Bone Turnover is not Influenced by Serum 25-Hydroxyvitamin D in Pubertal Healthy Black and White Children

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Abstract

Low serum 25-hydroxyvitamin D [25(OH)D] is common in healthy children particularly in blacks. However, serum 25(OH)D concentrations for optimal bone turnover in children is unknown and few data exist that describe effects of increasing serum 25(OH)D on bone turnover markers during puberty. The purpose of this study was to determine the relationships between serum 25(OH)D and changes in serum 25(OH)D and bone turnover in white and black pubertal adolescents. Bone turnover markers were measured in 318 healthy boys and girls from Georgia (34°N) and Indiana (40°N) who participated in a study of oral vitamin D_3 supplementation (0 to 4000 IU/d). Serum 25(OH)D, osteocalcin, bone alkaline phosphatase, and urine N-telopeptide cross-links were measured at baseline and 12 weeks. Relationships among baseline 25(OH)D and bone biomarkers, and between changes over 12 weeks were determined and tested for effects of race, sex, latitude, and baseline 25(OH)D. Median 25(OH)D was 27.6 ng/mL (n=318, range 10.1–46.0 ng/mL) at baseline and 34.5 ng/mL (n=302, range 9.7–95.1 ng/mL) at 12 weeks. Neither baseline nor change in 25(OH)D over 12 weeks were associated with bone turnover. The lack of association was not affected by race, sex, latitude, or baseline serum 25(OH)D. Serum 25(OH)D in the range of 10-46 ng/mL appears to be sufficient for normal bone turnover in healthy black and white pubertal adolescents.

Keywords

bone turnover; adolescence; vitamin D; race; 25-hydroxyvitamin D

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1. Introduction

A high prevalence of low serum 25-hydroxyvitamin D [25(OH)D] has been reported in healthy children and adolescents [1-4], which has driven the concept that it is important to improve vitamin D status in this population. But, there are few data to support a level of serum 25(OH)D for optimal bone turnover in children above that which is necessary to prevent rickets (> 10 ng/mL). Further, American black children compared with white children have lower serum 25(OH)D [1-3], but generally higher bone mass and strength [5-7], indicating that optimal vitamin D status in blacks, at least for bone health outcomes, may be different from that for whites.

In the absence of functional outcomes such as bone mineral density and fracture, which require large sample sizes and relatively long study durations, biochemical markers of bone formation and bone resorption are potentially useful to gauge the influence of vitamin D supplementation on bone. During puberty, serum concentrations of bone turnover markers are high compared with adult values [8]. Cross-sectional data linking serum 25(OH)D and bone turnover markers in children and adolescents are conflicting. A study in adolescent Chinese girls [9] reported a negative association between serum 25(OH)D and markers of both bone formation and resorption, whereas, two studies in European children across pubertal stages [8, 10] reported lack of associations between serum 25(OH)D and markers of bone formation and resorption. Though few prospective data exist that describe the effects of increasing serum 25(OH)D on bone turnover markers during puberty in healthy children, several studies [11-13] have shown that vitamin D supplementation in children increases serum 25(OH)D, but has little influence on bone turnover markers. These studies, however, used relatively low doses of vitamin D supplementation (200 – 600 IU/d) with result that the magnitude of change in serum 25(OH)D may not have been great enough to elicit measurable changes in bone turnover. In addition, data are lacking comparing bone turnover markers in whites and blacks following vitamin D_3 supplementation.

This study aimed to determine the baseline relationships between serum 25(OH)D and bone turnover markers in American black and white children who participated in a 12-week vitamin D study, using doses up to 4000 IU/day. Further, the study aimed to determine the effect of change in serum 25(OH)D over 12 weeks on corresponding change in bone turnover.

2. Participants and Methods

Healthy children in sexual maturation stages 2 or 3 as described by Tanner[14] were recruited from the community by advertisement to participate in a 12-week randomized, placebo-controlled trial of vitamin D_3 supplementation, stratified for race (black and white), sex, and geographical location [U.S. states of Indiana ("northern site," 40°N) and Georgia ("southern site," 34°N)]. Sexual maturation stage [14] was determined by self-staging (breast stage for girls and genital stage for boys) with the assistance of a parent. Three-hundred twenty-three pubertal boys (ages 8 to 13 years) and girls (ages 9 to 12 years) enrolled in the parent study (83 black girls [43 northern and 40 southern], 82 black boys [42 northern and 40 southern], 78 white girls [38 northern and 40 southern], 80 white boys [40

northern and 40 southern]). All participants were enrolled during winter (October to January) to recruit children whose serum 25D had reached the seasonal nadir. In the parent study, participants were randomized to receive 0, 400, 1000, 2000, or 4000 IU/d of oral vitamin D_3 supplements (Douglas Laboratories, Pittsburgh, PA). Vitamin D_3 supplement doses were confirmed by an independent lab (Covance, Inc.). Compliance with supplementation was assessed by pill count. The study protocol was approved by the Institutional Review Boards of the University of Georgia, Purdue University, and Indiana University School of Medicine. All participants and parents/guardians provided informed assent and consent, respectively.

2.1 Serum and Urine Biochemistries

Fasting serum and urine samples were collected at baseline and 12 weeks and stored at -80°C until analysis. Each analyte was measured at a single laboratory for all participants and blocked such that baseline and 12-week samples for each participant were measured at the same time. Serum 25(OH)D (ng/mL) was measured by RIA (Diasorin, Stillwater, USA) in a DEQAS participant laboratory (CV 5.5 %). Bone turnover markers were measured by ELISA: Serum osteocalcin (OC, ng/mL) and bone alkaline phosphatase (BAP, U/L) (MicroVue™, Quidel Corporation, San Diego, CA; CV 8.0 % and 4.1 %, respectively), and urine N-telopeptide cross-links (Ntx, nM bone collagen equivalents; Osteomark®, Wampole Laboratories, Princeton, NJ, CV 4.6 %). Urine creatinine (mM) was measured by colorimetric assay using a COBAS MIRA clinical analyzer (Roche Diagnostic, Indianapolis, IN). Urine Ntx was corrected for urine creatinine and values reported are in nM bone collagen equivalents/mM creatinine.

2.2 Statistical Analyses

Statistical Analysis Software (SAS 9.2, Cary, NC) was used for statistical analyses. Seven outliers for urine Ntx (all > 4 SD above the mean) were excluded from the analyses. Transformed values for bone turnover markers were used in the statistical analyses to satisfy the assumption of normally distributed errors: square-root BAP, natural log OC, natural log Ntx. For change from baseline to 12 weeks, a natural log transformation for OC was used and the other variables remained in original units. Independent samples *t*-tests were used to determine race differences in baseline variables. Because the aim of the analysis was to determine the relationship between serum 25(OH)D and bone turnover the effect of dose of vitamin D supplement on serum 25(OH)D was not examined. Simple regression was used to determine relationships among baseline values for serum 25(OH)D, BAP, OC, and urine Ntx. Then, to determine if change in 25(OH)D over 12 weeks impacted bone turnover, simple regressions were performed among change in 25(OH)D and change in BAP, OC, and Ntx. Regressions were also performed by month of enrollment to investigate any intra-seasonal variation in the relationships among variables. Multivariate regression was used to determine if the relationships among variables were affected by adjusting for sexual maturation stage, race, sex, latitude, and baseline serum 25(OH)D. The effect of race on the relationship between 25(OH)D and bone turnover was also investigated by repeating the simple regressions in whites and blacks separately. The Bonferroni method was used to adjust the overall significance level of $\alpha = 0.05$ for multiple tests, which gave an adjusted significance level of $\alpha = 0.002$.

3. Results

3.1 Relationships among Baseline Variables

Baseline demographic and biochemical variables are presented for 318 participants in Table 1. Five participants were excluded due to lack of baseline biochemical data. Median serum 25(OH)D was 27.6 ng/mL at baseline (Figure 1A, Table 1). There were 47 participants (14.7 %) with 25(OH)D in the insufficiency range (< 20 ng/mL) and no participants in the deficiency range (< 10 ng/mL). Blacks had lower 25(OH)D (p < 0.0001) and higher OC (p < 0.0001) than whites. Baseline serum 25(OH)D was not associated with baseline serum BAP or urine Ntx, and there was a modest negative association with serum OC ($R^2 = 0.05$, p < 0.0001), which was no longer statistically significant after adjusting for race or multivariate adjustment for sexual maturation, race, sex, and latitude (Table 2A). The analysis of blacks and whites separately yielded similar results (Figures 2A-C). Relationships among baseline variables were not affected by month of enrollment (data not shown).

3.2 Relationships among 12-week Change

Data from 302 participants who completed the 12-week study are included in the prospective analysis. The range of vitamin D_3 doses from 0 to 4000 units shifted median serum 25(OH)D at 12 weeks to 34.5 ng/mL (range 9.7 to 95.1 ng/mL; Figure 1B, Table 1) and produced changes in serum 25(OH)D ranging from -5.9 to +62.4 ng/mL at 12 weeks (Figure 1C). There were 26 (9%) participants at 12 weeks in the vitamin D insufficiency range (< 20 ng/mL) and 1 (<1%) in the vitamin D deficiency range (< 10 ng/mL). Mean change in serum 25(OH)D was higher with each increasing level of vitamin D_3 dosage: (-3.5, +1.8, +7.6, +14.1, +31.2 ng/mL for 0, 400, 1000, 2000, 4000 IU/d, respectively, p < 0.002 for all doses).

Similar to baseline results, 12-week serum 25(OH)D was not related to 12-week bone turnover markers (data not shown). Change in serum 25(OH)D was not correlated with change in serum BAP ($R^2 = 0.001$, p = 0.56), serum OC ($R^2 = 0$, p = 0.71), or urine Ntx ($R^2 = 0.01$, p = 0.15; Table 2B, Figures 2D-F).

Adjustment for race alone, or multivariate adjustment for sexual maturation, race, sex, latitude, and baseline serum 25(OH)D did not affect the lack of association between change in serum 25(OH)D and change in bone turnover markers, nor did analyses of blacks and whites separately (Figures 2D-F). Relationships among change variables were not affected by month of enrollment nor level of vitamin D_3 dose (data not shown).

4. Discussion

This study did not show a positive relationship between serum 25(OH)D levels and biochemical markers of bone turnover in healthy pubertal adolescents either at baseline or with change over 12 weeks. The lack of a relationship was present in both white and black children despite the fact that black adolescents had lower serum 25(OH)D and higher bone turnover than whites. These findings corroborate the results of previous cross-sectional studies [8, 10] and intervention studies in adolescents using lower doses of oral vitamin D [11-13, 15] which found no relationship between serum 25(OH)D and bone turnover. Our

study extends these reports by using a larger sample of healthy boys and girls, and showing that perturbing baseline serum 25(OH)D through a wide range from zero to 60 ng/ml using daily oral vitamin D_3 supplementation did not change bone turnover.

The lack of relationship between serum 25(OH)D and bone turnover, and additionally the lack of relationship between change in serum 25(OH)D and change in bone turnover following 12 weeks of vitamin D supplementation indicates that at baseline these adolescents were within the range of serum 25(OH)D required for healthy bone turnover. This contrasts with findings in classically defined vitamin D deficiency (serum 25(OH)D < 10 ng/mL) that produces rickets with increased bone turnover, and which responds to vitamin D treatment with a reduction in bone turnover [16]. In a study of Chinese girls [9] where a third of the participants had serum 25(OH)D in the deficient range, girls categorized as vitamin D deficient (< 10 ng/mL) had higher BAP than those categorized as insufficient (<20 ng/mL and > 10 ng/mL) or sufficient (>20 ng/mL), but there were no differences between those categorized as insufficient and sufficient. In the present study, median serum 25(OH)D was 27.6 ng/mL, which is well above the deficiency level of 10 ng/mL, but also above the insufficiency level of 20 ng/mL defined by the Institute of Medicine [17]. None of the participants in this study had a baseline serum 25(OH)D level defined as deficient. Therefore, a limitation of the present study is that it cannot exclude either an association between serum 25(OH)D and bone turnover or an effect of improving serum 25(OH)D through vitamin D supplementation on bone turnover in children with deficient baseline serum 25(OH)D.

Race did not affect the results of this study, despite the finding that blacks had lower serum 25(OH)D and higher OC compared with whites. Neither adjusting for race in the multivariate analyses nor repeating the analyses in whites and blacks separately revealed that the lack of relationship between serum 25(OH)D and bone turnover might be race-dependent.

There were several strengths of this study including the relatively large sample of black and white boys and girls from northern and southern U.S. latitudes where each race and sex from both latitudes were equally represented. The participants in this study were studied over winter months to limit confounding by seasonal sun exposure causing dermal vitamin D production, and to ensure that vitamin D supplements would have the greatest effect on low serum 25(OH)D. In addition, separate analyses by month of enrollment did not suggest any seasonal effect on the relationship between serum 25(OH)D and bone turnover markers in this study. The study occurred over 12 weeks, which is sufficient time to observe effects of vitamin D on bone turnover [18]. Bone turnover in this study was measured by three biochemical markers of bone turnover, which we have shown to be reliable markers of bone turnover measured by skeletal calcium retention during calcium balance studies in adolescents [19]. The large range of vitamin D doses in this study, extending up to the Tolerable Upper Intake Level [17] of 4000 IU/d, produced a wide range of change in serum 25(OH)D over the 12-week study, which allowed us to investigate the relationship between change in 25(OH)D and change in bone turnover. Our study sample represents a relatively narrow range of sexual maturity, minimizing confounding from sexual maturation, but does limit the results from being generalized to all of childhood. Furthermore, analysis of the data

using sexual maturation stage as a covariate did not affect the results. However, self-staging of sexual maturation and as use of sexual maturation stage as only a screening measure are limitations of the study.

Our sample size was similar to that in the aforementioned Chinese girls study [9] including vitamin D deficient girls, which showed a relationship between serum 25(OH)D status and bone turnover. Therefore, we were likely adequately powered to detect a relationship. In addition, the weakness (R^2 ranging from 0.00 to 0.03) of the non-significant relationships between serum 25(OH)D and bone turnover markers indicates that even if our sample size were larger to produce significant results, these relationships would be of little clinical relevance.

5. Conclusions

The results of this study indicate that serum 25(OH)D ranging from 10 to 46 ng/mL during winter appears to be sufficient in healthy black and white adolescents for bone turnover, and increasing serum 25(OH)D does not affect bone turnover in these children. Our results suggest that there is no apparent benefit of increasing serum 25(OH)D for bone turnover in children when a winter baseline serum 25(OH)D is above the deficiency level of 10 ng/mL.

Acknowledgments

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Highlights (3-5 bullet points, max 20 words per point)

- In healthy black and white pubertal children, bone turnover markers are not associated with serum 25(OH)D;

- increasing serum 25(OH)D does not affect bone turnover markers;

  and serum 25(OH)D ranging from 10-46 ng/mL is sufficient for normal bone turnover
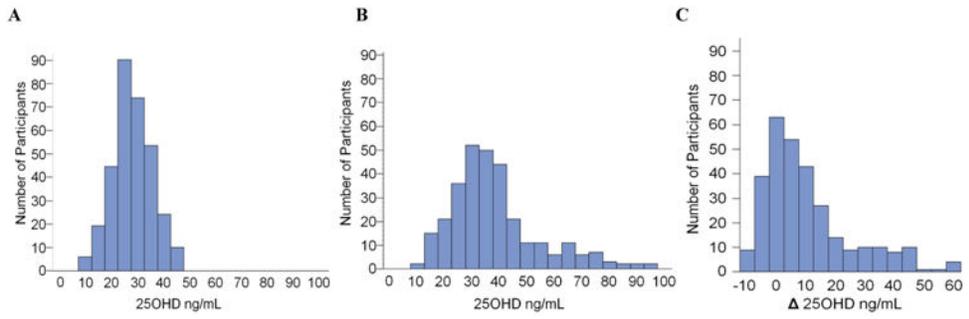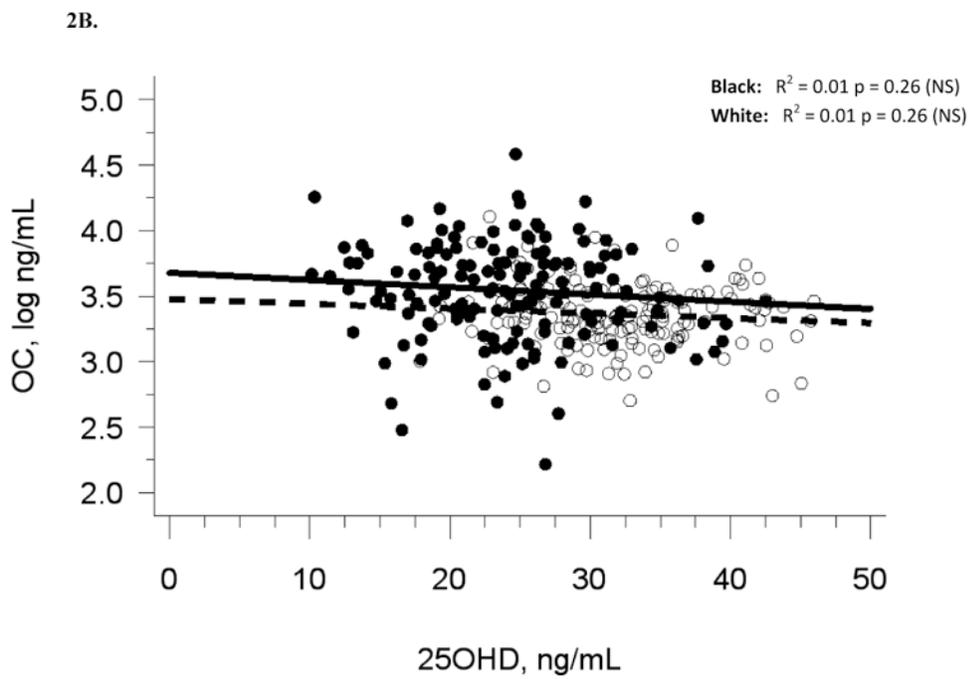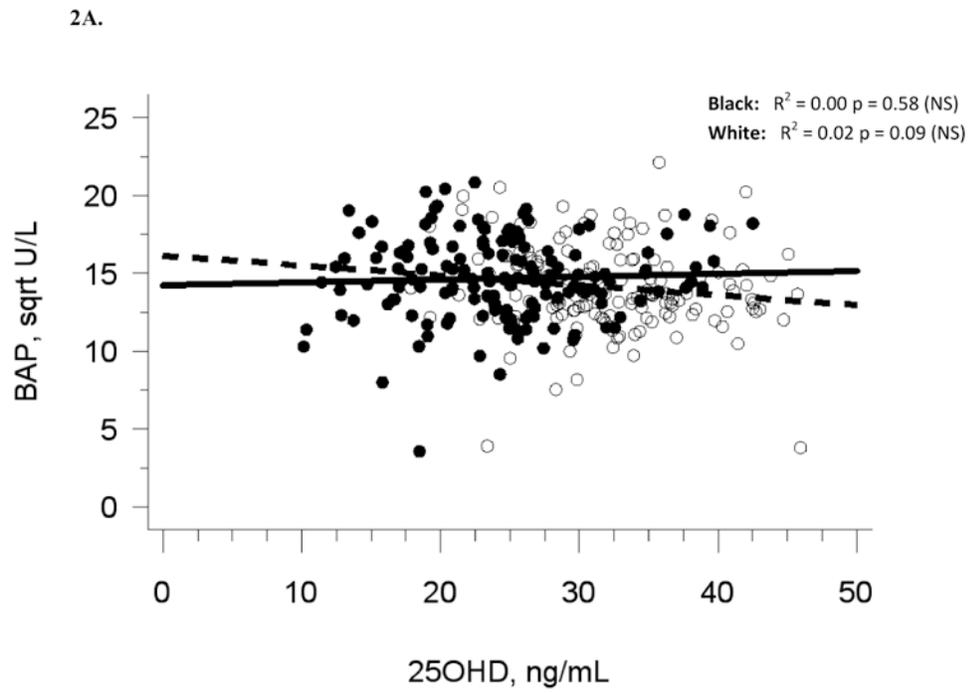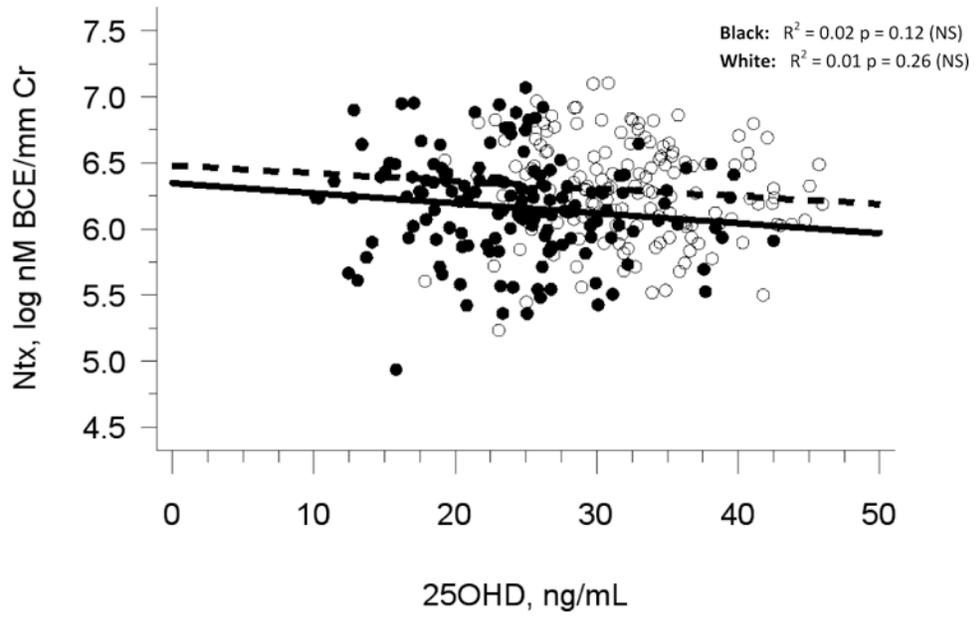
Figure 1. Distribution of serum 25OHD (A) at baseline, (B) 12 weeks, and (C) as change (Δ) from baseline to 12 weeks

2A.

Black: $R^2 = 0.00$ $p = 0.58$ (NS)
White: $R^2 = 0.02$ $p = 0.09$ (NS)

2B.

Black: $R^2 = 0.01$ $p = 0.26$ (NS)
White: $R^2 = 0.01$ $p = 0.26$ (NS)

2C.

2D.

2E.

2F.
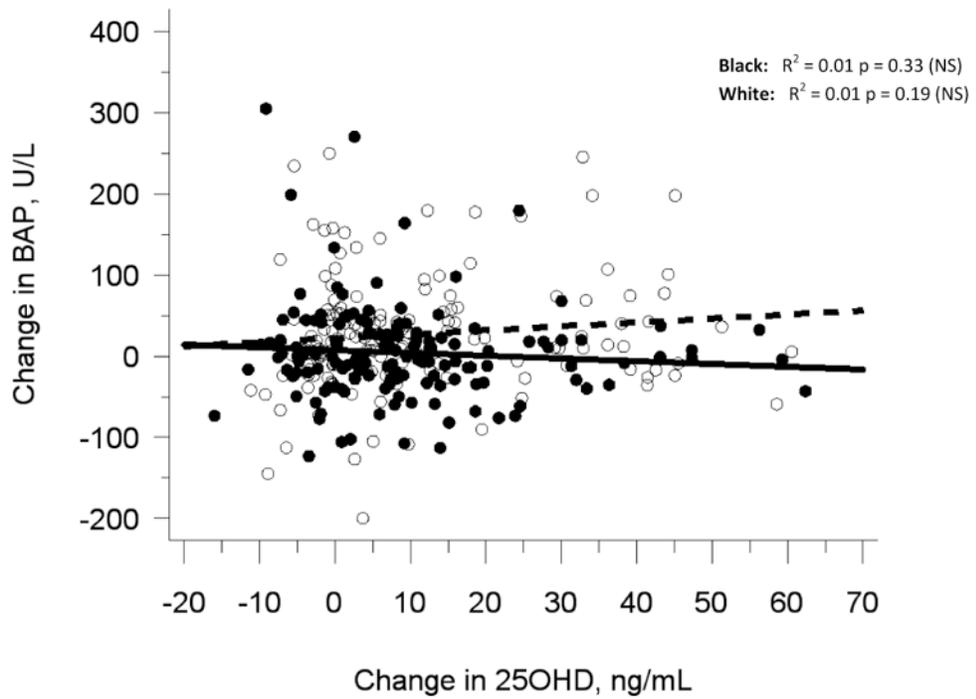
Figure 2.
The relationship between serum 25OHD and (A) serum BAP at baseline, (B) serum OC at baseline, and (C) urine NTX at baseline, and the relationship between change in serum 25OHD after 12 weeks of vitamin D supplementation versus (D) change in serum BAP, (E)

change in serum OC, and (F) change in urine NTX. Blacks are represented by filled circles and solid lines and white are represented by open circles and dashed lines.
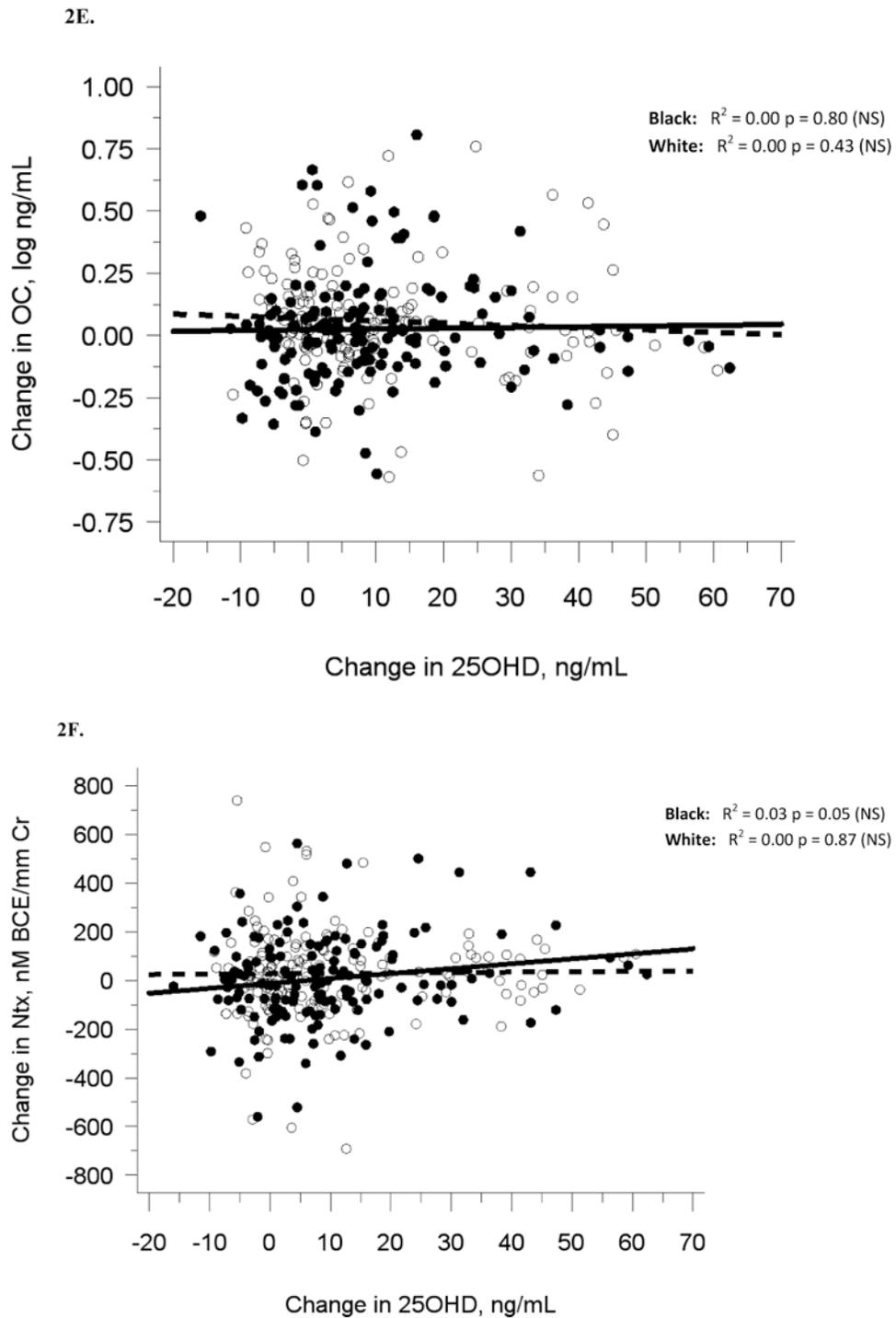
Table 1

Participant Demographics and Baseline and 12-week Biochemistries[a]

	All Participants	White	Black	p-value
Demographics				
n	318	156	162	
Latitude (North/South)	158/160	76/80	82/80	
Sex (Girls/Boys)	157/161	77/79	80/82	
Sexual Maturation stage (2/3)[b]	207/111	107/49	100/62	
Age (yr)	11.4 ± 0.1	11.6 ± 0.1	11.2 ± 0.1	0.004
	11.3 (8.9 - 14.0)	*11.7 (9.1 - 14.0)*	*11.1 (8.9 - 14.0)*	
Biochemistries				
Baseline				
Serum 25OHD (ng/ml)	28.0 ± 0.4	32.0 ± 1.3	24.1 ± 0.5	**<0.0001**
(n = 318)	*27.6 (10.2 - 46.0)*	*31.9 (17.9 - 46.0)*	*24.5 (10.2 - 42.5)*	
Serum BAP (U/L)	214.3 ± 4.5	206.3 ± 6.3	222.5 ± 6.3	0.07
(n = 303)	*202.8 (12.8 - 490.0)*	*188.0 (14.5 - 490.0)*	*212.8 (12.8 - 434.3)*	
Serum OC (ng/ml)	33.3 ± 0.7	29.7 ± 0.6	37.0 ± 1.1	**<0.0001**
(n = 303)	*31.3 (9.2 - 98.1)*	*28.6 (14.9 - 60.8)*	*35.2 (9.2 - 98.1)*	
Urine Ntx (nM BCE/mM Creat)	544.7 ± 12.0	577.9 ± 17.2	511.3 ± 16.3	0.004
(n = 299)	*512.1 (139.5 - 1219.2)*	*542.8 (187.7 - 1219.2)*	*483.0 (139.5 - 1177.1)*	
12-week				
Serum 25OHD (ng/ml)	38.2 ± 0.9	42.5 ± 1.3	33.5 ± 1.3	**<0.0001**
(n = 302)	*34.5 (9.7 - 95.1)*	*37.6 (12.7 - 95.1)*	*30.7 (9.7 - 92.4)*	
Serum BAP (U/L)	231.5 ± 4.9	236.3 ± 7.4	226.5 ± 6.5	0.38
(n = 302)	*214.7 (63.3 - 597.0)*	*219.7 (88.1 - 597.0)*	*212.5 (63.3 - 448.2)*	
Serum OC (ng/ml)	34.9 ± 0.7	31.7 ± 0.7	38.4 ± 1.3	**<0.0001**
(n = 302)	*32.6 (13.1 - 104.6)*	*30.9 (13.1 - 62.5)*	*35.8 (15.4 - 104.6)*	
Urine Ntx (nM BCE/mM Creat)	565.6 ± 13.3	608.0 ± 18.3	521.1 ± 18.7	**0.0002**
(n = 301)	*530.1 (99.4 - 1562.4)*	*557.4 (99.4 - 1562.4)*	*489.2 (114.9 - 1542.6)*	

[a]Values are presented as mean ± SE and *median (min-max)*; t-tests for bone turnover markers were performed using transformed variables: square-root BAP, log OC, log Ntx. Original units are presented in the table. P-values in **bold** are statistically significant.

[b]12 girls reached menarche during the study.

Table 2

Serum 25OHD and Bone Turnover Markers - Baseline and Change Relationships[a]

A. BASELINE

Simple Regression

	R-squared	p-value
Sqrt (Serum BAP)	0.01	0.1
log (Serum OC)	0.05	**0.0001**
log (Urine Ntx)	0.0003	0.48

Race Adjusted

	R-squared	p-value
Sqrt (Serum BAP)	0.002	0.43
log (Serum OC)	0.01	0.11
log (Urine Ntx)	0.02	0.02

Multivariate Adjusted[b]

	R-squared	p-value
Sqrt (Serum BAP)	0.001	0.65
log (Serum OC)	0.01	0.09
log (Urine Ntx)	0.01	0.08

B. CHANGE OVER 12 WEEKS

Simple Regression

	R-squared	p-value
Serum BAP	0.001	0.56
log Serum OC	0.001	0.71
Urine Ntx	0.01	0.15

Race Adjusted

	R-squared	p-value
Serum BAP	0.001	0.64
log Serum OC	0.001	0.67
Urine Ntx	0.01	0.16

Multivariate Adjusted[c]

	R-squared	p-value
Serum BAP	0.002	0.45
log Serum OC	0.001	0.65
Urine Ntx	0.004	0.26

[a] p-values in **bold** are statistically significant.   = change from baseline to 12 weeks;

[b] adjusted for sexual maturation stage, race, sex, and latitude;

[c] adjusted for sexual maturation stage, race, sex, latitude, baseline 25OHD.