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Original Article

Biochemical Responses of Bone Metabolism to 1,25-Dihydroxyvitamin D Administration in Black and White Women

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Abstract. The basis for the racial difference in bone mass between black and white women is not known. Lower bone turnover, better renal calcium conservation, and decreased sensitivity to parathyroid hormone (PTH) have been proposed as explanations. A dynamic comparison of osteoblast function, utilizing stimulation by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], has not been tested between these two ethnic groups. We compared well-matched black (n = 15) and white (n = 15) premenopausal women, before and during 5 days of 1,25$(OH)_2$D administration (1.0 μg/day) in order to assess dynamic indices of bone metabolism. As expected, at baseline, black women had lower levels of serum 25-hydroxyvitamin D and biochemical markers of bone turnover with slightly higher levels of PTH. Black women also had superior renal calcium conservation than white women at baseline. In response to 1,25$(OH)_2$D administration, black women had a slightly greater increase in serum calcium and greater decrement in PTH. Moreover, black women showed a lesser increment in urinary calcium than white women and a more robust increase in two markers of bone formation – osteocalcin and carboxyterminal propeptide of type 1 procollagen – than white women. There were no changes in bone resorption indices in either race upon 1,25$(OH)_2$D administration. These data provide preliminary evidence that black women conserve calcium more efficiently under both static and dynamic conditions, and also appear to have better osteoblastic functional reserve than white women.

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Introduction

It is generally well accepted that black women have a greater bone mass [1–6] and lower incidence of osteoporotic fracture [7–9] than white women. This higher prevalence of osteoporosis in whites has overshadowed the importance of the disease in the aging black population. Although the development of the disease may be delayed in black women, a substantial decline in hip, radial and vertebral bone density [3] and an age-related exponential rise in the risk of hip fractures has also been demonstrated in black women [8]. It has been proposed that differences in peak bone mass, bone turnover and age-related rate of bone loss underlie the differences in bone mass which exist between races [2,3,5,6]. The underlying mechanism of these differences has not been well established.

Osteocalcin, a protein produced by osteoblasts, is distinguished by the presence of gamma-carboxyglutamic acid, and its relatively specific presence in bone is directly correlated with the degree of mineralization [10] and rate of bone turnover [11–13]. Vitamin D modulation of osteocalcin synthesis by osteoblasts and therefore circulating osteocalcin levels is well established. Administration of physiologic levels of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] results in a measurable increase in serum osteocalcin concentrations in healthy adult males [14] and postmenopausal women

[15–17]. Since a trend toward greater increases in serum osteocalcin during $1,25(OH)_2D$ administration was seen in women with osteoporosis compared with nonosteoporotic postmenopausal women, the authors concluded that defective osteoblast function is not the major cause of bone loss in postmenopausal osteoporosis [16].

Overall, circulating osteocalcin levels have been reported to be lower in black than white women [5,18,19], but differences have been small and there is significant overlap of individual values. We reasoned that a stimulation test for this osteoblast product might provide greater discrimination between white and black subjects than would be possible with static measurements of circulating levels. A greater response of osteocalcin to $1,25(OH)_2D$ in black women might indicate greater bone formation, due to greater sensitivity of the osteoblast to this hormone, and would provide an alternate hormonal mechanism for the higher bone mass in black women. Elevation of $1,25(OH)_2D$ has been known to reduce circulatory levels of parathyroid hormone (PTH) [20]. We and others have previously proposed that black women may have reduced sensitivity to PTH-induced resorption activity [21,22]. Differences in the PTH response to $1,25(OH)_2D$ administration, therefore, between the two racial groups, could offer further insights into the possible mechanisms of bone mass variation between the two races.

Subjects and Methods

Subjects

Healthy premenopausal black (n = 15) and white (n = 15) women between the ages of 25 and 40 years were recruited from the New York City and Rockland County, New York areas. Ethnic group assignment was based both on self-identification and on the racial identity of three of the subject's four grandparents through a questionnaire. After an initial telephone interview of potential study subjects to assure premenopausal status and to exclude subjects with certain diseases, eligible subjects were screened by detailed medical history and physical examination. Pregnant women and subjects suffering from malignancy, eating disorders, alcoholism, endocrine, renal, hepatic, gastrointestinal or other chronic diseases, were excluded. Use of medication such as oral contraceptive, steroid or anticonvulsant therapy, or other drugs known to affect bone metabolism, in the previous 6 months, were similarly excluded. Baseline calcium intake was assessed in all participants using a food frequency questionnaire. The study was approved by the Institutional Review Board of Helen Hayes Hospital and all subjects gave informed consent.

$1,25(OH)_2D$ Stimulation Test Protocol

Study subjects reported to the Clinical Research Center each morning for 5 consecutive days after an overnight fast. On day 1, beginning at 8:00 a.m., two basal serum samples were collected over a 20 min period. A second morning fasting urine sample was also obtained. Subjects were then administered 0.5 μg of $1,25(OH)_2D$ (Rocaltrol, Roche Laboratories, Nutley, NJ) orally each day between 8:30 and 9:00 a.m., following a protocol similar to one used by us previously [17]. Serum and urine samples were obtained 1 h after the a.m. Rocaltrol dose each day while subjects remained fasting. Subjects took a second dose of Rocaltrol, 0.5 μg each evening between 8:30 and 9:00 p.m. Subjects were placed on a moderate calcium and phosphorus diet, under the guidance of a nutritionist, during the 5-day investigation in order to avoid possible development of hypercalcemia during $1,25(OH)_2D$ administration.

Biochemical Assays

Serum intact PTH molecules were assayed with an immunoradiometric assay (Intact PTH, Nichols Institute, San Juan Capistrano, CA). Serum 25-hydroxyvitamin D [25(OH)D] and $1,25(OH)_2D$ were analyzed by competitive protein binding and radioreceptor assays [23,24]. Serum ionized calcium was analyzed by a standard technique (Nova 8 ionized calcium analyzer, Nova Biomedical, Newton, MA). Urinary and serum calcium (Ca) and creatinine (Cr) were assayed with a standard chemistry machine. Calcium clearance (per creatinine clearance) was calculated using the following equation (urine Ca/serum Ca divided by urine Cr/serum Cr).

Samples from the basal, day 4 and day 5 time points were also analyzed for the following assays. Serum intact osteocalcin (OC) was assayed by immunoradiometric assay (Human osteocalcin, Nichols Institute, CA). Serum bone specific alkaline phosphatase (BSAP) was assayed with an IRMA assay (Ostase, Ostex International, San Diago, CA). Carboxyterminal propeptide of type I collagen (PICP) was determined by radioimmunoassay (INCSTAR, Stillwater, MN). Urinary free pyridinoline (F-PYD) was assayed by EIA assay (Pyrilink, Metra Biosystem, Palo Alto, CA). Urinary cross-linked N-telopeptide of type 1 collagen (Ntx) was assayed by an EIA assay (Osteomark, Ostex, Seattle, WA). Quality control information for these assays have been published previously [17]. All interassay coefficients of variation were less than 10% and all intra-assay coefficients of variation were less than 8%.

Statistical Analysis

All statistical analyses were performed using the SAS statistical program (SAS Institute, Cary, NC). The mean and standard error for each variable are reported. Repeated measurement analysis of variance was used to determine significance of time trends in each group (race), group differences and possible interactions between race and time for variables of calcium homeostasis. Racial differences between baseline variables and

in responsivity of bone turnover variables to 1,25(OH)$_2$D administration were analyzed by two-tailed *t*-tests. The significance of change in bone turnover from baseline within each group was tested using paired *t*-tests.

Results

Subjects

There were no significant differences between the two groups in age, height, educational level, parity, body weight or height. Baseline mean daily calcium intake was 630 mg in black women and 650 mg in white women (NS). Mean body mass index of the black women (24.5 kg/m^2) was slightly higher than that of the white women (22.7 kg/m^2; $p<0.05$). Routine chemistry, thyroid, gonadal and hematology profiles were normal in all women and similar in the two groups (data not shown). Bone density was higher at all sites (3.5–8.5%) in black versus white women (anteroposterior lumbar spine, black mean 1.313 vs white mean: 1.268 g/cm^2, $p = 0.16$; femoral neck, black mean 1.089 vs white mean 1.004 g/cm^2, $p = 0.05$).

Basal Biochemistry (Table 1)

Black women showed significantly lower levels of serum 25(OH)D ($p<0.0001$), urinary calcium/creatinine ($p<0.05$) and calcium clearance ($p<0.05$), when compared with white women. Mean basal level of PTH was higher in black women than white women but the difference missed statistical significance ($p = 0.08$). The basal 1,25(OH)$_2$D level was higher in black women than in white women ($p = 0.03$). All bone turnover markers, serum OC, PICP, BSAP, and urinary F-PYD and NTX, were lower in black women than white women at baseline, but only the difference in F-PYD was statistically significant.

Table 1. Biochemical parameters at baseline

	Blacks	Whites
Serum ionized Ca (mM)	1.208 ± 0.011	1.231 ± 0.011
Serum total Ca (mM)	2.29 ± 0.04	2.35 ± 0.02
Serum PTH (pM)	4.992 ± 0.368	4.125 ± 0.318
Serum 25(OH)D (nM)	37.12 ± 5.77	77.95 ± 6.91*
Urine Ca/Cr (mg/mg)	0.158 ± 0.021	0.306 ± 0.064*
Calcium clearance (%)	0.48 ± 0.06	0.71 ± 0.11*
Serum OC (nM)	1.062 ± 0.076	1.174 ± 0.084
Serum PICP (ng/ml)	103.7 ± 6.9	123.8 ± 10.2
Serum BSAP (ng/ml)	9.25 ± 0.66	9.76 ± 0.68
Urine F-PYD (nmol/mmol Cr)	26.87 ± 1.18	32.00 ± 1.74*
Urine Ntx (nmol BCE/mmol Cr)	40.56 ± 4.80	45.93 ± 4.74

Values are the mean ± SEM.
Ca, calcium; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; Cr, creatinine; OC, osteocalcin; PICP, carboxyterminal propeptide of type I collagen; BSAP, bone-specific alkaline phosphatase; F-PYD, urinary free pyridinoline; Ntx, urinary cross-linked N-telopeptide of type 1 collagen.
*$p<0.05$ versus black group.

1,25(OH)$_2$D Stimulation Test

Calcium Homeostasis (Fig. 1). There was a significant increase in serum 1,25(OH)$_2$D level after its administration in both black and white women. No group difference or time/group interaction was observed. Mean serum 1,25(OH)$_2$D concentrations were elevated after the first dose of 1,25(OH)$_2$D and reached a plateau from days 2 through 5 of 1,25(OH)$_2$D administration. Levels of serum ionized and total calcium remained in the normal range throughout the study. Serum ionized and total calcium increased more in black than white women with a significant time trend found only for ionized calcium in the black women. There were, however, no significant group or time/group differences for either ionized or total calcium.

A highly significant reduction in serum PTH over time was seen in black women ($p = 0.0001$), but the reduction was of smaller magnitude and not quite statistically significant in white women ($p = 0.073$; group difference $p = 0.045$). Mean serum 25(OH)D level did not change in either group throughout the study but remained persistently lower in black women than white women.

Urinary calcium/creatinine increased significantly by day 5 in white women, but not black women, and both a group difference ($p = 0.015$) and a time/group interaction ($p = 0.005$) were observed. Furthermore, calcium clearance increased only in white women ($p = 0.0038$). Group differences ($p = 0.07$) and racial/time interactions ($p = 0.09$) just missed statistical significance for this variable.

Bone Turnover Markers (Fig. 2). The changes in five markers of bone turnover – serum OC, PICP and BSAP, and urinary F-PYD and NTX – were examined on days 4 and 5 of 1,25(OH)$_2$D administration. There were no significant increments in either of the bone resorption markers (F-PYD or NTX) in either black or white women and excretion of F-PYD and NTX remained lower in black than white women throughout the study. No increases were observed in one bone formation marker, serum BSAP, in either black or white women; however, serum OC and PICP levels increased significantly in black but not white women on both days 4 and 5 of the study.

Discussion

Our basal biochemical results are typical of racial differences in calcium metabolism shown previously, with lower levels of 25(OH)D [18,25–28] and urinary calcium [5,26,29,30] but slightly higher levels of PTH [5,18,27] and 1,25(OH)$_2$D [18,29] in black women compared with white women. Our data also agree with the concept that elevated PTH may play a role in increased 1,25(OH)$_2$D levels in black women and that black women have evidence of somewhat lower bone turnover than white women [5,18,19,21].

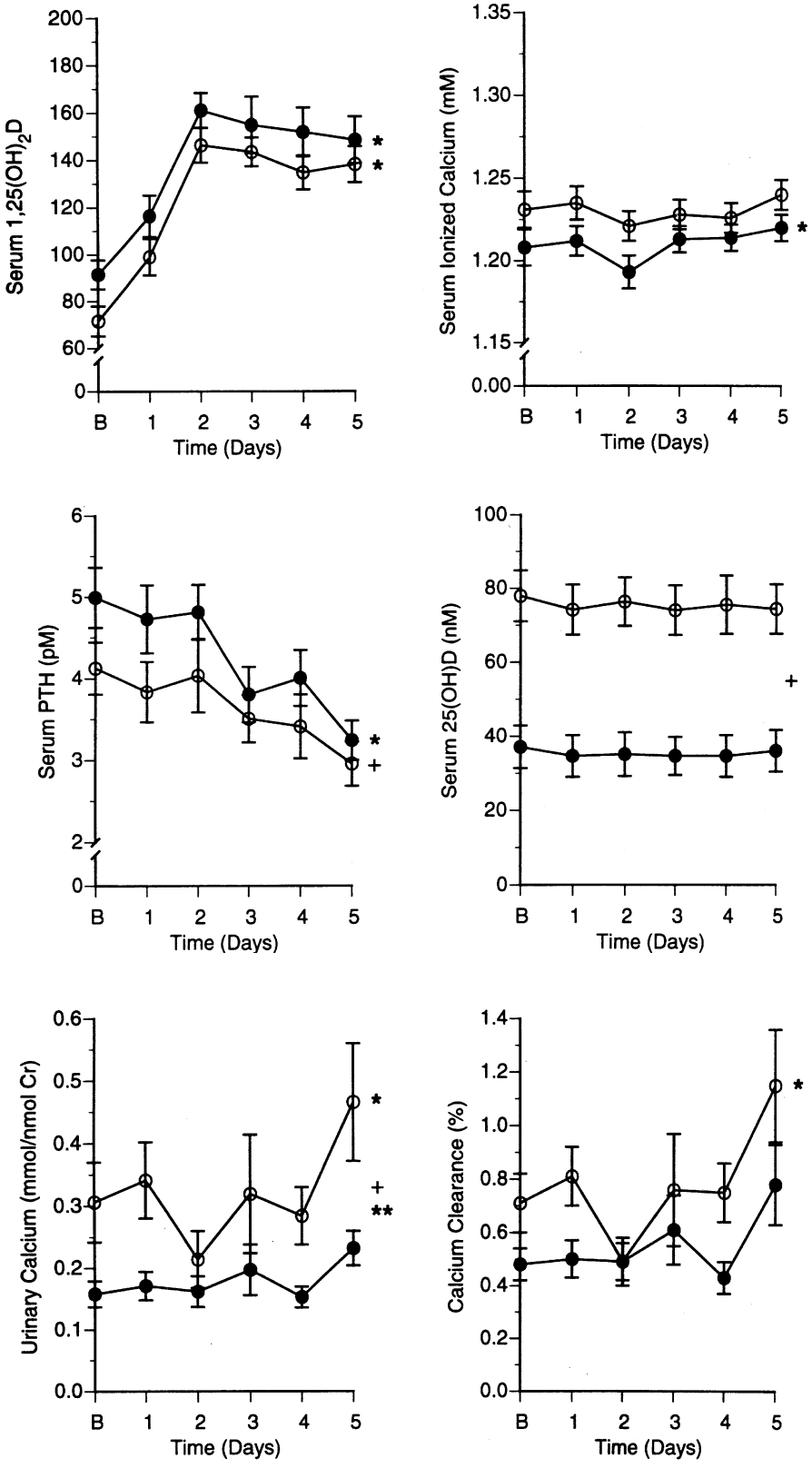

Fig. 1. Calcium homeostasis variables in black (*filled circles*) and white (*open circles*) premenopausal women during administration of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. **Top:** Serum levels of 1,25$(OH)_2$D and ionized calcium. **Middle:** serum levels of PTH and 25(OH)D. **Bottom:** urinary calcium and calcium clearance. Time trends are shown for each group separately *($p<0.05$). Group differences are shown by $^{+}p<0.05$. Time/group interactions are shown by **($p<0.05$). (All analyses were by repeated measures ANOVA.)

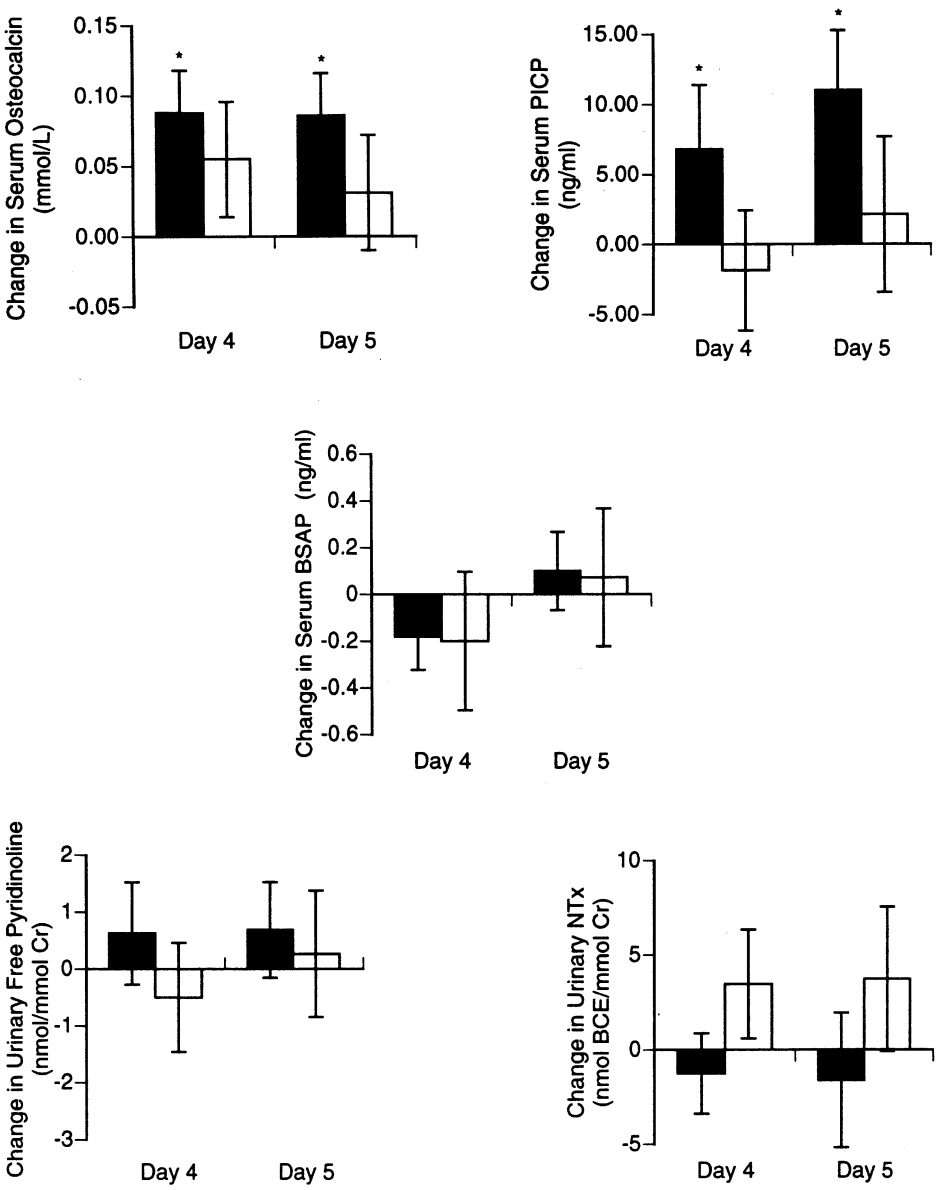

Fig. 2. Changes in bone turnover markers in black (*filled columns*) and white (*open columns*) premenopausal women after 1,25(OH)$_2$D administration. The results are shown as mean ± standard error. *p<0.05 at day 4 or 5 compared with the basal value by paired t-test. *BSAP*, bone-specific alkaline phosphatase; *PICP*, carboxyterminal propeptide of type I collagen.

It is known that increasing serum calcium and 1,25(OH)$_2$D have an inhibitory effect on PTH production [20]. Increments in 1,25(OH)$_2$D were the same in black and white women, whereas the expected increase in serum calcium with this protocol was slightly greater in black women. This could be due to greater calcium absorption in black women in response to the increase in 1,25(OH)$_2$D, although this study did not directly evaluate this. Prior studies have been controversial with regard to differences in absorption efficiency between black and white people [31–34]. Decrements in PTH were larger in black than white women, presumably due largely to the greater increments in serum calcium in the former.

The finding of a comparatively lower increment in calcium clearance under dynamic conditions in this study in black women, supports the thesis that renal calcium handling is a major racial difference in skeletal metabolism. Even in the face of a greater increase in serum calcium and more prominent suppression of PTH, both of which should increase urinary calcium more dramatically in black than in white women, black women had substantially less urinary calcium excretion. This finding also agrees with racial differences in renal calcium conservation when challenged by PTH infusion [22].

1,25(OH)$_2$D is known to be a very potent agent to induce bone resorption in vitro [35]; however, this action

has not clearly been shown in vivo. Duda et al. [16] showed a very mild stimulation of bone resorption in osteoporotic and postmenopausal normal women assessed by urine hydroxyproline with a daily dose of 2 μg/day, but Geusens et al. [36], who administered an even higher dose of $1,25(OH)_2D$ (4 μg/day) to patients with osteoporosis and osteoarthritis, showed no hydroxyproline increment. With the same dose of $1,25(OH)_2D$ (1 μg/day) used in this study, we did not observe any changes in biochemical indices of bone resorption in postmenopausal women with osteoporosis [17]. In this study of young healthy premenopausal women we also observed no change in bone resorption in either black or white women after $1,25(OH)_2D$ administration. It is possible that the 1,25(OH)D-induced bone resorptive stimulus is limited in vivo by the acute suppression of PTH.

If $1,25(OH)_2D$ administration stimulates bone formation, then one would expect to see increases in all the bone formation markers measured in this study. Serum osteocalcin levels increased during $1,25(OH)_2D$ administration in postmenopausal women with osteoporosis, but serum total alkaline phosphatase [16,17] and PICP [17] levels did not change. Similarly, in this study, using the bone-specific alkaline phosphatase assay, levels of the enzyme did not increase with $1,25(OH)_2D$ stimulation. Although $1,25(OH)_2D$ has been shown to stimulate alkaline phosphatase gene transcription and mRNA production in vitro [37], it is thus likely that mature osteoblasts, in vivo, do not respond to $1,25(OH)_2D$ administration. This raises the question as to whether the $1,25(OH)_2D$ effect on osteocalcin might be gene specific, rather than an indication of bone formation stimulation. Both serum osteocalcin and PICP increased after $1,25(OH)_2D$ administration in black women, but in white women the increments were smaller and not statistically significant. The findings are not surprising in white premenopausal women, since we observed similar effects in postmenopausal white women previously [17]. The surprising finding, however, that black women could respond to $1,25(OH)_2D$ administration with an increase in the bone matrix component, PICP, is of significance. The PICP increase in black women also supports the concept that a greater increase in osteocalcin seen here in black females may actually be related to bone organic matrix formation rather than solely to osteocalcin gene-specific stimulation by $1,25(OH)_2D$. Furthermore, this increase in osteocalcin and PICP is likely to be independent of prior resorption events in the normal coupling process since there is no time lag and there was no change in bone resorption shown after $1,25(OH)_2D$ administration. These results indicate that either black women have a greater capacity of mature osteoblasts to respond to $1,25(OH)_2D$ or that the response is more vigorous because of the lower basal levels in blacks. By either explanation, one could speculate that the ability to respond to $1,25(OH)_2D$ more robustly with osteocalcin and PICP could confer some advantages in blacks to achieve higher bone mass than their white counterparts. It is important to note, however, that black women still have lower osteocalcin and PICP levels than white women, even with the observed $1,25(OH)_2D$-induced increase.

In conclusion, we have shown that black women have superior renal calcium conservation under static and dynamic conditions and perhaps a better ability of the osteoblast to respond to a formation stimulus.

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