

Calcium Absorption Responses to Calcitriol in Black and White Premenopausal Women

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ABSTRACT

On the basis of recent findings that adult black women had similar calcium absorption but higher levels of 1,25-dihydroxyvitamin D [1,25-(OH)₂D] than white women, we hypothesized that blacks have a gut resistance to the action of calcitriol. To test this, we studied 11 black [age, 32.4 ± 5.7 (±SD) yr] and 12 white women (28.4 ± 5.5 yr). The women were maintained on a constant 500-mg calcium diet for 4 weeks, and each received calcitriol (0.25 μg) four times daily for the last 2 weeks. After 2 and 4 weeks, each subject had measurements of fractional ⁴⁵Ca absorption index and blood and urine tests. At 2 weeks, the black women had similar calcium absorption indexes [18.7

± 1.9% (±SEM)/L vs. 20.0 ± 1.8%/L; age adjusted], borderline higher 1,25-(OH)₂D levels [95.7 ± 6.4 (±SEM) vs. 78.2 ± 6.2 pmol/L; P = 0.071; age adjusted], higher serum PTH levels, and lower urinary calcium excretion. Calcitriol therapy induced similar increments in plasma 1,25-(OH)₂D levels in the two groups, but a smaller increment in calcium absorption in the black women (18.4 ± 8.6% vs. 44.6 ± 7.8%; P = 0.043; means adjusted for age and initial absorption index). These findings support the hypothesis that, compared with whites, healthy premenopausal black women have gut resistance to the action of calcitriol. (*J Clin Endocrinol Metab* 80: 3068–3072, 1995)

THIS STUDY tests the hypothesis that, compared with white women, adult black women have a gut resistance to the action of calcitriol. The hypothesis stems from a recent metabolic study in which we observed that black women had similar fractional ⁴⁷Ca whole body retention (an index of calcium absorption), but higher levels of 1,25-dihydroxyvitamin D [1,25-(OH)₂D] than white women (1). In that study, the black women had higher 1,25-(OH)₂D levels on high (2000 mg/day) and low (300 mg) calcium diets and a significantly greater increase in the plasma 1,25-(OH)₂D concentration after dietary calcium restriction (1). No other studies of calcium absorption in black and white adults are available for comparison, but 2 comparative studies of calcium absorption in black and white children have been reported (2, 3). Bell *et al.* (2) found absorption to be similar in a group of 33 black and white boys and girls aged 9–18 yr. In contrast, Abrams *et al.* (3), studying 62 girls, aged 7–14 yr, found calcium absorption to be greater in the black girls. In the study that measured vitamin D levels (2), serum 1,25-(OH)₂D was higher in the black children. Among adults, 1,25-(OH)₂D levels have been reported to be higher in blacks in some studies (1, 4–6) and similar to those in whites in others (7–9).

Other racial differences in calcium-regulating hormones

have been reported. The serum PTH concentration has been found to be higher in blacks than whites in some (5–7), but not other (1, 8, 10), studies. With dynamic testing involving calcium and calcium citrate infusions, El-Hajj Fuleihan *et al.* (9) identified a mild hyperparathyroidism in black adults. Lower urinary calcium excretion has been widely reported in blacks (5–8, 11–13); however, in several of these studies, calcium intake was either not assessed (7, 12, 13) or was estimated to be lower in the blacks (11).

Racial differences in calcium-regulating hormones are important because blacks have higher bone mass (14–16) and lower fracture rates (17, 18) than whites. This study was conducted to examine racial differences in the 1,25-(OH)₂D-calcium absorption axis. We approached this by comparing calcium absorption and the absorption responses to exogenous calcitriol in adult black and white women maintained on constant metabolic diets. Other objectives were to compare urinary calcium excretion and calcium-regulating hormone levels in the two groups before and after calcitriol treatment.

Subjects and Methods

Subjects

Twenty-four healthy premenopausal women, 12 black and 12 white, were enrolled in this study. To be eligible, the women were required to have both parents be either black or white. Good health was determined by physical examination and screening laboratory tests. Women taking glucocorticoids, diuretics, estrogen, or calcium and vitamin D supplements were excluded. None of the women was pregnant (each had a negative pregnancy test 1 week before enrollment) or lactating. The research protocol was approved by the radiation safety and human investigation review committees at Tufts University, and written informed consent was obtained from each subject.

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TABLE 1. Mean nutrient contents (\pm SD) of the 3-day cycle diets

	Black	White	P
No.	11	12	
Cal/day	2302 \pm 219	2453 \pm 209	0.106
Protein (g/day)	51.4 \pm 0.3	51.5 \pm 0.2	0.248
Fat (g/day)	104.2 \pm 12.1	111.2 \pm 14.4	0.216
Carbohydrate (g/day)	298.4 \pm 29.4	320.8 \pm 25.2	0.066
Crude fiber (g/day)	4.0 \pm 0.1	4.1 \pm 0.1	0.057
Calcium (mg/day)	503.6 \pm 10.4	511.4 \pm 7.3	0.054
Magnesium (mg/day)	282.3 \pm 9.7	287.4 \pm 4.3	0.135
Phosphorus (mg/day)	697.4 \pm 11.6	704.2 \pm 9.4	0.142
Sodium (mg/day)	2526 \pm 43	2554 \pm 42	0.134

TABLE 2. Mean fractional ^{45}Ca absorption indices and percent CV of paired measurements made 5 days apart in 12 subjects over age 60

Time after dosing (h)	Mean fractional ^{45}Ca absorption index (%L) ^a	Mean % CV of paired measures
2	16.0 \pm 4.2	10.4 \pm 7.0
3	16.7 \pm 3.9	9.1 \pm 7.1
4	16.6 \pm 4.1	9.4 \pm 8.5

Each subject was treated with 600 mg calcium and 400 IU vitamin D daily for 2 weeks before and during the precision study to reduce physiological variation related to changing calcium and vitamin D intakes. Values are the mean \pm SD.

^a First of paired measurements.

Study design

During the 4-week study, volunteers consumed a constant metabolic diet. Each woman came to the Metabolic Research Unit daily to be weighed, eat a meal, and pick up two packaged meals and water. After 2 weeks on the metabolic diet, each woman took calcitriol tablets (0.25 μg) four times per day (upon arising, at lunch, dinner, and bedtime) for 2 weeks. Weight was maintained within 1.5 kg of the enrollment weight by adjustment of intake, as needed, of carbohydrate and fat. To reduce variations in vitamin D intake and eliminate seasonal variations in circulating 25-hydroxyvitamin D (25OHD) levels, each woman took a multivitamin containing 400 IU vitamin D daily for 3 weeks before and throughout the study. The multivitamins and calcitriol tablets were obtained from the same lots. At enrollment, the date of onset of the last menstrual period was recorded.

Measurements were made after 2 weeks on the metabolic diet and again after 2 weeks of calcitriol therapy (and 4 weeks on the metabolic diet). On both occasions, subjects came to the Metabolic Unit at 0800 h after an 8-h fast. Each brought a 24-h urine collection. A blood sample was drawn by venipuncture at 0815 h, and at 0830 h, subjects ingested the ^{45}Ca tracer (see below). Blood samples were analyzed for calcium (total), phosphorus, PTH, 25OHD, 1,25-(OH) $_2\text{D}$, and osteocalcin. Twen-

ty-four-hour urine samples were analyzed for calcium, sodium, phosphorus, and creatinine.

Intake assessment and metabolic diet

Self-selected intakes of calcium and vitamin D were estimated at enrollment with a general food frequency questionnaire [food frequency questionnaire version 06.10.88 (1988), Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA]. Throughout the study, the women were fed the same constant diets as a 3-day cycle menu designed to contain a fixed fiber intake and 500 mg calcium, 700 mg phosphorus, 280 mg magnesium, 50 g protein, and 2.5 g sodium. Average intakes of these nutrients during the study are shown in Table 1. All water consumed was deionized.

Laboratory assays

Serum intact PTH was measured with Allegro intact PTH RIA kits from Nichols Institute (San Juan Capistrano, CA) with intra- and inter-assay coefficients of variation of 5.6% and 6.6%, respectively. Plasma 1,25-(OH) $_2\text{D}$ was measured by the competitive protein-binding method of Reinhardt *et al.* (19), with intra- and interassay coefficients of variation of 4.9% and 7.7%, respectively. Plasma 25OHD was measured by the method of Preece *et al.* (20), with intra- and interassay coefficients of variation of 5.0% and 7.3%, respectively. Serum osteocalcin was measured with two-site immunoradiometric assay kits from Nichols Institute, with intra- and interassay coefficients of variation of 4.0% and 6.0%, respectively. Serum calcium was measured with a Nova 7 analyzer (Nova Biomedical, Waltham, MA). Serum phosphorus and serum and urinary creatinine were assayed by colorimetry with a Cobas Fara centrifugal analyzer (Roche Instruments, Belleville, NJ). Urinary calcium, phosphorus, and sodium were measured by direct current plasma emission spectroscopy with a Spectrospan 6 (Beckman Instruments, Palo Alto, CA). All samples for individual subjects were measured in a single assay.

Densitometry

Bone mineral density (BMD) of the spine (L2-L4) and femoral neck were measured with a model DPX dual energy x-ray absorptiometer (Lunar Radiation, Madison, WI), with coefficients of variation in our laboratory of 1% and 2%, respectively (21).

Calcium absorption

Fractional ^{45}Ca absorption was estimated, as described previously (22), from the appearance of ^{45}Ca in the blood after ingestion of 100 mL of an aqueous solution containing 3 μCi ^{45}Ca and 100 mg cold calcium, as the chloride. This was followed immediately by ingestion of three 50-mL deionized water rinses. Before ingestion, duplicate 60- μL standards were removed from each test dose for counting. Exactly 3 h after ingestion, 15 mL whole blood were drawn. Two 2-mL aliquots of serum

TABLE 3. Clinical characteristics

Measure	Black	White	Significance level (P value) of difference
No.	11	12	
Age (yr)	32.4 \pm 5.7	28.4 \pm 5.5	0.106
Ht (cm)	165 \pm 7	167 \pm 7	0.380
Wt (kg)	68.9 \pm 10.9	66.2 \pm 8.5	0.508
Body mass index (kg/m 2)	25.4 \pm 3.5	23.8 \pm 3.5	0.266
Dietary calcium (mg/day)	462 \pm 190 (10) ^a	982 \pm 325	<0.001
Dietary vitamin D (IU/day)	110 \pm 68 (10)	238 \pm 94	0.001
Bone mineral density (g/cm 2)			
Spine, L2-L4	1.30 \pm 0.13 (10)	1.19 \pm 0.11	0.044
Femoral neck	1.03 \pm 0.16	1.02 \pm 0.05	0.778

Values are the mean \pm SD.

^a Sample size given in parentheses.

TABLE 4. Fractional ⁴⁵Ca absorption index and laboratory values (mean ± SEM) in 11 black and 12 white women before and after 2 weeks of calcitriol therapy

Measure and significance (P) of group difference	Before calcitriol	After calcitriol
Fr ⁴⁵ Ca absorption index (percent/L)		
Black (10) ^a	18.7 ± 1.9	22.3 ± 1.9
White	20.0 ± 1.8	26.8 ± 1.8
P	0.658	0.112
Serum calcium (mmol/L)		
Black	2.21 ± 0.02	2.23 ± 0.02
White	2.24 ± 0.02	2.27 ± 0.02
P	0.239	0.304
Serum phosphorus (mmol/L)		
Black	1.03 ± 0.04	1.06 ± 0.04
White	1.04 ± 0.03	1.09 ± 0.04
P	0.867	0.661
Serum PTH (ng/L)		
Black	48.1 ± 4.0	33.9 ± 3.7
White	31.7 ± 3.8	23.4 ± 3.6
P	0.010	0.065
Plasma 25 OHD (nmol/L)		
Black	49.6 ± 9.7	54.2 ± 12.9
White	88.5 ± 9.3	96.8 ± 12.3
P	0.011	0.031
Plasma 1,25-(OH) ₂ D (pmol/L)		
Black	95.7 ± 6.4	108.5 ± 10.0
White	78.2 ± 6.2	105.4 ± 9.5
P	0.071	0.828
Serum osteocalcin (nmol/L)		
Black	0.92 ± 0.08	0.98 ± 0.09
White	0.90 ± 0.07	1.00 ± 0.08
P	0.862	0.835
24-h urinary calcium/creatinine (mmol/mol)		
Black (n = 10)	143.7 ± 31.7	396.2 ± 51.3
White	313.8 ± 28.8	601.5 ± 46.5
P	0.001	0.010
24-h urinary phosphorus/creatinine (mmol/mol)		
Black (10)	1378 ± 124	1536 ± 150
White	1927 ± 112	1974 ± 136
P	0.005	0.051
24-h urinary sodium/creatinine (mmol/mol)		
Black (10)	8358 ± 1115	7559 ± 645
White ^b	11,110 ± 1010	10,685 ± 585
P	0.095	0.003

Values are means adjusted for age.

^a One subject had an invalid postcalcitriol absorption measurement because of an error in the timing of her blood draw.

^b Includes one woman with urinary sodium/creatinine excretions of 20,464 and 12,446 mmol/mol.

and the standards were each added to 18 mL scintillation fluid, and β-emissions were counted in a scintillation counter (model LS3801, Beckman Instruments, Fullerton, CA). Counts were corrected for quenching. In accordance with the procedure of Marshall and Nordin (23), the fraction of ⁴⁵Ca counts per L serum was multiplied by 15% of the body weight to yield an estimate of the fraction of the dose circulating in extracellular fluid. The reproducibility of the method was previously evaluated in our laboratory in 12 subjects, each measured 2, 3, and 4 h after treatment, twice, 5 days apart (Table 2). The 3 h point with a

precision of 9.1 ± 7.1% (±SD) was selected for this study. Results are reported as the fraction of the dose circulating in the extracellular fluid at 3 h and referred to as the fractional ⁴⁵Ca absorption index.

⁴⁵Ca was purchased from Amersham Corp. (Arlington Heights, IL), and a complete spectral analysis was carried out on each batch before its use to ensure purity. Radiation exposure from the two ⁴⁵Ca absorption studies totaled 150 mrem to bone, the critical organ, and 15 mrem to the whole body.

Statistical analysis

Simple and partial correlation coefficients were computed with standard parametric procedures in SPSS (24). The mean calcium absorption index and biochemical values of the race groups were adjusted for age by analysis of covariance. The mean percent changes in these measures were adjusted for both age and precalcitriol values. Analysis of covariance procedures were performed with the SAS general linear models least square means procedure (25). Significance tests for correlations and adjusted means were conducted at the two-tailed 0.05 level.

Results

Of the 24 subjects enrolled, 1 black woman was excluded from the analyses. Although her calcium absorption indexes were similar to those of the other black women (*i.e.* 16.7%/L before and 22.2%/L after calcitriol), she was presumed to have a renal leak of calcium on the basis of a high normal preenrollment urinary calcium excretion, high postenrollment urinary calcium, high serum PTH and osteocalcin levels, and low normal postenrollment serum calcium and BMD. The clinical characteristics of the remaining 23 women are shown in Table 3. The black women were an average of 4 yr older, had lower self-selected calcium and vitamin D intakes, and had higher spinal BMD. The numbers of black and white women who had their first calcium absorption measurements during the follicular phase of their menstrual cycle (8 blacks and 7 whites) or during the luteal phase (3 blacks and 5 whites) were similar (by χ^2 test, $P = 0.469$).

After 2 weeks on the metabolic diet, the mean fractional ⁴⁵Ca absorption index was similar in the black and white women despite borderline higher levels of 1,25-(OH)₂D in the blacks (Table 4). The absorption index increased after calcitriol therapy in both racial groups, but the mean increase was less in the black than in the white women [18.4 ± 8.6% (±SE) *vs.* 44.6 ± 7.8%; $P = 0.043$; Table 5]. Increments in plasma 1,25-(OH)₂D after calcitriol were similar in the two groups, and inclusion of the increment in 1,25-(OH)₂D in the adjustments did not substantially alter the mean fractional ⁴⁵Ca absorption responses to calcitriol [20.5 ± 8.9% (±SE) in blacks and 42.7 ± 8.0% in whites]. Plasma 25OHD and the ⁴⁵Ca absorption index were not correlated.

The black women had lower urinary calcium/creatinine excretion than the white women before and after calcitriol therapy (Table 4). Both groups responded to calcitriol with similar large increases in urinary calcium excretion (Table 5). Serum PTH, initially higher in the black women (Table 4), declined similarly in the two groups after calcitriol therapy (Table 5). Correlations between change in urinary calcium/creatinine excretion and changes in the serum PTH concentration were of borderline significance (in the groups combined: $r = -0.39$; $P = 0.093$; in blacks: $r = -0.31$; and in

TABLE 5. Change in fractional ^{45}Ca absorption index and laboratory values after calcitriol therapy, expressed as a percentage of the initial value

	Black	White	P
No.	11	12	
^{45}Ca absorption index	18.4 \pm 8.6 (n= 10) ^a	44.5 \pm 7.8	0.043
Serum calcium	0.58 \pm 1.07	1.81 \pm 1.02	0.437
Serum phosphorus	3.61 \pm 3.24	5.19 \pm 3.09	0.737
Serum PTH	-22.7 \pm 10.7	-26.8 \pm 10.1	0.807
Plasma 1,25-(OH) ₂ D	21.4 \pm 10.3	29.4 \pm 9.8	0.607
24-h urinary calcium/creatinine	129.2 \pm 27.6 (n=10) ^b	161.5 \pm 24.4	0.460
24-h urinary phosphorus/creatinine	6.16 \pm 8.63 (n=10) ^b	9.26 \pm 7.68	0.815

Values are means adjusted for age and baseline value.

^a Minus woman with late blood draw.

^b One woman did not return her week 4 urine collection.

whites: $r = -0.47$; after controlling for differences in initial PTH and 24-h urinary calcium/creatinine values).

Initially, urinary phosphorus/creatinine excretion was lower in the black than the white women despite similar phosphorus intakes and higher levels of 1,25-(OH)₂D and PTH in the blacks. Changes in phosphorus excretion after calcitriol therapy were modest and similar in the two groups.

After 5 and 7 weeks of daily use of a multivitamin reported to contain 400 IU vitamin D, serum 25OHD levels were lower in the black than the white women (Table 4). Plasma 25OHD and 1,25-(OH)₂D levels were not significantly correlated in either racial group.

Serum calcium, phosphorus, and osteocalcin levels were similar in the two racial groups and stable throughout the study; urinary sodium excretion was higher in the white women.

Discussion

The blunted calcium absorption response to oral calcitriol in the black women in this study provides support for the hypothesis that adult black women have a gut resistance to the action of calcitriol. The similar absorption indexes of the two racial groups and the borderline higher 1,25-(OH)₂D level in the black women at baseline are similar to findings in our previous study (1) and to those of Bell *et al.* (2). No studies of absorption responses to calcitriol in blacks and whites are available for comparison. Although the study is limited by sample size, its design has several strengths, including the use of the same constant diets for both racial groups [in contrast to the matched calcium intakes used in our previous study (1)], maintenance of the women on the metabolic diets for the 2 weeks needed for gastrointestinal adaptation to the metabolic conditions before each measurement (1, 26), and use of a reproducible index of calcium absorption. In addition to its reproducibility, this absorption index is highly correlated with values from the more elaborate double isotope and balance methods (23, 27).

Oral calcitriol in split doses was used because it produces a more prolonged increase in 1,25-(OH)₂D blood levels than intermittent iv doses. The plasma responses to oral calcitriol were similar in the two racial groups. Of course, we cannot exclude the possibility of a racial difference in nongenomic effects of oral calcitriol. The absorption response to calcitriol is dose related (28, 29). A dose of 1

$\mu\text{g}/\text{day}$ was selected because it stimulates calcium absorption in a high proportion of responders (28), but does not provide a maximal stimulus (5) that might diminish the ability to detect a racial difference. As expected (30), none of the subjects in our study became hypercalcemic or hypercalciuric (except the woman with a renal leak who was hypercalciuric), presumably because calcium intake was set at the low level of 500 mg/day.

Gut resistance to the action of 1,25-(OH)₂D in blacks might be expected to impact negatively on bone mass and result in lower, not higher, bone mass. One consequence of the gut resistance is secondary hyperparathyroidism. In whites, higher PTH levels are widely associated with increased bone resorption and lower bone mass, but blacks appear to have a skeletal resistance to the action of PTH (8). Further study is needed to identify other racial differences that may contribute to the higher skeletal mass in blacks.

This study agrees with others that urinary calcium excretion is lower in black than white women. The finding cannot be attributed to differences in dietary intake of calcium, differences in intake of other nutrients that influence calcium bioavailability, or differences in initial calcium absorption index. The most obvious explanation for the lower urinary calcium in the black women is their higher PTH level. Previously, we did not detect a significant racial difference in serum PTH levels, perhaps because the diets were not as closely matched (1). In this study, calcitriol caused a 14% greater increment in urinary calcium excretion in whites than in blacks, a difference that was not significant. In contrast, Bell *et al.* (5) found the urinary calcium response to calcitriol to be significantly greater in white than in black men and women. This difference may be related to the higher dose of calcitriol (4 vs. 1 μg) used by Bell or to differences in PTH status of the two study populations.

The lower initial urinary phosphorus excretion in the blacks despite their higher PTH levels and similar phosphorus intakes is consistent with a gut resistance to calcitriol in the blacks, as calcitriol stimulates the active intestinal transport of phosphorus as well as calcium. However, this study cannot exclude the possibility of a racial difference in renal handling of phosphorus.

On daily supplementation with a multivitamin, plasma 25OHD levels remained lower in the black women. The multivitamins were not assayed for vitamin D content, but they appear to have contained approximately the stated amount

of 400 IU cholecalciferol, because pills known to contain 400 IU vitamin D produced a mean 25OHD level in another group of white women (31) that was similar to that in the white women in this study. Although blacks have less efficient photosynthesis of vitamin D than whites (32), the 25OHD levels achieved in the blacks in this study were lower than those in white women measured here in the winter (31), a season when sun exposure does not induce skin synthesis of the vitamin (33). Lower 25OHD levels in blacks have been widely reported (1, 2, 5, 6, 9). The persistent low 25OHD levels during oral supplementation suggests that the absorption and/or metabolism of ingested vitamin D differ in black and white women.

In conclusion, their blunted calcium absorption response to calcitriol supports the hypothesis that healthy adult black women have a gut resistance to the action of 1,25-(OH)₂D. Other racial differences in this study include higher levels of PTH, lower levels of 25OHD, and lower urinary calcium excretion in the black women.

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