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Original Article

Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy, adolescent females

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Abstract This study aimed to investigate the relationship between 25-hydroxyvitamin D [25(OH)D)] and parathyroid hormone (PTH) levels in adolescent females residing in a northern climate. Concern regarding vitamin D status in this population is due to limited sunlight exposure in northern latitudes, decreased outdoor recreational activities, as well as decreased conversion in black girls from increased skin pigmentation. In this cross-sectional analysis, serum samples were assayed for 25(OH)D using competitive protein binding (CPB) assay and PTH with immuno-radiometric (RIA) procedures. Four hundred postmenarcheal females (12–18 years) residing in northeastern Ohio were recruited. Subjects were excluded if they had a history of bone, kidney, or liver disease, or used medications that affect bone. The primary goal was to determine serum 25(OH)D concentrations in relation to circulating PTH levels in a population of adolescent girls. The Spearman correlation test was used to compare PTH and 25(OH)D. Fit multiple split models were run to determine change in slope of the regression line when 25(OH)D and PTH were plotted. Analysis of variance was determined using modeled means with differences by race and

season in the final model. Unadjusted mean serum 25(OH)D and PTH levels were 55.0±30.4 nmol/l and 39.4±20.6 ng/l, respectively. Blacks had lower 25(OH)D and higher PTH compared with non-blacks (P<0.0001), especially during the winter months. Decreasing 25(OH)D was inversely correlated with PTH (r=−0.314) (P<0.0001), and at concentrations of 25(OH)D ≤90 nmol/l, an increase in PTH was observed. Adolescents are at risk for decreased serum 25(OH)D concentrations, especially black girls. We found that the widely used cutoff for vitamin D deficiency is associated with increasing PTH levels and is below the inflection point for a change in the slope of the regression line. Our results support the need for further research to establish optimal vitamin D status in adolescent girls.

Keywords 25-Hydroxyvitamin D · Adolescent · Female · Parathyroid hormone · Vitamin D

Introduction

Vitamin D is essential for maintaining serum calcium levels for optimal bone mineralization. A deficiency of vitamin D leads to decreased dietary calcium absorption, secondary hyperparathyroidism, and poor skeletal mineralization. In children, vitamin D deficiency leads to increased risk of altered bone development and rickets. In individuals residing in northern climates where sunlight exposure is variable based on seasonality and for individuals with increased skin pigmentation, risk for altered vitamin D status is high [1].

Currently, there is a notable lack of consensus regarding the optimal serum 25-hydroxyvitamin D [25(OH)D] level needed to define vitamin D deficiency and vitamin D insufficiency [2]. For older adults, vitamin D deficiency has been defined as serum 25(OH)D ≤37.5 nmol/l, since this is associated with secondary hyperparathyroidism, decreased serum calcium, and increased serum alkaline phosphatase [3]. For children and adolescents, the criterion for deficiency is different, and in fact, lower than in the older adult population. The currently accepted minimum serum cutoff level for deficiency is <27.5 nmol/l for the prevention of bone abnormalities and rickets [4].

It has been previously reported that in young adults (17–35 years) residing in the northern part of the United States (US), there are alterations in parathyroid hormone (PTH) at low normal concentrations of vitamin D [5]. Furthermore, in a number of adolescent studies completed in Europe, it has been determined that a rise in PTH concentration occurs at vitamin D levels between 25.0 and 83.0 nmol/l [6,7,8,9]. Similar results have been reported in older adults [3,10].

To date, minimal information exists about the threshold level of serum 25(OH)D that will prevent a rise in serum PTH levels in older children in the US [11,12]. Thus, in this cross-sectional analysis, we investigated serum 25(OH)D and PTH levels in a large sample of adolescent females (12–18 years) residing at 41° N throughout the year. In addition, we proposed to use PTH as marker of vitamin D status and to statistically define the 25(OH)D concentrations at which circulating levels of PTH begin to increase in postmenarcheal adolescent girls over several seasons and across different racial groups.

Materials and methods

Subjects

Four hundred postmenarcheal females (12–18 years of age) requesting contraceptives agents, who were eligible for participation in a larger National Institutes of Health (NIH) sponsored clinical trial, were recruited from four Adolescent Medicine Clinics in the Greater Cleveland, Ohio area (41° N latitude). Subjects were excluded from the study for the following reasons: history of bone, kidney, thromboembolic or liver disease, current medication with known effect on bone, current pregnancy, oral contraceptive use in the previous 3 months, previous use of depot medroxyprogesterone acetate, or breastfeeding or pregnancy within the past 6 months. Subjects self-reported racial and ethnic classification. Racial/ethnic group classifications were black (n=254) and non-black (n=146). The non-black category included the following racial/ethnic mix: white non-Hispanic (n=131), Asian (n=2), Native American (n=1), and white Hispanic (n=12).

Between April 2000 and February 2003, 400 study participants were identified and underwent baseline testing for serum 25(OH)D and PTH levels. Serum samples were collected during study enrollment, prior to initiation of contraceptive medications, between the hours of 1400–1800. Study enrollment was continuous across three spring/summer and three fall/winter seasons. Seasons were categorized as spring/summer (May–October) and fall/winter (November–April). The study was approved by the MetroHealth Medical Center Institutional Review Board and all participants and their parents provided written informed consent.

Serum analysis

Prior to centrifugation at 5°C, serum samples were allowed to coagulate for 30 min. Serum samples for PTH were frozen at −70°C and stored for batch analysis. Separate aliquots of serum were

frozen to –70°C and immediately shipped on dry ice to Specialty Laboratories (Santa Monica, Calif., USA), where they were analyzed for 25(OH)D.

Serum 25(OH)D samples (*n*=400) were assayed using a competitive protein binding assay (CPB) employing automated chemiluminescence (Nichols Institute, San Clemente, Calif., USA). Since serum 25(OH)D values for the pediatric population have not been established by the Nichols Institute, the performance characteristics for this assay were based on results from healthy adults (*n*=159) (19–76 years of age). The manufacturer's reported within run and total precision coefficients of variation are 3.0–4.5% and 6.4–14.5%, respectively. These characteristics are for the Nichols CPB assay only, as estimates of serum levels can vary by laboratory and assay type. When the CPB method was compared with radioimmunoassay (RIA), it produced results that were approximately 30% higher than the RIA [13].

Serum PTH (*n*=393) was assayed by DSL-8000 ACTIVE Intact PTH Immuno-radiometric (IRMA) Kit (Diagnostic Systems Laboratories, Webster, Tex., USA) at our General Clinical Research Center core chemistry laboratory. Normal pediatric ranges for adolescents for serum PTH have not been established in our core laboratory. The normal range for adults (*n*=59), established by Diagnostic Systems Laboratories, for this assay is 9–55 ng/l. The manufacturer's stated intra-assay precision is 2.8–5.7% and inter-assay precision is 4.3–10.4%.

Statistical analysis

Data were expressed as means (95% confidence limits) unless otherwise indicated. The comparison of modeled means was determined by using ANOVA to test for differences by season and race (SAS Version 8.2, SAS Institute, Cary, N.C., USA). Data were log transformed, and the models were analyzed using a stepwise progression approach with non-significant covariates and non-significant interaction effects removed in the final models for race and season. Backlog transformed estimates were generated from each model. Spearman correlation coefficients were obtained when correlation analysis was preformed on PTH and 25(OH)D. Fit multiple split models with a moving window on the split were used to determine the point where the slope of the regression line for 25(OH)D and PTH changed from negative to positive.

Results

Three hundred and ninety-three subjects were included in the analysis for vitamin D and PTH status. The mean (SD) age of the study population was 15.5 (1.6 years) with a mean (SD) body

weight of 65.6 (16.9 kg). Mean (SD) serum calcium and phosphorus levels were 2.4 (0.1) mmol/l and 1.2 (0.2 nmol/l), respectively. Unadjusted mean (SD) serum 25(OH)D concentrations were 55.02±30.4 nmol/l and unadjusted mean (SD) serum PTH levels were 39.4±20.6 ng/l.

There was a significant difference in adjusted mean serum 25(OH)D levels between the black and non-black girls, with values being significantly lower in black girls (43.5 nmol/l) (95% CI 40.2, 46.8) than in non-black girls (75.1 nmol/l) (95% CI 70.8, 79.5) (P<0.0001). Mean serum PTH was significantly higher in the black girls compared with the non-black girls (P<0.0001) and higher during the fall/winter compared with during the spring/summer (P<0.01) (Table 1). Figure 1 shows that during both seasonal time points (spring/summer and fall/winter), black girls had significantly lower adjusted mean serum 25(OH)D compared with non-black (P<0.0001). Mean PTH in the black girls was significantly higher during the fall/winter compared with non-black (P<0.001) (Fig. 2).

[Table 1 will appear here. See end of document.]

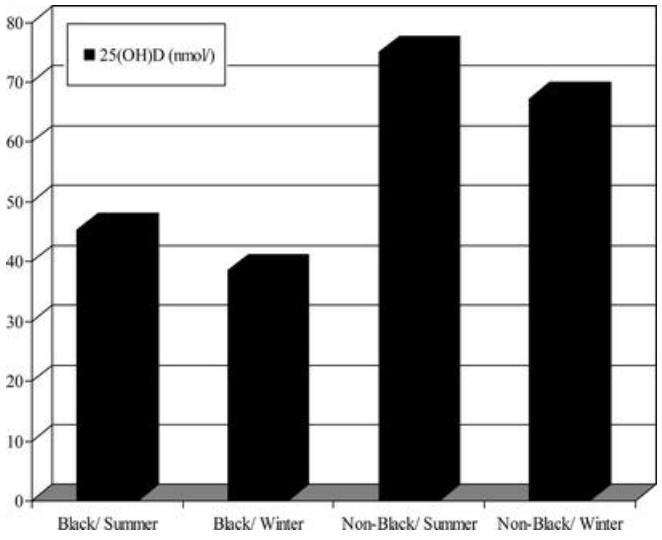

Fig. 1 Adjusted mean serum 25(OH)D levels for 400 adolescent girls (12–18 years of age) stratified by race and season. Race is categorized as black and non-black and season as summer (May–October) and winter (November–April). Significant differences in serum 25(OH)D levels between black/summer vs black/winter (P<0.001), black/winter vs non-black/winter (P<0.0001), and black/summer vs non-black/summer (P<0.0001)

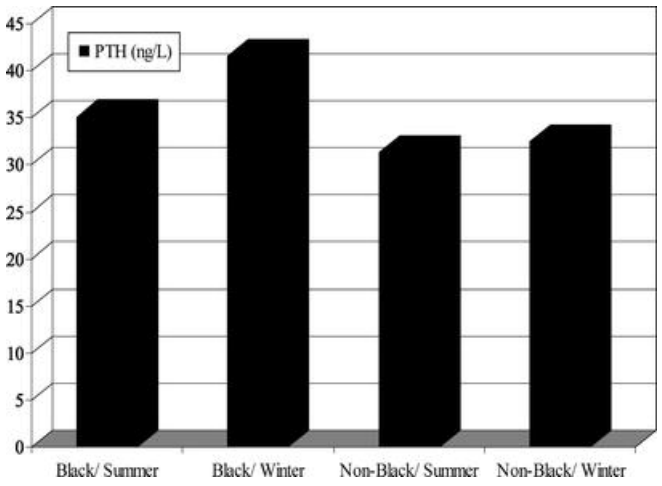

Fig. 2 Adjusted mean serum parathyroid hormone levels for 393 adolescent girls (12–18 years of age) stratified by race and season. Race is categorized as black and non-black and season as summer (May–October) and winter (November–April). Significant differences for serum PTH levels between black/summer vs black/winter ($P<0.001$) and black/winter vs non-black/winter ($P<0.001$)

There was a significant correlation between serum 25(OH)D and PTH levels ($r=-0.314$) ($P<0.0001$). Parathyroid hormone and 25(OH)D were correlated for both racial groups, with a stronger association for the non-black girls ($r=-0.28$, $P<0.001$) compared with the black girls ($r=-0.19$, $P<0.01$).

The regression lines for the relationship between vitamin D and PTH are plotted in Fig. 3. Using fit multiple split models, the data suggested that the point of inflection for the change in the slope from negative to positive occurred at serum 25(OH)D=90 nmol/l and PTH=30 ng/l. The following are the regression equations for the change in the slope for the two models:

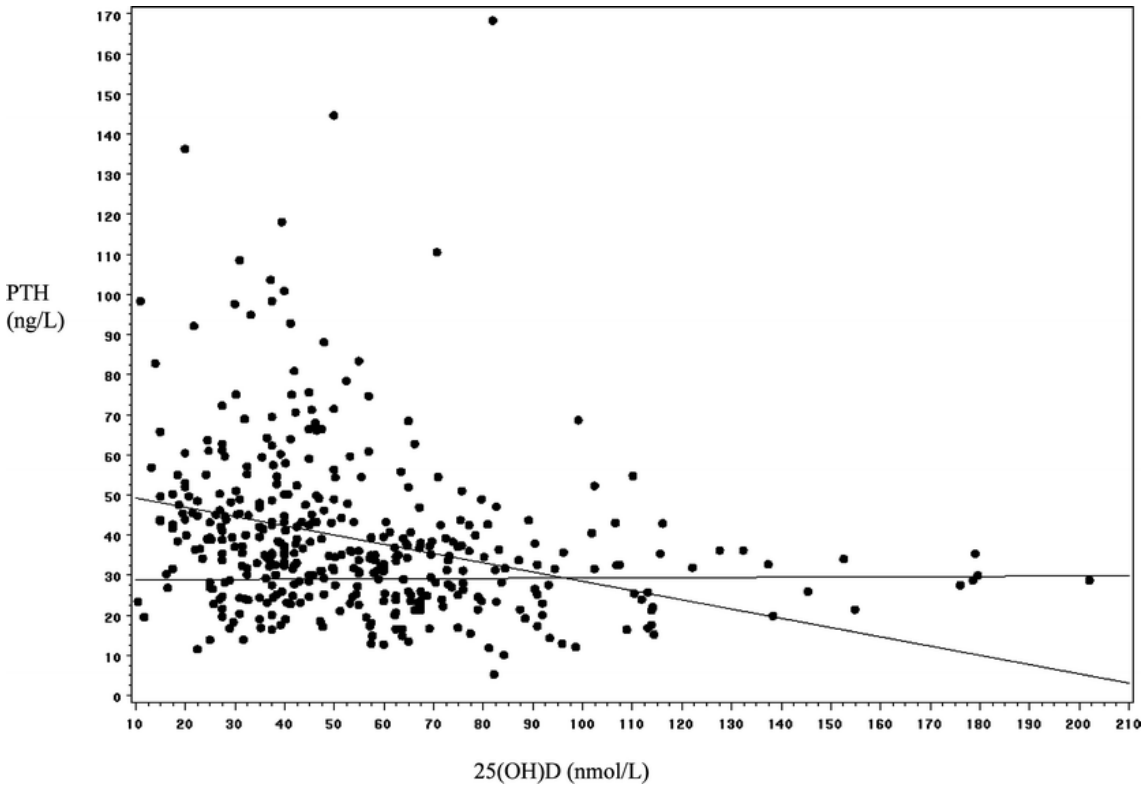

Fig. 3 Serum PTH×25(OH) D levels in 393 adolescent females (12–18 years). Figure depicts the regression lines for the change in the slope from negative to positive at a point where serum 25(OH)D=90 nmol/l and PTH=30 ng/l

$$25(\mathrm{OH})\mathrm{D} \leqslant 90\ \mathrm{nmol/L}: \ \mathrm{PTH} = 51.49829 - 0.231226 * 25(\mathrm{OH})\mathrm{D}$$
$$25(\mathrm{OH})\mathrm{D} > 90\ \mathrm{nmol/L}: \ \mathrm{PTH} = 28.83255 + 0.005241 * 25(\mathrm{OH})\mathrm{D}$$

Discussion

There was a significant inverse relationship between serum 25(OH)D and PTH levels in this large sample of adolescent females, as well as within each racial group. In addition, the data suggested that the change in the slope of the plot for serum 25(OH)D from negative to positive occurred at serum 25(OH)D=90 nmol/l. These results are consistent with previously published data. For example, Outila and colleagues [9] noted that a serum 25(OH)D concentration ≥40 nmol/l (assayed with RIA methods) in 178 Finnish female adolescents (14–16 years) was needed to maintain low PTH levels and determined that a 1 unit increase in serum 25(OH)D concentrations related to a decrease in PTH by 0.198 units. Furthermore, Guillemant and colleagues [6] found that when 25(OH) levels fell below 83 nmol/l when assayed using the CPB method, the increase in PTH concentration accelerated, and Chapuy and colleagues [14] reported that in French adults (35–60 years), serum PTH levels were stable at a 25(OH)D concentration of 78 nmol/l (CPB assay).

Seasonal variations were significant in our study, with higher PTH levels occurring during the fall/winter months corresponding to decreased 25(OH)D concentrations. This finding has been previously reported in adolescents [8,15]. Guillemant and colleagues [8] found seasonal changes in 25(OH)D and PTH in 54 French male adolescents (13–16 years), with a significant increase in winter-time PTH and a concurrent decrease in 25(OH)D (using CPB assay).

Several studies have found increased PTH levels and lower 25(OH)D concentrations in black women compared with white women [16,17]. Similarly, the black girls in our study had lower 25(OH)D levels and higher PTH levels compared with the non-black girls. In addition, 25(OH)D levels in the black girls were significantly lower during both seasonal time points, and PTH levels were significantly higher during the fall/winter. There was a significant correlation between PTH and 25(OH)D in the black girls, although not as strong as in the non-black girls.

It has been previously noted that vitamin D insufficiency is associated with an increase in serum concentrations of PTH that are within the threshold of the level considered to be normal for adults [18,19,20]. Our data support this statement, and suggest that vitamin D insufficiency does exist in adolescent females and is associated with increasing concentrations of PTH. Supplementation studies with children and adolescents have shown that providing a dietary vitamin D supplement does increase serum 25(OH)D while causing a decrease in serum PTH [7,8]. In addition, previous research has suggested a clinical impact of vitamin D insufficiency. In female adolescents, Outila and colleagues noted that serum 25(OH)D levels ≤40 nmol/l were associated with low forearm bone mineral density [9].

This study had a number of limitations. We recognize that the cross-sectional analysis of serum 25(OH)D and PTH is not optimal and that the best approach is a prospective, longitudinal design. In addition, it is important to note that a criticism in the literature has been the differences in assay methods to determine serum 25(OH)D concentrations and the variability with the commonly used assay methods for 25(OH)D. Competitive protein binding assay has been shown to produce estimates that are approximately 30% higher than those reported using RIA [13]. In this study, we used CPB assay to measure serum 25(OH)D levels, since this study was part of a larger NIH sponsor clinical trial. We attempt to clarify these differences in our paper by noting the specific assay type for each cited reference. Additional limitations include the lack of assessment of sunlight exposure and dietary calcium and vitamin D intake. The addition of these variables to the model would have been useful. Since we did not measure dietary variables, sunlight exposure, and skin pigmentation, we cannot determine if the low levels of 25(OH)D and increased PTH are associated with these factors. We can postulated that poor dietary calcium and vitamin D intake coupled with

lack of adequate sunlight exposure and increased skin pigmentation contributed to the high prevalence of altered 25(OH)D and PTH concentrations, especially in the black girls, since symptomatic rickets has been reported in adolescents who live in colder environments [21]. Moreover, rickets has been observed in sunny climates when vitamin D intake was below the recommended level. Narchi and colleagues [22] found 21 cases of symptomatic rickets in Saudi Arabian adolescents, who had an estimated median sunlight exposure of 15 min/day with median vitamin D intake of 2.8 μg/day.

Based on our results of this cross-sectional analysis, we conclude vitamin D insufficiency is associated with a rise in PTH concentrations in adolescent girls. Consideration should be given to re-evaluating the currently accepted serum threshold for vitamin D deficiency in older children. Further work needs to be done to evaluate 25(OH)D and PTH concentrations in context with dietary calcium and vitamin D intake and cutaneous conversion in adolescents.

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References

1. Calvo MS (2003) Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of current food fortification and dietary supplement use. Nutr Rev 61:107–113
2. Vieth R (1999) Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 69:842–856
3. Lips P, Wiersinga A, VanGinkel FC, Jongen MJM, Netelenbos JC, Hackeng WHL, Delmas PD, VanDerVijgh WJF (1988) The effects of vitamin D supplementation on vitamin D status and parathyroid hormone function in elderly subjects. J Clin Endocrinol Metab 67:644–650
4. Institute of Medicine, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1997) Vitamin D. In: Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academy Press, Washington D.C., pp 250–287
5. Tangpricha V, Pearce EN, Chen TC, Holick MF (2002) Vitamin D insufficiency among free-living healthy young adults. Am J Med 112:659–662
6. Guillemant J, Taupin P, Le HT, Taright N, Allemandou A, Pérès G, Guillemant S (1999) Vitamin D status during puberty in French healthy male adolescents. Osteoporos Int 10:222–225
7. Docio S, Riancho JA, Pérez A, Olmos JM, Amado JA, González-Macías J (1998) Seasonal deficiency of vitamin D in children: potential target for osteoporosis-preventing strategies? J Bone Miner Res 13:544–548
8. Guillemant J, Le H-T, Maria A, Allemandou A, Pérès G, Guillemant S (2001) Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D supplements. Osteoporos Int 12:875–879

9. Outila TA, Kärkkäinen MUM, Lamberg-Allardt CJE (2001) Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. Am J Clin Nutr 74:206–210
10. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF (1998) Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporosis Int 8:222–830
11. Heaney RP (1999) Lessons for nutritional science from vitamin D. Am J Clin Nutr 69:825–826
12. Malabanan A, Veronikis IE, Holick MF (1998) Redefining vitamin D insufficiency. Lancet 351:805–806
13. Lips P, Chapuy MC, Dawson-Hughes B, Pols HAP, Holick MF (1999) An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporos Int 9:394–397
14. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ (1997) Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 7:439–443
15. Oliveri MB, Ladizesky M, Mautalen CA, Alonso A, Martinez L (1993) Seasonal variations of 25 hydroxyvitamin D and parathyroid hormone in Ushuaia (Argentina), the southernmost city in the world. Bone Miner 20:99–108
16. Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S, Shary J (1985) Evidence for alteration of the vitamin D-endocrine system in blacks. J Clin Invest 76:470–473
17. Parisien M, Cosman F, Morgan D, Schnitzer M, Liang X, Nieves J, Forese L, Luckey M, Meier D, Shen V, Lindsay R, Dempster DW (1997) Histomorphometric assessment of bone mass, structure, and remodeling: a comparison between healthy black and white premenopausal women. J Bone Miner Res 12:948–956
18. Scharla SH (1998) Prevalence of subclinical vitamin D deficiency in different European countries. Osteoporos Int 8:S7-S12
19. Peacock M (1998) Effects of calcium and vitamin D insufficiency on the skeleton. Osteoporos Int 8:S45-S51
20. Zitterman A (2003) Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 89:552–572
21. Moncrieff MW, Lunt HR, Arthur LJ (1973) Nutritional rickets at puberty. Arch Dis Child 48:221–224
22. Narchi H, El Jamil M, Kulaylat N (2001) Symptomatic rickets in adolescents. Arch Dis Child 84:501–503

Table 1 Parathyroid hormone (PTH) levels in 393 black and non-black adolescent females (12–18 years of age) by race and season. Values are adjusted mean (95% confidence limits)[a]

Category	Number of subjects (*n*)	PTH (ng/l)	95% confidence limits
Black	248	43.6****	41.1, 46.1
Non-black	145	34.1****	30.8, 37.4
Spring/summer	246	35.9**	33.4, 38.5
Fall/winter	147	41.8**	38.5, 45.1

[a]Adjusted for race and season in the final model

****Significant difference between adjusted means for black vs non-black ($P<0.0001$)

**Significant difference between adjusted means for spring/summer vs fall/winter ($P<0.01$)