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Changes in Vitamin D and PTH Metabolism in Incident Pediatric Crohn Disease

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Abstract

Background and Aims—Prior studies of vitamin D metabolism in Crohn disease (CD) did not include controls or examine changes following diagnosis. This study examined associations among 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)₂D] and parathyroid hormone (PTH) levels in incident pediatric CD, compared with controls, and following diagnosis.

Methods—Serum vitamin D and PTH were measured at diagnosis (n = 78), 6, 12, and a median of 43 months (n = 52) later in CD participants, and once in 221 controls. Multivariate regression was used to examine baseline associations, and quasi-least squares regression to assess subsequent changes.

Results—At diagnosis, 42% of CD participants were 25(OH)D deficient (<20 ng/mL). The odds ratio for deficiency was 2.1 (95% CI 1.1, 3.9; p<0.05) vs. controls, adjusted for age, race, and season. 1,25(OH)₂D was lower in CD vs. controls (p<0.05), adjusted for 25(OH)D, tumor necrosis factor- α (TNF- α) and PTH. TNF- α was associated with lower 1,25(OH)₂D (p<0.05), and the positive association between PTH and 1,25(OH)₂D in controls was absent in CD (interaction p=0.02). Among participants with 25(OH)D <30 ng/mL, CD was associated with lower PTH (p<0.05) vs. controls. Following diagnosis, 25(OH)D and 1,25(OH)₂D improved (p<0.001). At the final visit, 3% were 25(OH)D deficient, PTH was no longer low relative to 25(OH)D, and 1,25(OH)₂D was significantly elevated (p<0.001), compared with controls.

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Conclusions—Incident CD was associated with 25(OH)D and 1,25(OH)₂D deficiency and a relative hypoparathyroidism that resolved following diagnosis. Inflammatory cytokine suppression of PTH and renal 1- α -hydroxylase may contribute to these alterations.

Keywords

Crohn disease; children; 25(OH)D; 1,25(OH)₂D; parathyroid hormone

INTRODUCTION

Adequate serum Vitamin D levels are essential for musculoskeletal health.¹⁻² Circulating 25-hydroxyvitamin D [25(OH)D] is converted by renal 1- α -hydroxylase to 1,25-dihydroxyvitamin D [1,25(OH)₂D]. This process is largely regulated by parathyroid hormone (PTH) to maintain mineral homeostasis. The recent recognition that most cells in the body have the vitamin D receptor and many possess 1- α -hydroxylase has provided new insights into non-classical autocrine/paracrine actions of vitamin D. Vitamin D deficiency may also play a role in cardiovascular disease, diabetes, malignancy, infectious diseases, and abnormalities in innate and adaptive immunity.³

Crohn disease is an idiopathic, chronic inflammatory condition of the gastrointestinal tract associated with defective innate immune regulation of the intestinal bacterial load. Recent studies suggest that vitamin D deficiency may contribute to the pathogenesis and progression of inflammatory bowel disease (IBD).⁴⁻⁶ In addition, inflammatory cytokines in IBD may alter vitamin D metabolism by inhibiting the expression of renal 1- α -hydroxylase⁷, stimulating the expression of colonic 1- α -hydroxylase⁸, and upregulating the parathyroid calcium-sensing receptor, leading to relatively lower PTH levels.⁹⁻¹⁰ The relations among circulating 25(OH)D, 1,25(OH)₂D and PTH levels in patients with Crohn disease have not been addressed.

Prior studies of vitamin D deficiency in children with IBD are largely limited to cross-sectional studies in heterogeneous samples of children with variable disease severity and duration.¹¹⁻¹⁶ Given the high prevalence of vitamin D deficiency among otherwise healthy children and adults, studies of vitamin D deficiency in Crohn disease should include healthy controls in order to determine the independent effects of Crohn disease. We recently reported that 26% of otherwise healthy children had vitamin D deficiency [25(OH)D <20 ng/mL]; significant independent risk factors for vitamin D deficiency included black race, winter season, low vitamin D intake, and older age.¹⁷

The present study improves upon previous investigations of vitamin D status in pediatric Crohn disease by following an incident cohort of participants from diagnosis through an extended period of follow-up exceeding two years, and including healthy controls. Additional covariates include serum cytokine levels and measures of body composition. The aims of this study are to characterize serum 25(OH)D, 1,25(OH)₂D, and PTH levels at Crohn disease diagnosis, compared with controls, and to identify correlates of changes in vitamin D and PTH levels following diagnosis and therapy.

MATERIALS AND METHODS

Study Participants

Incident Crohn disease participants, ages 5-21 years, were recruited from the IBD Center of the Children's Hospital of Philadelphia between 2002-2005, as previously described.¹⁸⁻²⁰ Baseline visits were completed within two weeks of diagnosis, and follow-up visits were completed six and twelve months later. A long-term follow-up (LTFU) visit was completed a median(range) of 43(24-63) months after diagnosis between 2006-2007. Healthy control participants were enrolled from general pediatric clinics in the greater Philadelphia area between 2002-2007 and completed one visit. Controls were excluded for any chronic illness or medication affecting growth and development.

The study protocol was approved by the Institutional Review Board at the Children's Hospital of Philadelphia. Informed consent was obtained from young adult participants and the parents or guardians of participants under the age of 18 years. Assent was obtained from participants between 7 and 18 years of age. Study participants and their parents identified participant race according to the National Institute of Health categories.

Crohn disease Characteristics and Definitions

Crohn disease was confirmed by radiographic, endoscopic, histologic, and clinical parameters, as previously described.¹⁸⁻²⁰ Crohn disease activity was assessed at the time of the study visit using the Pediatric Crohn disease Activity Index (PCDAI), with scores ranging from 0-100.²¹ Data on duration of symptoms at diagnosis, extraintestinal manifestations, medications, and nutritional supplements at each visit were collected. Vitamin D supplementation (ergocalciferol or cholecalciferol as part of a multivitamin, single supplement, or caloric supplement) was ascertained through a questionnaire and review of medical records, and summarized as the average international units (IU) per day. Dietary vitamin D intake was not assessed.

Anthropometry, Body Composition, and Pubertal Development

Weight and height were measured using a digital scale to the nearest 0.1 kg (Scaltronix, White Plains, NY) and a wall-mounted stadiometer to the nearest 0.1 cm (Holtain Ltd., Crosswell, Crymmych, UK), respectively. Pubertal status was assessed with a validated self-assessment questionnaire²² and classified according to the method of Tanner.²³ Whole body lean mass (kg) and fat mass (kg) were assessed by dual energy x-ray absorptiometry (DXA) using a Hologic Delphi densitometer (Bedford, MA) with a fan beam in the array mode (software version 12.4).²⁴ The baseline and longitudinal anthropometry, maturation and body composition data have been reported.^{18, 20}

Laboratory Studies

Laboratory studies included serum hematocrit, erythrocyte sedimentation rate, albumin, creatinine in the Crohn disease participants and magnesium in a subset of Crohn disease participants; assays were performed using standard techniques in the clinical laboratories at the Children's Hospital of Philadelphia. Estimated glomerular filtration rate (eGFR) was calculated using the pediatric Schwartz formula.²⁵ Serum 25(OH)D and 1,25(OH)₂D levels

were performed by radioimmunoassay (RIA) with I¹²⁵-labeled tracer.²⁶ The intra-assay coefficient of variation (CV) for 25(OH)D was 2.2% and for 1,25(OH)₂D was 7-11%.²⁷ Vitamin D samples were batched, and assays were not performed in real time. Given this lag, the levels were not provided to the clinicians. Serum intact PTH and total calcium were measured by Quest Diagnostics (Nichols Institute, San Juan Capistrano, California) using a chemiluminescence assay with an interassay CV of 7-9% and spectrophotometry, respectively. Serum tumor necrosis factor- α (TNF- α) (sensitivity: 0.05 pg/mL, inter-assay variation: 8.3%) and interleukin-6 (IL-6) (sensitivity: 0.10 pg/mL, inter-assay variation: 7%) levels were measured using the LuminexTM platform and the human cytokine six-plex high sensitivity antibody bead kit (Millipore, Billerica, MA). Serum BSAP was performed using a two-site immunoradiometric assay with an inter-assay coefficient of variation (CV) of 8.5%, and the ratio of urine deoxypyridinoline (DPD) to creatinine from a second morning void was assayed using high performance liquid chromatography with an inter-assay CV between 6.2% and 7.8% at Quest Diagnostics Laboratories (San Juan Capistrano, CA).

All of the above laboratory parameters were obtained in the Crohn disease participants at each study visit, except for serum cytokines, which were obtained at baseline, 6 months, and LTFU. Serum 25(OH)D, 1,25(OH)₂D, PTH, cytokine, and bone biomarker levels were obtained once in the controls using the assays and laboratories described above.

Statistical Analysis

Analyses were conducted using Stata 11.0 (Stata Corporation, College Station, TX). A two-tailed p-value of <0.05 was the criterion for statistical significance. Differences in continuous variables were assessed using Student's t-test or the Wilcoxon's rank sum test, as appropriate. Correlations between continuous variables were assessed by Pearson product moment correlations or Spearman rank correlation tests, as appropriate. Log-transformed values for serum 25(OH)D, 1,25(OH)₂D, PTH, and cytokine levels were used in the linear regression models.

Height and body mass index (BMI, kg/m²) were converted to age- and sex-specific Z-scores using national reference data.²⁸ Whole body lean mass and fat mass were assessed as race- and sex-specific Z-scores relative to height, using the LMS method, as previously described.^{18, 20, 29} Z-scores were created for BSAP and DPD relative to sex and Tanner stage of maturation, as previously described.³⁰ Given the strong association between bone biomarker Z-scores and growth, the associations between PTH levels and bone biomarker Z-scores at baseline were assessed adjusted for height Z-score.³¹

Vitamin D deficiency was defined as serum 25(OH)D level <20 ng/mL consistent with the 2011 Institute of Medicine Report.² Multivariable logistic regression examined the odds ratio (OR) of vitamin D deficiency in Crohn disease participants compared with controls in a stepwise fashion. First, possible covariates were analyzed using logistic regression models that included an indicator variable for Crohn disease status in addition to the factor under analysis. Tested covariates included age, sex, race (black vs. all others), winter season (November-March vs. April-October), total vitamin D supplementation (IU/day), and use of vitamin D supplements (categorized as <200 IU/day vs. or 200 IU/day), as previously described.¹⁷ Age was categorized as <12, 12 to <15 and 15 years based on our prior

assessment of the association between age and 25(OH)D levels.¹⁷ Prior studies have demonstrated an association between adiposity and low vitamin D levels using both BMI and DXA-derived measures of fat mass.³²⁻³⁵ Therefore, BMI and fat mass-for-height Z-scores were also included in these analyses. Covariates for which the unadjusted OR had a p-value of <0.20 were eligible for inclusion in the multivariable model.

Differences in baseline levels of serum 1,25(OH)₂D and PTH between Crohn disease participants and controls were also examined using multivariable linear regression models in a stepwise fashion. First, possible covariates were analyzed using linear regression models that included an indicator variable for Crohn disease status in addition to the factor under analysis. Tested covariates for 1,25(OH)₂D included age, sex, race, season, baseline 25(OH)D, and baseline cytokine levels. Serum PTH level was added to the model to determine if PTH levels were positively associated with 1,25(OH)₂D levels – consistent with greater renal 1- α -hydroxylation of 25(OH)D. Tested covariates for PTH included age, sex, race, Tanner stage, season, baseline 25(OH)D and 1,25(OH)₂D, and total serum calcium. In order to determine if 25(OH)D deficiency was associated with a comparable elevation in PTH in Crohn disease and controls, the model for PTH was limited to those participants with 25(OH)D <30 ng/mL, based on prior studies in adults that showed 30 ng/mL was the level of 25(OH)D above which vitamin D supplementation no longer decreased PTH.³⁶⁻³⁷ Covariates which had a p-value of <0.20 in the unadjusted analyses were eligible for inclusion in the multivariable models for baseline 1,25(OH)₂D and PTH levels. Multiplicative interaction terms were included to determine if the impact of Crohn disease status differed according to other covariates in each of these models.

Quasi-least squares (QLS) regression using the xtqls function in STATA 10.0 assessed (1) changes over time in 25(OH)D, 1,25(OH)₂D, and PTH levels and (2) correlates of changes in these parameters within the Crohn disease participants.³⁸ QLS models allow for a variable number of measurements per subject and the implementation of the Markov correlation structure, which is appropriate for modeling associations among measurements that are unequally spaced in time. Because these laboratory parameters did not increase linearly during the study period, variables for time and (time)² were added to the models examining changes over time. All models involving changes in variables used absolute changes from baseline, adjusted for baseline values. Continuous variables were not log-transformed since QLS analyses do not require normally distributed data.

Circulating vitamin D is >99% bound to vitamin D binding protein and albumin and the assay measures free and bound vitamin D.³⁹ Because hypoalbuminemia may result in an overestimation of deficiency in participants with severe Crohn disease, all baseline and longitudinal models were repeated excluding participants with albumin levels <3.0 g/dl.

RESULTS

Participant Characteristics and Laboratory Results

Table 1 summarizes baseline subject characteristics. The sex and race distributions of the Crohn disease participants are consistent with the demographics of the disease.⁴⁰ Crohn disease participants had significantly lower height, BMI, and lean mass-for-height Z-scores

compared with controls. Vitamin D supplementation (200 IU/day) rates were significantly greater in Crohn disease participants at diagnosis (50%), compared with controls (22%). The majority of Crohn disease participants presented with growth failure, moderate to severe disease activity, and ileocolonic disease. Of the 78 participants originally enrolled in the study, 73, 67, and 52 completed 6-month, 12-month, and LTFU (24-63 months from baseline) study visits, respectively. There were no differences at baseline in sex, race, PCDAI score, and height Z-score distributions between participants who did and did not complete the LTFU visit. PCDAI improved significantly over the study interval ($p < 0.001$), with a median score of 40 at baseline, 15 at 6 months, 10 at 12 months, and 5 at LTFU within participants who completed all 4 visits.

Table 2 summarizes laboratory parameters in controls and Crohn disease participants. Serum albumin, ESR, hematocrit, TNF- α , and IL-6 improved significantly (all, $p < 0.001$) following diagnosis. All Crohn disease participants had normal renal function ($\text{eGFR} > 90 \text{ ml/min/1.73 m}^2$) at each study visit. CD subjects had significantly decreased BSAP and DPD Z-scores compared with controls, ($p < 0.001$ and $p < 0.005$, respectively). BSAP and DPD Z-scores increased significantly during the study interval (both, $p < 0.001$). Total serum calcium levels, adjusted for albumin and available in 70 participants at baseline, were (mean $\pm$ SD) $9.6 \pm 0.5 \text{ mg/dL}$. Clinical serum magnesium levels were available in 27 participants at baseline; none were $< 1.5 \text{ mg/dL}$.

Vitamin D Deficiency and PTH Levels at Baseline

Within the controls and Crohn disease participants at baseline, 25(OH)D levels were significantly lower in black compared with non-blacks participants, (both $p < 0.001$). Therefore, serum 25(OH)D levels are summarized according to race (Table 2). Within control participants, the proportions of non-blacks and blacks with vitamin D deficiency were 26 and 69%, respectively; and within Crohn disease participants, the proportions of non-blacks and blacks with vitamin D deficiency were 36 and 100%, respectively. The multivariate logistic regression model in Crohn disease participants, compared with controls (Table 3) indicates that the odds ratio for 25(OH)D deficiency was 2.1 ($p < 0.05$). Crohn disease, black race, a winter study visit, and older age were independently associated with vitamin D deficiency. Within CD participants, univariate analyses demonstrated an association between 25(OH)D levels and PCDAI at baseline ($\rho = -0.30$, $p < 0.05$.) Vitamin D levels were not associated with reported use of supplements at each visit. The majority of vitamin D supplements were administered as a multivitamin in Crohn disease and control participants. Given that fact that these subjects were diagnosed with a chronic illness characterized by malabsorption and malnutrition, it is likely that their treating physicians recommended multivitamins. Excluding the Crohn disease participants with serum albumin levels $< 3.0 \text{ g/dL}$ did not change the magnitude, direction, or significance of the covariates in the baseline 25(OH)D analyses.

As shown in Table 2, unadjusted 1,25(OH) $_2$ D levels were significantly lower in Crohn disease at baseline, compared with controls ($p < 0.001$). In a model adjusted for age and race only, serum log transformed 1,25(OH) $_2$ D levels were lower in Crohn disease, compared with controls [$\beta = -0.19$ (95% C.I. $-0.23, -0.14$); $p < 0.001$]. In order to determine if the

lower 1,25(OH)₂D levels in Crohn disease were related to the lower 25(OH)D levels, the model was further adjusted for 25(OH)D: this did not attenuate the Crohn disease effect [$\beta = -0.18$ (95% C.I. $-0.22, -0.13$); $p < 0.001$]. Serum 1,25(OH)₂D levels were associated with IL-6 ($\rho = -0.30$, $p < 0.05$) and TNF- α ($\rho = -0.27$, $p < 0.05$) levels in univariate analyses. Therefore, the model was further adjusted for each cytokine individually. IL-6 was not significant in the multivariate model. TNF- α was inversely and independently associated with 1,25(OH)₂D ($p < 0.05$); however, adjustment for TNF- α did not significantly attenuate the Crohn disease effect [$\beta = -0.17$ (95% C.I. $-0.23, -0.12$); $p < 0.001$]. The final model was adjusted for PTH levels (Table 4). The significant Crohn disease-PTH interaction term indicated the following: among the controls, a one unit greater log-transformed PTH level was associated with a 0.16 greater log-transformed 1,25(OH)₂D level ($p = 0.001$). However, within Crohn disease participants, log-transformed PTH was not associated with log-transformed 1,25(OH)D level, estimated from linear combinations of estimators: [$\beta = -0.02$ (95% C.I. $-0.11, 0.08$); $p = 0.8$]. There was no association between 1,25(OH)₂D and PCDAI at baseline.

Log-transformed PTH did not differ significantly between controls and Crohn disease participants at baseline in unadjusted analyses (Table 2). The multivariable model for log-transformed PTH at baseline, adjusted for Tanner stage, demonstrated that log-transformed 25(OH)D was negatively associated with log-transformed PTH levels ($p < 0.05$) and Crohn disease was associated with marginally lower log-transformed PTH levels [$\beta = -0.10$ (95% C.I. $-0.21, 0.0100$; $p = 0.07$], compared with controls. In a model restricted to participants with 25(OH)D levels < 30 ng/mL at baseline, Crohn disease was associated with significantly lower PTH levels, compared with controls [$\beta = -0.15$ (95% C.I. $-0.28, -0.03$); $p < 0.05$], adjusted for 25(OH)D levels. Excluding the Crohn disease participants with albumin < 3.0 g/dL did not change the results. Total serum calcium, adjusted for albumin, was not associated with PTH at baseline. Additionally, at baseline, PTH levels were not associated with bone biomarker Z-scores in univariate analyses or in multivariate analyses including adjustment for height Z-score.

Correlates of Changes in Vitamin D and PTH Levels

Figure 1 illustrates the significant changes in 25(OH)D and 1,25(OH)₂D levels in the Crohn disease participants during the study interval. The median (interquartile range) increase in 25(OH)D levels over the entire study interval was 13.8 (8.5-22.1) ng/mL, with no significant differences in changes between non-blacks and blacks. At the final visit, 3% of Crohn disease participants were vitamin D deficient, a significant decrease from the baseline proportion of 42% ($p < 0.001$). The proportions of CD participants receiving vitamin D supplementation 200 IU/day were 50%, 67%, 56%, and 47%, at diagnosis, 6 months, 12 months, and LTFU, respectively. Among participants on vitamin D supplementation at LTFU, the median (range) dose was 400 (60-1160) IU/day. Although there were no significant changes in PTH over the entire study interval, there was a transient increase in PTH from baseline to the 6 month visit ($p < 0.05$).

The multivariable QLS regression model of the correlates of changes in 25(OH)D levels from baseline within Crohn disease participants demonstrated that lower baseline PCDAI [$\beta = -0.11$ (95% C.I. $-0.21, -0.003$); $p < 0.05$] and lower baseline serum 25(OH)D levels [$\beta =$

−0.45(95% C.I.−0.65,0.25); $p<0.001$] were associated with greater increases in 25(OH)D over time, while a follow-up visit during a winter month was associated with a lesser increase [$\beta = -3.92(95\% \text{ C.I.} -7.14, -0.07)$; $p<0.05$]. Changes in PCDAI were not associated with changes in 25(OH)D levels. A multivariate model of predictors of changes in PCDAI did not show an association with baseline 25(OH)D levels or changes in 25(OH)D levels over the study interval. We previously reported significant improvements from baseline to LTFU in BMI ($p<0.001$), and fat mass-for-height Z-scores ($p<0.005$) in this cohort;²⁰ however, changes from baseline in 25(OH)D levels were not associated with changes in these covariates.

The multivariable QLS regression model of the correlates of changes in 1,25(OH)₂D levels from baseline within Crohn disease participants demonstrated that lower baseline 1,25(OH)₂D levels [$\beta = -0.65$ (95% C.I. −0.86, −0.45); $p < 0.001$] and greater increases in PTH from baseline [$\beta = 0.14$ (95% C.I. 0.04, 0.24); $p < 0.01$] were associated with greater increases in 1,25(OH)₂D levels. Changes in 1,25(OH)₂D levels following diagnosis were not significantly associated with baseline 25(OH)D or changes in 25(OH)D levels. A multivariate model of predictors of changes in PCDAI did not show an association with baseline 1,25(OH)₂D levels or changes in 1,25(OH)₂D levels.

The multivariable QLS regression model of changes in PTH levels from baseline within Crohn disease participants demonstrated that higher baseline PTH levels [$\beta = -0.69$ (95% C.I.−0.90,−0.48); $p<0.001$] and greater increases in serum 25(OH)D levels [$\beta = -0.51$ (95% C.I.−0.79 −0.23); $p<0.001$] were associated with greater declines in PTH levels, adjusted for baseline 25(OH)D levels and season. Additionally, greater increases in 1,25(OH)₂D levels were not associated with declines in PTH levels. Total serum calcium did not change significantly during the study interval.

Limiting the three models above to participants with albumin levels greater than 3.0 g/dL did not change the magnitude, direction, or significance of the correlates in these analyses.

At the time of the LTFU visit Crohn disease participants had significantly higher 25(OH)D and 1,25(OH)₂D levels, compared with controls (Table 2). Crohn disease was associated with significantly lower odds of 25(OH)D deficiency [OR: 0.06 (95% C.I. 0.02, 0.23); $p<0.001$], compared with controls. This was not explained by differences in vitamin D supplementation rates or amounts. At the time of the LTFU visit, PTH levels did not differ between Crohn disease and control participants.

DISCUSSION

This study is the first to examine changes in vitamin D and PTH levels in an incident cohort of Crohn disease participants (pediatric or adult), and the first to include healthy controls. In addition to identifying vitamin D deficiency [25(OH)D <20 ng/mL] in 42% of participants at the time of Crohn disease diagnosis, multivariate analyses demonstrated that Crohn disease participants had significantly greater odds of 25(OH) vitamin D deficiency, and lower mean 1,25(OH)₂D levels, compared with concurrent controls. The lower 1,25(OH)₂D levels in Crohn disease were not explained by the lower 25(OH)D levels and greater TNF- α levels,

although both were significantly associated with lower 1,25(OH)₂D levels. Greater disease activity was associated with lower vitamin D levels at baseline; however, the analyses did not demonstrate that greater vitamin D levels were associated with improvements in disease activity. The expected positive association between PTH and 1,25(OH)₂D levels observed in the controls was absent in the Crohn disease participants (significant test for interaction). Furthermore, within participants with 25(OH)D levels < 30 ng/mL, PTH levels were lower relative to 25(OH)D levels in Crohn disease compared with controls. Longitudinal analyses revealed significant improvements in both 25(OH)D and 1,25(OH)₂D levels during median follow-up of 43 months, with transient increases in PTH during the first six months. Greater increases in PTH levels were associated with greater increases in 1,25(OH)₂D levels. Together, these data provide novel insights into abnormalities in vitamin D metabolism in Crohn disease and suggest 25(OH)D deficiency, impaired renal 1- α -hydroxylation of 25(OH)D and relative hypoparathyroidism at the time of diagnosis, with resolution following initiation of Crohn disease therapies.

Prior reports of vitamin D status in children with IBD are limited to cross-sectional studies with a wide range of disease duration^{11-14, 16} and one report in children at diagnosis.¹⁵ These studies provide conflicting estimates of the prevalence of vitamin D deficiency. For example, Pappa, et al examined a heterogeneous cohort of 94 pediatric CD participants (3% black) with a mean duration since diagnosis of 30 months (range 0-12 years): 36% had 25(OH)D levels <15 ng/mL.¹⁴ Prevalent cohorts may capture children with greater disease severity, accounting for the greater prevalence of vitamin D deficiency than observed in our participants over time. Of note, Sylvester, et. al., reported a mean 25(OH)D level of 22 ± 7 ng/mL in 58 white children with Crohn disease at diagnosis, similar to our findings in non-black children (24 ± 9 ng/mL) at diagnosis.¹⁵ Given the marked increases in vitamin D following diagnosis in our cohort, comparison across prevalent cohorts may be limited by differences in disease severity and duration.

The perturbations in the vitamin D-PTH axis described here suggest abnormalities in renal 1- α -hydroxylase activity and in the PTH response to low 25(OH)D levels. Liu, et al examined these relations in a mouse model of colitis.⁷ Mice treated with dextran sodium sulfate showed weight loss, histological evidence of colitis, and increased expression of inflammatory cytokines. The colitis was associated with a five-fold decrease in the expression of renal 1- α -hydroxylase and significant reductions in circulating 1,25(OH)₂D levels, compared with controls. We hypothesize that the lower 1,25(OH)₂D levels observed at Crohn disease diagnosis after adjustment for serum 25(OH)D, TNF- α , and PTH levels indicates impaired renal 1- α -hydroxylation of 25(OH)D (despite normal renal function) and resistance to PTH effects.

At the time of the LTFU visits, 25(OH)D and 1,25(OH)₂D levels were greater in Crohn disease participants, compared with controls. The higher 25(OH)D levels in Crohn disease were not explained by differences in vitamin D supplementation at the study visit. However, the Crohn disease participants had greater supplementation rates at earlier visits and the high levels at LTFU may have been due to more sustained vitamin D supplementation over the preceding months, compared with controls. Prior studies have demonstrated that greater fat mass is associated with lower 25(OH)D levels.^{32-35, 41} Given the increases in fat mass Z-

scores previously reported in this cohort,²⁰ we do not believe that changes in fat mass contributed to the changes in vitamin D levels observed here. The lower vitamin D levels in older participants may be related to lower physical activity and sun exposure.⁴²

Vitamin D deficiency likely contributes to the pathogenesis of Crohn disease through effects on T and B lymphocyte, macrophage, and dendritic cell regulation.⁴³⁻⁴⁵ The greater 1,25(OH)₂D levels at LTFU may represent a compensatory increase in intestinal 1- α -hydroxylase activity. Data in the murine model of colitis described above suggest that the increased intestinal 1- α -hydroxylase activity is a protective response;⁷ mice lacking the 1- α -hydroxylase gene were more susceptible to dextran sodium sulfate-induced colitis. Our observation of significantly higher 1,25(OH)₂D levels in Crohn disease participants at LTFU, compared with controls, is consistent with a prior report of elevated 1,25(OH)₂D levels in an adult Crohn disease cohort with mean disease duration of 12 years.⁸ Colonic biopsies in 14 participants demonstrated increased intestinal expression of the 1- α -hydroxylase in epithelial cells, macrophages and multinucleated giant cells, compared with normal colonic biopsies. The higher 1,25(OH)₂D levels seen in our study at LTFU may be due to greater availability of 25(OH)D substrate for intestinal conversion to 1,25(OH)₂D and restoration of renal 1- α -hydroxylase activity as the disease was treated. The interpretation of the bone biomarker Z-scores at baseline is difficult given the potential competing effects of impaired growth and lower PTH levels to result in decreased biomarker levels, and inflammatory cytokines to increase bone resorption.⁴⁶⁻⁴⁷ The impact of the lower PTH levels on calcium and bone metabolism in Crohn disease at baseline is unknown; however we hypothesize that this leads to lower 1,25(OH)₂D levels through decreased activation of 1- α -hydroxylase.

Vitamin D deficiency normally results in secondary hyperparathyroidism. The data presented here suggest that new-onset Crohn disease is associated with a relative hypoparathyroidism that resolves after initiation of therapy. Among participants with suboptimal 25(OH)D levels, PTH levels were lower relative to 25(OH)D levels in Crohn disease, compared with controls. Also, the transient increase in PTH levels following Crohn disease diagnosis was associated with subsequent increases in 1,25(OH)₂D levels. The marked increases in BSAP Z-scores suggest accelerated bone accrual; therefore, greater calcium uptake may have contributed to increases in PTH during the study interval. Previous studies have demonstrated an up-regulation of the calcium-sensing receptor of the parathyroid gland by IL-1 β , IL-6, and TNF- α in an effort to explain the hypocalcemia and altered vitamin D metabolism of septic patients.⁹⁻¹⁰ It is conceivable that pro-inflammatory cytokines associated with Crohn disease upregulate the parathyroid gland calcium-sensing receptor, preventing an appropriate PTH response. Further investigations are needed to test the hypotheses proposed here.

The limitations of this study include the lack of data on sunlight exposure, dietary intake of calcium, serum ionized calcium levels, and urinary calcium excretion rates in Crohn disease control participants. In addition, vitamin D fortification of the food supply has increased in recent years, and the study is limited by lack of data on dietary vitamin D intake. Given that fact that these subjects were diagnosed with a chronic illness characterized by malabsorption and malnutrition, it is likely that the greater use of vitamin D supplements was related to

their chronic disease. The analyses of changes in the vitamin D–PTH axis after diagnosis may be limited by incomplete follow-up in a subset of participants. However, subject demographics, disease activity, and baseline 25(OH)D, 1,25(OH)₂D, and PTH levels did not differ between those that did and did not complete all four study visits, suggesting minimal bias.

In summary, this novel study demonstrates that pediatric Crohn disease patients experience significant abnormalities in vitamin D and PTH metabolism at diagnosis. Although these deficits improve following diagnosis and therapy, vitamin D deficiency may contribute to disease activity and the complications of Crohn disease. Future studies are needed to examine the impact of vitamin D levels on clinical outcomes in pediatric Crohn disease.

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Abbreviations

BMI	body mass index
CD	Crohn disease
ESR	erythrocyte sedimentation rate
IL	interleukin
LTFU	long-term follow-up
PTH	parathyroid hormone
QLS	quasi-least squares
TNF-α	tumor necrosis factor-α

REFERENCES

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr.* 2004; 80:1678S–88S. [PubMed: 15585788]
2. Institute of Medicine 2011 Dietary reference intakes for calcium and vitamin D. The National Academies Press; 2011.
3. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr.* 2008; 88:491S–499S. [PubMed: 18689389]
4. Zehnder D, Bland R, Chana RS, et al. Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephrol.* 2002; 13:621–9. [PubMed: 11856765]
5. Lim WC, Hanauer SB, Li YC. Mechanisms of disease: vitamin D and inflammatory bowel disease. *Nat Clin Pract Gastroenterol Hepatol.* 2005; 2:308–15. [PubMed: 16265284]
6. Wang TT, Dabbas B, Laperriere D, et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. *J Biol Chem.* 2010; 285:2227–31. [PubMed: 19948723]

7. Liu N, Nguyen L, Chun RF, et al. Altered endocrine and autocrine metabolism of vitamin D in a mouse model of gastrointestinal inflammation. *Endocrinology*. 2008; 149:4799–808. [PubMed: 18535110]
8. Abreu MT, Kantorovich V, Vasiliauskas EA, et al. Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density. *Gut*. 2004; 53:1129–36. [PubMed: 15247180]
9. Canaff L, Hendy GN. Calcium-sensing receptor gene transcription is up-regulated by the proinflammatory cytokine, interleukin-1 β . Role of the NF-kappaB PATHWAY and kappaB elements. *J Biol Chem*. 2005; 280:14177–88. [PubMed: 15684428]
10. Canaff L, Zhou X, Hendy GN. The proinflammatory cytokine, interleukin-6, up-regulates calcium-sensing receptor gene transcription via Stat1/3 and Sp1/3. *J Biol Chem*. 2008; 283:13586–600. [PubMed: 18348986]
11. Issenman R, Atkinson S, Radoja C, et al. Longitudinal assessment of growth, mineral metabolism, and bone mass in pediatric Crohn's disease. *J.Ped.Gastroenterol.Nutr*. 1993; 17:401–406.
12. Gokhale R, Favus MJ, Karrison T, et al. Bone mineral density assessment in children with inflammatory bowel disease. *Gastroenterology*. 1998; 114:902–11. [PubMed: 9558278]
13. Sentongo T, EJ S, Stettler N, et al. Vitamin D status in children, adolescent, and young adults with Crohn disease. *American Journal of Clinical Nutrition*. 2002; 76:1077–1081. [PubMed: 12399281]
14. Pappa HM, Gordon CM, Saslowsky TM, et al. Vitamin D status in children and young adults with inflammatory bowel disease. *Pediatrics*. 2006; 118:1950–61. [PubMed: 17079566]
15. Sylvester FA, Wyzga N, Hyams JS, et al. Natural history of bone metabolism and bone mineral density in children with inflammatory bowel disease. *Inflamm Bowel Dis*. 2007; 13:42–50. [PubMed: 17206638]
16. Levin AD, Wadhwa V, Leach ST, et al. Vitamin d deficiency in children with inflammatory bowel disease. *Dig Dis Sci*. 2011; 56:830–6. [PubMed: 21222159]
17. Weng FL, Shults J, Leonard MB, et al. Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. *Am J Clin Nutr*. 2007; 86:150–8. [PubMed: 17616775]
18. Thayu M, Shults J, Burnham JM, et al. Gender differences in body composition deficits at diagnosis in children and adolescents with Crohn's disease. *Inflamm Bowel Dis*. 2007; 13:1121–8. [PubMed: 17427245]
19. Dubner SE, Shults J, Baldassano RN, et al. Longitudinal assessment of bone density and structure in an incident cohort of children with Crohn's disease. *Gastroenterology*. 2009; 136:123–30. [PubMed: 19026647]
20. Thayu M, Denson LA, Shults J, et al. Determinants of changes in linear growth and body composition in incident pediatric Crohn's disease. *Gastroenterology*. 2010; 139:430–8. [PubMed: 20417635]
21. Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr*. 1991; 12:439–47. [PubMed: 1678008]
22. Morris NN, Udry JR. Validation of a self-administered instrument to assess stage of adolescent development. *J Youth Adolesc*. 1980:271–80. [PubMed: 24318082]
23. Tanner, JM. *Growth at Adolescence*. Blackwell Scientific Publication; 1962.
24. Burnham JM, Shults J, Semeao E, et al. Body-composition alterations consistent with cachexia in children and young adults with Crohn disease. *Am J Clin Nutr*. 2005; 82:413–20. [PubMed: 16087987]
25. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. *Pediatr Clin North Am*. 1987; 34:571–90. [PubMed: 3588043]
26. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. *Methods Enzymol*. 1997; 282:174–86. [PubMed: 9330287]
27. Clive DR, Sudhaker D, Giacherio D, et al. Analytical and clinical validation of a radioimmunoassay for the measurement of 1,25 dihydroxy vitamin D. *Clin Biochem*. 2002; 35:517–21. [PubMed: 12493579]

28. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics*. 2002; 109:45–60. [PubMed: 11773541]
29. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr*. 1990; 44:45–60. [PubMed: 2354692]
30. Terpstra AM, Kalkwarf HJ, Shults J, et al. Bone Density and Cortical Structure after Pediatric Renal Transplantation. *J Am Soc Nephrol*. 2012
31. Tuchman S, Thayu M, Shults J, et al. Interpretation of Biomarkers of Bone Metabolism in Children: Impact of Growth Velocity and Body Size in Healthy Children and Chronic Disease. *J Pediatr*. 2008; 153:484–90. [PubMed: 18555484]
32. Gordon CM, DePeters KC, Feldman HA, et al. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med*. 2004; 158:531–7. [PubMed: 15184215]
33. Rockell JE, Green TJ, Skeaff CM, et al. Season and ethnicity are determinants of serum 25-hydroxyvitamin D concentrations in New Zealand children aged 5–14 y. *J Nutr*. 2005; 135:2602–8. [PubMed: 16251618]
34. Alemzadeh R, Kichler J, Babar G, et al. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism*. 2008; 57:183–91. [PubMed: 18191047]
35. Snijder MB, van Dam RM, Visser M, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab*. 2005; 90:4119–23. [PubMed: 15855256]
36. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet*. 1998; 351:805–6. [PubMed: 9519960]
37. McKenna MJ, Freaney R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. *Osteoporos Int*. 1998; 8(Suppl 2):S3–6. [PubMed: 10197175]
38. Shults J, Ratcliffe SJ, Leonard MB. Improved generalized estimating equation analysis via xtqls for implementation of quasi-least squares in Stata. *The STATA Journal*. 2007; 7:147–166.
39. Haddad JG. Plasma vitamin D-binding protein (Gc-globulin): multiple tasks. *J Steroid Biochem Mol Biol*. 1995; 53:579–82. [PubMed: 7626513]
40. Andres PG, Friedman LS. Epidemiology and the natural course of inflammatory bowel disease. *Gastroenterol Clin North Am*. 1999; 28:255–81. vii. [PubMed: 10372268]
41. Kremer R, Campbell PP, Reinhardt T, et al. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab*. 2009; 94:67–73. [PubMed: 18984659]
42. Absoud M, Cummins C, Lim MJ, et al. Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One*. 2011; 6:e22179. [PubMed: 21799790]
43. Maruotti N, Cantatore FP. Vitamin D and the immune system. *J Rheumatol*. 37:491–5. [PubMed: 20080911]
44. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006; 311:1770–3. [PubMed: 16497887]
45. White JH. Vitamin D signaling, infectious diseases, and regulation of innate immunity. *Infect Immun*. 2008; 76:3837–43. [PubMed: 18505808]
46. Schonau, E.; Rauch, F. Biochemical Markers of Bone Metabolism.. In: Glorieux, FH., editor. *Pediatric Bone: Biology and Diseases*. Academic Press; San Diego: 2003. p. 339–357.
47. Lee SE, Chung WJ, Kwak HB, et al. Tumor necrosis factor- α supports the survival of osteoclasts through the activation of Akt and ERK. *J Biol Chem*. 2001; 276:49343–9. [PubMed: 11675379]

Figure 1. 25(OH)D, 1,25(OH)₂D, and intact PTH levels following disease diagnosis in controls and incident Crohn disease participants. The data in the figure are limited to the 47 Crohn disease participants with 25(OH)D levels at the first and fourth study visits. P-values for time and (time)² are reported jointly to capture the nonlinear relations for changes in parameters in the Crohn disease participants..

Table 1

Baseline Characteristics in Controls and Incident Crohn Disease Participants

	Controls	Crohn Disease	p
N	221	78	
Age, years	13.5 ± 4.4	12.7 ± 2.8	0.23
Median (range)	13.4 (5.3-22.0)	12.7 (5.5-18.0)	
Male, N(%)	112 (51%)	44 (56%)	0.43
Black race, N(%)	62 (28%)	8 (10%)	<0.001
Tanner stage 1-2, N(%)	79 (36%)	42 (54%)	0.007
Height Z-score	0.35 ± 0.90	-0.26 ± 1.06	<0.001
BMI Z-score	0.33 ± 1.04	-0.55 ± 1.15	<0.001
Lean mass-height Z-score	0.11 ± 1.06	-0.88 ± 0.96	<0.0001
Fat mass-height Z-score	-0.02 ± 1.09	-0.23 ± 0.88	0.12
Vitamin D supplements 200 IU/day, N(%)	46 (22%)	38 (50%)	<0.001
Symptom duration at diagnosis, months		12.0 ± 11.8	
Median (range)		7.1 (0.1-52.6)	
History of growth failure, N(%)		62 (84%)	
PCDAI		38.4 ± 18.0	
Median (Range)		36.2(5-80)	
0-10 (No Disease), N(%)		3 (4%)	
11-30 (Mild Disease), N(%)		28 (38%)	
>30 (Moderate To Severe Disease), N(%)		43 (58%)	
Site of Disease, N(%)			
Isolated ileal disease		2 (3%)	
Isolated colonic disease		7 (9%)	
Ileocolonic disease		46 (59%)	
Isolated upper gastrointestinal disease		5 (7%)	
Perianal Involvement		28 (36%)	

PCDAI, Pediatric Crohn disease Activity Index

Continuous variables presented as mean ± SD

Table 2

Laboratory Values in Control and Crohn Disease Participants

	Controls	Crohn Disease Participants			
		Baseline	6 mo	12 mo	LTFU
N(total)	221	78	73	67	52
Albumin, g/dL		3.4 ± 0.7 3.5 (1.8- 4.7)	3.8 ± 0.5 3.9 (2.3-4.5)	4.0 ± 0.4 4.0 (3.1-4.9)	4.2 ± 0.5 4.2 (3.0-5.3)
ESR, mm/hr		27 ± 21 21 (0-128)	17 ± 10 15 (1-46)	20 ± 15 17 (0-60)	14 ± 14 8 (0-51)
Hematocrit, %		35.0 ± 5.0 34.3 (21.4-44.4)	37.2 ± 3.9 37.3 (15.6-43.7)	37.6 ± 3.4 37.8 (26.3-46.5)	39.0 ± 4.0 38.8 (26.1-46.6)
TNF-α, pg/mL	6.7 ± 6.1 [*] 6.2 (0.8-73.5)	8.9 ± 4.2 ^c 8.2 (2.5-18.5)	10.2 ± 5.5 ^c 9.6 (1.1-26.2)		3.9 ± 2.7 ^c 4.0 (0.7-12.0)
IL-6, pg/mL	17.8 ± 21.1 [*] 9.2 (0.4-102.7)	28.6 ± 27.3 ^c 21.6 (4.5-182.8)	29.5 ± 23.4 ^c 23.0 (2.0-102.6)		18.6 ± 23.0 11.9 (0.6-94.8)
eGFR, ml/min/1.73m ²		143 ± 26 143 (95-252)	147 ± 22 144 (106-204)	151 ± 29 149 (100-218)	151 ± 29 146 (99-247)
25(OH)D, ng/mL					
Non-black participants	25.3 ± 8.7 24.5(6.2 to 57.1)	23.5 ± 9.2 22.1(7.6 to 52.6)	29.7 ± 12.2 ^a 26.6(9.3 to 74.9)	28.7 ± 10.3 ^a 27.7(7.1 to 58.9)	37.0 ± 12.1 ^c 36.7(9.9 to 72.2)
Black participants	15.8 ± 7.9 15.0 (3.7-39.2)	10.5 ± 4.6 9.1 (4.5-19.1)	25.4 ± 16.1 20.0 (12.7 to 43.5)	18.9 ± 8.9 21.5 (8.9-26.2)	20.7 ± 7.5 22.7 (8.0-27.8)
1,25(OH) ₂ D, pg/mL	41.5 ± 12.3 38.8 (11.4-101.4)	30.0 ± 11.9 ^c 28.0 (4.0-64.0)	44.5 ± 13.6 ^a 43.7 (12.2-93.4)	43.2 ± 11.6 43.2 (9.4-66.1)	51.1 ± 20.4 ^c 49.2 (5.0-105.2)
PTH, pg/mL	32.3 ± 15.6 29.0 (4.0-92.0)	29.8 ± 15.8 28.0 (4.0-83.0)	36.9 ± 24.8 31.0 (4.0-135.0)	35.6 ± 20.2 31.5 (9.0-105.0)	34.12 ± 16.4 30.0 (12.0-87.0)
BSAP Z-score	-0.20 ± 1.1	-1.87 ± 2.02 ^c	-0.96 ± 1.56 ^a	-0.37 ± 1.68	-0.12 ± 1.09
DPD Z-score	-0.08 ± 1.0	-0.60 ± 1.40 ^b	0.65 ± 1.29 ^c	0.39 ± 1.58 ^a	0.35 ± 1.06 ^a

ESR, erythrocyte sedimentation rate; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; BSAP, bone specific alkaline phosphatase; DPD, deoxypyridinoline.

Continuous variables presented as mean ± SD and median (range).

P-values denote comparison to controls:

^a p<0.05

^b p<0.01

^c p<0.001

^{*} Data available in 153 participants

Table 3

Multivariate Logistic Regression Model of Vitamin D deficiency (<20 ng/mL) in Controls and in Crohn Disease Participants at Baseline

Variable	Odds Ratio (95% CI)	p
Crohn disease (vs. controls)	2.1 (1.1, 3.9)	<0.05
Black race	10.4 (5.3, 20.7)	<0.001
Study visit in winter months	2.4 (1.4, 4.1)	<0.005
Age (vs. <12)		
12 to <15	2.7 (1.4, 5.4)	<0.005
>15	3.2 (1.7, 6.3)	0.001

Table 4

Multivariable Linear Regression Model of Log-Transformed 1,25(OH)₂D concentrations in Controls and Crohn Disease Participants at Baseline

	β (95% CI)	p-value
Crohn disease (vs. controls)	0.49 (0.19, 0.95)	<0.05
Crohn disease by log-transformed PTH interaction term	−0.17 (−0.30, −0.04)	0.009
Log-transformed PTH	0.16 (0.08, 0.24)	<0.001
Log-transformed 25(OH)D	0.28 (0.13, 0.42)	<0.001
Log-transformed TNF-α	−0.08 (−0.14, −0.02)	<0.05
Black race (vs. non-black)	0.08 (0.01, 0.15)	<0.05
Age (vs. <12)		
12 to <15	−0.036 (−0.09, 0.02)	0.202
>15	−0.06 (−0.12, −0.01)	0.020