC accrual would attenuate, or even eliminate the BSAP deficit in children with Crohn's disease. Interestingly, the magnitude of the BSAP deficit in Crohn's disease compared with controls (Table IV) was unchanged in the fully adjusted model. Similarly, we anticipated that the same adjustments would attenuate the elevations in DPD seen in children with Crohn's disease. In contrast, the magnitude of the DPD excess increased in Crohn's disease with the sequential addition of covariates (Table V).

The opposing effects of Crohn's disease on bone formation and resorption may reflect the effects of glucocorticoid therapy or inflammatory cytokines to suppress osteoblasts and promote osteoclastogenesis.(26) *In vitro* studies have shown that serum from newly diagnosed pediatric subjects with Crohn's disease was associated with lower *in vitro* rat osteoblast activity when compared with serum from healthy controls.(27) Furthermore, rat bone cultures exposed *in vitro* to sera of patients with Crohn's disease manifested evidence of decreased mineralization and disorganized osteoid deposition.(28) Experiments using human osteoblastic cell cultures have confirmed decreased BSAP levels associated with exposure to the inflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-1 β .(29) The effects of inflammatory cytokines on osteoclasts have also been well elucidated. Patients with Crohn's disease have elevated levels of inflammatory cytokines including TNF- α , IL-1, and IL-6.(30) TNF- α stimulates osteoclast proliferation and differentiation through its effects on nuclear factor κ B (NF κ B) activity and its ability to increase receptor activator of the NF κ B ligand (RANKL) production; both of which are important for osteoclastogenesis.(31) Recent studies in adults with Crohn's disease have shown significant changes in biomarkers of bone metabolism with anti TNF- α therapy.(32,33) Of these, Franchimont et al. reported decreased bone formation and increased bone resorption markers at baseline when compared with healthy controls.(33) The observation that bone formation was low and bone resorption was high prior to treatment with anti TNF- α therapy is consistent with our observation of low BSAP and high DPD in Crohn's disease. The observation that greater disease activity (PCDAI score) was independently associated with greater reductions in BSAP levels in our study further supports the hypothesis that inflammatory cytokines contribute to suppressed bone formation in Crohn's disease.

Our finding of reduced BSAP levels in children with Crohn's disease is in agreement with the study by Sylvester et al of 23 children with Crohn's disease and 40 controls.(9) However, Sylvester, et al reported that Crohn's disease was associated with inconsistent alterations in biomarkers of bone resorption: urinary N-telopeptides of collagen levels were significantly lower in subjects with Crohn's disease compared with controls, but urinary DPD levels were not. There are multiple important differences between these studies that may explain the opposing results for bone resorption biomarkers. First, Sylvester et al examined subjects with Crohn's disease at the time of diagnosis. In contrast, our study was limited to prevalent subjects and the majority had been treated with corticosteroids. Therefore differences in disease activity and medication exposures may have contributed to the differences in bone resorption results.

However, in a subsequent study by Sylvester et al, bone resorption markers did not change significantly over the first year of therapy, despite marked reductions in disease activity and frequent corticosteroid use.(34) Possible explanations for the lack of elevated resorption markers in Crohn's disease in the Sylvester study relate to differences in study design and analytic approach. With our large cohort of healthy controls, we showed that bone resorption biomarkers were significantly associated with indicators of growth, maturation and skeletal size, and our analyses adjusted for these factors. The studies by Sylvester et al. did not have sufficient numbers of controls (22 males and 18 females across the entire pediatric age range) to adjust for the effects of sex and Tanner stage. Their analysis did not adjust for differences in growth and bone mass, and they likely had inadequate power to detect differences due to the small number of subjects with Crohn's disease and controls.

Our study has multiple limitations. First, biomarkers of bone metabolism are surrogate measures of bone turnover. Gold standard measures of bone formation such as double-labeled tetracycline bone biopsies were not obtained. However, numerous studies have demonstrated good correlations between biomarkers of bone metabolism and the actual rate of bone turnover, as quantified by calcium kinetics studies(35), or by histomorphometric analyses of labeled bone biopsies.(36) Second, biomarkers of bone metabolism in this study were only measured at a single time-point at study enrollment. These are dynamic markers of bone metabolism and would be expected to change with the changes in bone mass that occur with growth. However, we were able to explain 80% of the variability in turnover markers in the healthy controls. Third, an implicit assumption of this study is that the height velocity in the six months subsequent to the time the biomarkers of bone metabolism were measured was representative of the growth velocity at the time of the biomarker measures. Given the strong correlations between height velocity and turnover markers, this assumption is reasonable. Fourth, the single measures of biomarkers of bone metabolism in the subjects with Crohn's disease reflect recent disease activity and exposure to bone-active therapies. Our inability to demonstrate an association between corticosteroid exposure and biomarker levels likely reflects the opposing effects of disease activity and corticosteroid on bone metabolism. Future studies are needed examining repeated measures of bone biomarkers, WB-BMC, disease activity and concurrent therapies to determine the relations between changes in biomarkers and concurrent bone accretion rates in healthy children and children with chronic disease. Finally, the use of a self-assessment tool to assess Tanner stage is a limitation; however, we have validated this tool in children with Crohn's disease.(14)

These data illustrate that interpretation of biomarkers of bone metabolism results, especially within a research context, is complex and requires consideration of growth, skeletal size, pubertal maturation, and the presence of underlying chronic inflammation. In Crohn's disease, lower levels of bone formation markers cannot be explained entirely by the impaired growth, delayed pubertal maturation, and sub-optimal bone mass accrual that occurs in these patients. Given the suppressed bone formation and increased bone resorption observed in these subjects, therapeutic approaches may include anabolic and anti-resorptive agents. Future studies employing bone biopsies and imaging techniques, such as quantitative computed tomography are needed to determine the effects of Crohn's disease on remodeling and modeling, and to determine the impact of such therapies on bone accrual in children with Crohn's disease.

Acknowledgements

This study was supported by NIH grant R01 DK60030 and the Clinical and Translational Research Center (UL1-RR024134) at the Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine.

List of abbreviations

BMI, Body mass index
 BSAP, Bone specific alkaline phosphatase
 CV, Coefficient of variation
 DPD, Urine deoxypyridinoline to creatinine ratio
 DXA, Dual energy x-ray absorptiometry
 IL-1 β , Interleukin-1 β
 NF κ B, Nuclear factor κ B
 OPG, Osteoprotegerin
 PCDAI, Pediatric Crohn's Disease Activity Index
 RANKL, Receptor activator of the NF κ B ligand
 TNF- α , Tumor necrosis factor- α
 WB-BMC, Whole body bone mineral content
 WB-LM, Whole body lean mass

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Figure 1. Box-plot of log-transformed bone-specific alkaline Phosphatase (BSAP) by Tanner stage (1–5) stratified according to sex in healthy controls.

Figure 2. Box-plot of log-transformed urine deoxypyridinoline to creatinine(DPD) by Tanner stage (1–5) stratified according to sex in healthy controls.

Figure 3.
Plot of log-transformed bone-specific alkaline Phosphatase (BSAP) versus log-transformed height velocity in male and female healthy controls. (▲) = males, (○) = females, (—) = fitted regression line

Figure 4. Plot of log-transformed urine deoxypyridinoline to creatinine (DPD) versus log-transformed height velocity in male and female healthy controls. ($\blacktriangle$) = males, ($\circ$) = females, (—) = fitted regression line

Table I
Demographic and Anthropometric Characteristics in Subjects with Crohn's Disease and Healthy Controls

	Crohn Disease N = 110	Controls N = 202	p-value
Age (yr)	13.3 ± 2.9	12.6 ± 4.6	NS
Sex (% male)	57%	47%	NS
Race (% black)	5%	25%	< 0.001
Pubertal (% Tanner 4 & 5)	34%	46%	< 0.05
Height Z-score	-0.43 ± 1.07	0.31 ± 0.89	< 0.001
BMI Z-score	-0.12 ± 0.90	0.18 ± 0.92	< 0.01
WB-BMC-for-Height Z-score	-0.24 ± 1.11	0.05 ± 0.96	< 0.05

Table 2Determinants of BSAP Levels in Healthy Children and Adolescents Log Transformed Serum BSAP ($R^2 = 0.80$)

Covariates	β -coefficient (95% CI)	p-value
Tanner stage 4	0.40 (0.13, 0.68)	< 0.01
Tanner stage 5	0.33 (0, 0.66)	< 0.05
Female sex	0.03 (−0.06, 0.13)	0.5
Tanner stage 4 - sex interaction	−0.23 (−0.39, −0.07)	< 0.01
Tanner stage 5 - sex interaction	−0.22 (−0.39, −0.05)	< 0.05
Baseline WB-BMC	3.48 (−0.25, 7.2)	0.07
Baseline (WB-BMC) ²	−0.26 (−0.50, −0.01)	< 0.05
Height velocity (cm/yr)	0.66 (0.30, 1.01)	< 0.001
WB-BMC accrual velocity (g/yr)	0.79 (0.40, 1.19)	< 0.001

Table 3

Determinants of DPD Levels in Healthy Children and Adolescents Log Transformed Urine DPD:Creatinine Ratio ($R^2 = 0.77$)

Covariates	β -coefficient (95% CI)	p-value
Tanner stage 4	0.16 (-.10, 0.42)	0.2
Tanner stage 5	0.38 (0.08, 0.68)	< 0.05
Female sex	0.04 (-0.03, 0.12)	0.3
Tanner stage 4 – sex interaction	-0.05 (-0.20, 0.10)	0.5
Tanner stage 5 - sex interaction	-0.25 (-0.41, -0.09)	< 0.01
Baseline WB-BMC	5.52 (2.23, 8.80)	< 0.01
Baseline (WB-BMC) ²	-0.42 (-0.64, -0.19)	< 0.001
Height velocity (cm/yr)	0.74 (0.49, 1.37)	< 0.001
WB-BMC accrual velocity (g/yr)	0.93 (0.58, 1.49)	< 0.001

Table IV

Sequential Multivariate Model of the Independent Effect of Crohn's Disease on Log Transformed BSAP

Covariates	Crohn Disease Effect ^{**} β-coefficient (95% CI)	p-value ^{***}	R ²
Base model[*]	−0.12 (−0.19, −0.05)	<0.001	0.45
Base model[*] + WB-BMC + (WB-BMC)²	−0.13 (−0.19, −0.06)	<0.001	0.53
Base model[*] + WB-BMC + (WB-BMC)² +height velocity	−0.09 (−0.15, −0.03)	<0.01	0.64
Base model[*] + WB-BMC + (WB-BMC)² +height velocity + WB-BMC accrual velocity	−0.10 (−0.15, −0.05)	<0.001	0.67

* Base model: adjusted for sex, advanced Tanner stage, and sex –Tanner stage interactions.

** The β-coefficient represents the difference between log transformed bone biomarker levels in Crohn's disease compared with controls after the sequential addition of each covariate to the model.

*** This p-value represents the p-value associated with Crohn's disease status after the sequential addition of each covariate to the model.

Table V

Sequential Multivariate Model of the Independent Effect of Crohn's Disease on Log Transformed Urine DPD:Creatinine Ratio

Covariates	Crohn Disease Effect ** β -coefficient (95% CI)	p-value ***	R ²
Base model *	0.11 (0.04, 0.18)	<0.01	0.47
Base model * + WB-BMC + (WB-BMC)²	0.12 (0.05, 0.18)	<0.001	0.60
Base model * + WB-BMC + (WB-BMC)² + height velocity	0.15 (0.09, 0.21)	<0.001	0.66
Base model * + WB-BMC + (WB-BMC)² + height velocity + WB-BMC accrual velocity	0.15 (0.09, 0.21)	<0.001	0.67

* Base model: adjusted for sex, advanced Tanner stage, and sex –Tanner stage interactions.

** The β -coefficient represents the difference between log transformed bone biomarker levels in Crohn's disease compared with controls after the sequential addition of each covariate to the model.

*** This p-value represents the p-value associated with Crohn's disease status after the sequential addition of each covariate to the model.