

# Serum 1,25-dihydroxyvitamin D and calcium intake affect rates of bone calcium deposition during pregnancy and the early postpartum period<sup>1–3</sup>

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## ABSTRACT

**Background:** Factors affecting bone calcium deposition across pregnancy and lactation are not well characterized.

**Objective:** The impact of maternal age, calcium intake, race-ethnicity, and vitamin D status on the rate of bone calcium deposition (VO+) was assessed across pregnancy and lactation.

**Design:** Stable calcium isotopes were given to 46 women at pre- or early pregnancy (trimester 1), late pregnancy (trimester 3), and 3–10 wk postpartum. Three cohorts were included: 23 adolescents from Baltimore (MD), aged  $16.5 \pm 1.4$  y (mean  $\pm$  SD; Baltimore cohort); 13 adults from California, aged  $29.5 \pm 2.6$  y (California cohort); and 10 adults from Brazil, aged  $30.4 \pm 4.0$  y (Brazil cohort). The total exchangeable calcium pool, VO+, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D], parathyroid hormone, and calcium intake were evaluated.

**Results:** At trimester 3, inverse associations between 1,25(OH)<sub>2</sub>D and VO+ were evident in the Baltimore ( $P = 0.059$ ) and Brazil ( $P = 0.008$ ) cohorts and in the whole group ( $P = 0.029$ ); calcium intake was not a significant determinant of VO+ in any group during pregnancy. At postpartum, a significant positive association was evident between VO+ and calcium intake ( $P \leq 0.002$ ) and between VO+ and African ethnicity ( $P \leq 0.004$ ) in the whole group and within the Baltimore and Brazil cohorts.

**Conclusions:** Elevated 1,25(OH)<sub>2</sub>D was associated with decreased rates of bone calcium deposition during late pregnancy, a finding that was particularly evident in pregnant adolescents and adult women with low calcium intakes. Higher dietary calcium intakes and African ethnicity were associated with elevated rates of bone calcium deposition in the postpartum period. *Am J Clin Nutr* 2012;96:64–72.

## INTRODUCTION

Significant adaptations in maternal calcium homeostasis and bone turnover occur to support the demands of pregnancy and lactation. To date, these physiologic alterations and their possible determinants have not been fully characterized. The human fetus acquires  $\sim 30$  g Ca across gestation (1), which increases from only several milligrams per day in the first trimester to  $>250$  mg/d during the last trimester (2). During lactation, calcium losses into human breast milk represent  $\sim 260$  mg/L (3). These increased demands may involve alterations in calcium absorption and excretion and rates of bone calcium turnover.

Racial differences in rates of bone calcium deposition between nonpregnant white and African American females have been reported (4, 5), but possible differences during pregnancy are

largely unexplored. Alterations in bone turnover markers support an impact of age on bone turnover between pregnant and lactating adolescent and adult women (6). Similarly, longitudinal bone density studies in adult women have reported bone mineral density losses of 3.2–4.6% at trabecular sites across pregnancy (7, 8), with losses being more pronounced among adolescents (9). Although trabecular losses are frequently evident, increases in bone density at cortical bone sites may occur during pregnancy (10). During lactation, temporal losses of trabecular bone mineral at the spine and hip ( $\sim 3$ –5%) occur over the first 3–6 mo of lactation (11), and these losses appear to be unaffected by calcium supplementation (12).

Studies assessing longitudinal changes in 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]<sup>4</sup> across pregnancy and lactation have supported the concept that this hormone typically increases across pregnancy (13–17). Determinants of this increase are less well studied as are possible relations between serum 1,25(OH)<sub>2</sub>D and rates of bone calcium deposition across pregnancy and lactation. Longitudinal measures of parathyroid hormone (PTH) during pregnancy have reported decreases, increases, or no change in this hormone across gestation (6, 18, 19). Recent data highlight the increased risk of suboptimal 25-hydroxyvitamin D [25(OH)D] concentrations among pregnant women, with increased insufficiency being evident among minorities (16, 17, 20). Increasing data also link suboptimal concentrations of this prohormone with elevated concentrations of parathyroid hormone and/or 1,25(OH)<sub>2</sub>D (16, 17, 20), although all data do not support these associations (2, 21). The possible impact of these

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<sup>2</sup> Supported by the NIH (grants HD35191 and DK41732) and National Center for Research Resources/General Clinical Research Centers grant RR00052.

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<sup>4</sup> Abbreviations used: PTH, parathyroid hormone; TEP, total exchangeable pool; VO+, rate of bone calcium deposition; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Received November 2, 2011. Accepted for publication May 1, 2012.

First published online May 30, 2012; doi: 10.3945/ajcn.111.029231.

hormonal changes on bone calcium deposition during pregnancy remains largely uncharacterized.

To date, calcium isotopic studies across pregnancy and into the early postpartum/lactation period have focused primarily on changes in intestinal calcium absorption (6, 22–25). To our knowledge, only one longitudinal kinetic study has been published on bone calcium turnover across pregnancy and lactation (26). To address rates of bone deposition in response to the calcium challenges of pregnancy and lactation, we compiled our existing unpublished and published (26) longitudinal data with the use of intravenous stable calcium isotopes during both pregnancy and lactation as a function of habitual calcium intake, race-ethnicity, maternal age, and  $1,25(\text{OH})_2\text{D}$  concentration.

## SUBJECTS AND METHODS

### Subject recruitment

All women who were recruited were healthy nonsmokers. In the Baltimore cohort, pregnant adolescents ( $n = 23$ ;  $\leq 18$  y of age) were recruited from 1996 to 2002 from a Johns Hopkins Hospital clinic providing prenatal care to adolescents. Of the 23 adolescents, all were non-Hispanic; 20 were African American, and 3 were white. Adolescents were eligible to participate if they had a parity of 0 and intended to breastfeed their infant. Characteristics of the population at this clinic have been previously reported (27–29). The Johns Hopkins University Institutional Review Board approved the protocol, and informed written consent was obtained from all participants. Data on calcium absorption in this cohort have been published (30).

In the California cohort, women ( $n = 13$ ; 25–34 y of age) were recruited from the San Francisco Bay area from 1991 to 1993. Women were not eligible to participate if they were vegetarian, their prepregnancy BMI (in  $\text{kg}/\text{m}^2$ ) was  $<17$  or  $>27$ , their calcium intake was  $<800$  mg/d, or they consumed  $>3$  caffeine-containing beverages daily. All women were white, 2 women were Hispanic (15%), and all were of upper-middle-income economic status. Informed written consent was obtained, and the study was approved by the Committee of Human Subjects at the University of California. Calcium absorption data from this cohort have been published (25).

In the Brazil cohort, pregnant women ( $n = 10$ ; 20–35 y of age) were recruited from the Maternidade Escola of the Federal University of Rio de Janeiro from 1997 to 1999. All women were Hispanic, 4 women were white, and 6 were of African ethnicity; all were of low socioeconomic status. The study was approved by the Committee of Human Subjects at the University of California and by the Ethical Committee of the Maternidade Escola of the Federal University of Rio de Janeiro, Brazil. Data on calcium absorption and rates of bone calcium turnover from this cohort have been previously published (26, 31).

### Calcium kinetic studies

Studies were undertaken in a research unit after an overnight fast. Baseline anthropometric measures were obtained, fasting blood was collected, and women were given a standardized breakfast. In the Baltimore cohort, breakfast was self-selected to provide approximately one-third of the habitual daily calcium intake; in the California cohort, breakfast consisted of a toasted

English muffin and peanut butter; and in the Brazil cohort, breakfast consisted of white bread, butter, milk, and coffee. Midway through breakfast, women ingested an oral stable calcium isotope. Baltimore adolescents received either  $^{46}\text{Ca}$  (0.0075  $\mu\text{mol}/\text{kg}$ ) or  $^{44}\text{Ca}$  (0.005 mmol/kg) in  $\sim 60$  mL milk; California women received  $^{44}\text{Ca}$  ( $0.43 \pm 0.05$  mmol) in a small glass of a nonnutritive beverage (Crystal Light; Kraft General Foods); and Brazil women received  $^{46}\text{Ca}$  (0.25  $\mu\text{mol}$ ) in water. After breakfast, each woman received an intravenous injection of  $^{42}\text{Ca}$ . Intravenous amounts of stable calcium isotope given varied in relation to the analytic precision of the mass spectrometer and because of the increased quantity of stable calcium isotope required in adolescents (32). Intravenous amounts of stable calcium isotope administered were 0.025 mmol/kg or  $\sim 1.75$  mmol (Baltimore cohort),  $0.12 \pm 0.03$  mmol (California cohort), and 0.12 mmol (Brazil cohort). In all studies, timed blood samples were collected for 8 h after calcium isotope administration, a complete 24-h urine collection was obtained after administration, and then 3 daily spot urine samples were obtained 120 h after administration. In the Baltimore cohort, studies were undertaken during the third trimester of pregnancy (32–36 wk gestation) and in the early postpartum period (3–7 wk postpartum). In the California cohort, studies were undertaken at prepregnancy, during the second trimester (23–26 wk gestation), during the the third trimester (34–36 wk gestation), and in the early postpartum period (6–10 wk postpartum). In the Brazil cohort, studies were undertaken during the first (10–12 wk gestation) and third (34–36 wk gestation) trimesters of pregnancy and again in the postpartum period (7–8 wk postpartum).

Of the 23 teens in the Baltimore cohort, 15 returned for the postpartum study (3–7 wk after delivery). Of these, 8 of 15 (53%, all of whom were African American) were either partially ( $n = 4$ ) or exclusively ( $n = 4$ ) breastfeeding. In the California cohort, 10 women returned for the postpartum study (6–10 wk postdelivery). In the California cohort, 6 participants were exclusively breastfeeding and 4 were partially breastfeeding at postpartum. In the Brazil cohort, all women returned for the postpartum study from 7 to 8 wk after delivery. All Brazil women were exclusively breastfeeding at postpartum.

### Dietary intakes

The Baltimore adolescents were inpatients over the entire 120-h study. All dietary intakes were weighed before and after the study and analyzed for nutrient content with the use of the Minnesota Nutrient Database System (version 2.91; University of Minnesota). In the California cohort, a standardized diet was provided on the day before dosing, and a standardized breakfast, morning snack, and lunch were provided on the day of dosing. After lunch, California women adhered to their usual calcium intakes, and weighed food intakes were collected on 2 weekdays and on 1 weekend. Diet analysis was undertaken by using the Nutritionist III database (software version 8.5, Nutritionist III; N-Squared Computing). In the Brazil cohort, women were inpatients for 24 h after dosing then maintained on their usual calcium intakes after discharge. Dietary intakes in the Brazil cohort were weighed for 3 consecutive days before the study and were analyzed by using a Brazilian adaptation of the Food Processor nutrient database (33).

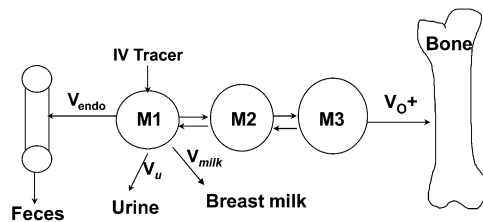
### Breast-milk calcium losses

The calcium content of the breast milk was measured in the Baltimore cohort by the nursing staff who weighed the infant before and after every feeding over one 24-h interval. The weight of milk produced was multiplied by the typical calcium content of breast milk (260 mg/L) (3). Of the 8 lactating adolescents, one did not complete the 120-h kinetic study; therefore, data from this individual were not available. In the California cohort, breast-milk intake was quantified by test-weighing the infant and by analysis of total calcium content of a complete 24-h breast-milk collection (25). In the Brazil cohort, breast-milk calcium was estimated (200 mg/d) by using Brazilian data from similar stages of lactation (34).

### Stable-isotope analysis and calculations

Calcium isotopes were measured in the Baltimore and Brazil cohorts by using magnetic sector thermal ionization mass spectrometry (Triton TI mass spectrometer; Thermoquest), and an earlier model of this mass spectrometer (MAT 261; Thermoquest) was used for the California cohort. The ratio of each administered tracer to either  $^{48}\text{Ca}$  or  $^{43}\text{Ca}$  was measured, and the degree to which this ratio increased over the natural abundance ratio was calculated (35).

Rates of bone calcium deposition ( $\text{VO}+$ ) were determined by the same individual (KOO) by using a multicompartmental model (Figure 1) and the Simulation, Analysis, and Modeling program as previously detailed (36).  $\text{VO}+$  was obtained by longitudinal measures of the change in the enrichment of the intravenous tracer in serum and urine over the 120-h postdosing period, after urinary and endogenous fecal calcium losses (and breast-milk calcium losses in lactating subjects) were accounted for.  $\text{VO}+$  was calculated as the product of the mass of compartment M3 multiplied by the fractional transfer coefficient of calcium loss from this compartment. We chose not to measure rates of bone



- $V_{\text{endo}}$  = Rate of endogenous fecal calcium excretion  
 $V_u$  = Rate of urinary calcium excretion  
 $V_{\text{milk}}$  = Rate of calcium loss into breast milk  
 $V_{\text{O}+}$  = Rate of bone calcium deposition  
 TEP = Total exchangeable calcium pool (M1 + M2 + M3)

**FIGURE 1.** To assess  $\text{VO}+$  and calcium absorption longitudinally across pregnancy and lactation, a stable calcium isotope ( $^{42}\text{Ca}$ ) was injected directly into compartment M1, which represents the mass of rapidly exchanging calcium pool. Clearance of the intravenous tracer in serum and urine over the 120-h interval postdosing was used to assess  $\text{VO}+$ . Compartments M2 and M3 are additional pools of calcium that represent time intermediaries in bone matrix formation. During pregnancy,  $\text{VO}+$  reflects the loss of calcium into both maternal and fetal bone. During lactation, additional calcium loss from compartment M1 is added into the model to account for calcium loss into breast milk. The TEP reflects the pool sizes of compartments M1, M2, and M3. The fraction of the TEP that is used for bone calcium deposition is defined as  $\text{VO}+/\text{TEP}$ .

calcium resorption in this study because this indirect measure is dependent on calcium absorption data and the variable study designs would limit interpretation between cohorts.

The total exchangeable pool (TEP) reflects the mass of calcium in metabolically active bone or along bone surfaces, which are in equilibrium with plasma and extracellular fluid. TEP was calculated as the sum of compartments M1, M2, and M3. The metabolic activity of bone was estimated on the basis of the amount of calcium transferred to bone as a fraction of the TEP ( $\text{VO}+/\text{TEP}$ ) (37, 38).

Endogenous fecal calcium losses were set at  $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . These losses are similar in adults and adolescents (39–41) and appear to be unaffected by pregnancy (42). Estimates of these losses will affect measures of net skeletal retention and bone calcium resorption but only minimally affect  $\text{VO}+$  as determined by following the disappearance of intravenous isotope over the 120-h interval after dosing. Calcium in 24-h urine was measured by using atomic absorption spectrophotometry.

### Hormone analysis

In the Baltimore cohort, 25(OH)D (sensitivity: 1.5 ng/mL; expected normal values: 9–37.6 ng/mL) and 1,25(OH) $_2$ D (sensitivity: 2 pg/mL; 95% CI: 25.1–66.1 pg/mL) were measured by using radioimmunoassays (Diasorin Inc); PTH analyses were not undertaken. In the California cohort, 1,25(OH) $_2$ D was measured by using a radioreceptor assay (Nichols Institute; 95% CI: 18–62 pg/mL; sensitivity: 2 pg/mL), serum 25(OH)D was determined by using a competitive protein binding assay (Nichols Institute; normal range: 16–74 ng/mL; sensitivity: 2.2 ng/mL), and PTH was measured by using an intact PTH assay (sensitivity: 2.82 pg/mL; Allegro; Nichols Institute) (25). In the Brazil cohort, 1,25(OH) $_2$ D was measured by using the same assay as for the California cohort (Nichols Institute Diagnostics), PTH was measured by ELISA (sensitivity: 1 pg/mL; normal range: 13–176 pg/mL; Diagnostic Systems Laboratories), and serum 25(OH)D was not measured.

### Statistical analyses

Data were analyzed by using SAS (version 9.2, 2007; SAS Institute). Descriptive statistics were computed for each of the variables by cohort. Initial analyses were based on analysis of variance models with repeated measures to assess relative differences between stages of pregnancy and during the postpartum period within each cohort. ANOVA models were then used along with the Tukey-Kramer method of adjustment for multiple comparisons to compare differences in study variables between cohorts at each stage of pregnancy and postpartum. Multiple regression models were developed to examine potential relations between bone calcium kinetic variables and other measured variables [maternal age, race-ethnicity, prepregnancy BMI, calcium intake, 25(OH)D, 1,25(OH) $_2$ D, PTH, breastfeeding practice]. All independent variables were initially included in the multiple regression models, and those that were nonsignificant were sequentially removed by using backward elimination. Variables remaining in the model were significant at the  $P < 0.10$  level. Significance was assessed at the  $P < 0.05$  level and considered to be borderline if  $P$  values were between 0.05 and 0.10.

## RESULTS

Prepregnancy height did not significantly differ between groups, and mean ( $\pm$ SD) values were  $162.8 \pm 5.1$  cm,  $159.6 \pm 6.4$  cm, and  $164.1 \pm 7.4$  cm in the Baltimore, Brazil, and California cohorts, respectively. Prepregnancy weight also did not significantly differ between groups and averaged  $62.8 \pm 12.7$  kg,  $60.3 \pm 7.2$  kg, and  $58.6 \pm 9.5$  kg in the Baltimore, Brazil, and California cohorts, respectively. Mean weight gain (and range in weight gain) over pregnancy was  $16.8 \pm 5.6$  kg (8–28 kg) and  $13.3 \pm 4.0$  kg (6.5–18.9 kg) in the California and Brazil cohorts, respectively. Total weight gain over pregnancy was not available for the Baltimore adolescents.

At entry into the study, the entire Baltimore cohort was nulliparous, non-Hispanic, and aged  $\leq 18$  y. Nearly all (87%) adolescents were African American, and they had calcium intakes similar to those of individuals in the California cohort ( $\sim 1200$  mg/d). Adolescents were recruited into the study only if they were nulliparous to avoid additional challenges to their calcium stores, which may have been depleted if they had more than one child during their adolescent years. Parity was not used as an inclusion or exclusion criterion in the adult cohorts. The entire California cohort was white; 15% were Hispanic. The majority (77%) of women were nulliparous at recruitment into the study. The entire Brazil cohort was Hispanic, and most were of African ethnicity (60%), were multiparous (90%), and had a mean calcium intake of  $\sim 450$  mg/d. All of the adult women breastfed their infants in the postpartum period compared with only 53% of the adolescents.

Serum 25(OH)D did not change from pregnancy to postpartum and did not differ significantly between the Baltimore (latitude  $39^\circ\text{N}$ ;  $48.2 \pm 24.2$  nmol/L) and California (latitude  $37^\circ\text{N}$ ;  $55.8 \pm 22.5$  nmol/L) cohorts; serum 25(OH)D was not measured in the Brazil cohort (latitude  $23^\circ\text{S}$ ) (Table 1). In the Baltimore cohort, 22% and 40% of teens had 25(OH)D concentrations  $< 30$  nmol/L at the third trimester and postpartum, respectively (the Institute of Medicine cutoff at which individuals are at an increased risk of deficiency) (43). In the California cohort, 25(OH)D concentrations  $< 30$  nmol/L were found in 15% of women both at the third trimester and postpartum. Concentrations of 25(OH)D in the Baltimore and California cohorts were not significantly associated with 1,25(OH)<sub>2</sub>D during pregnancy or lactation. Despite the small sample size and limited racial variability in the Baltimore cohort, serum 25(OH)D was significantly lower by  $-46 \pm 10$  (SE) nmol/L ( $P = 0.0001$ ) in African American ( $n = 20$ ) than in white ( $n = 3$ ) teens at T3 and by  $-54 \pm 14$  (SE) nmol/L ( $P = 0.002$ ) in African American ( $n = 13$ ) than in white ( $n = 2$ ) teens during the early postpartum period.

Serum 1,25(OH)<sub>2</sub>D increased across pregnancy in the California ( $P < 0.001$ ) and Brazil ( $P < 0.02$ ) adult groups and decreased significantly from the third trimester to the postpartum period in all 3 cohorts ( $P < 0.001$ ) (Table 1). The 1,25(OH)<sub>2</sub>D concentrations between groups did not significantly differ during pregnancy despite the variable calcium intake, different analytic approaches, and younger age of the Baltimore cohort. At postpartum, the Brazil cohort exhibited higher 1,25(OH)<sub>2</sub>D concentrations compared with the Baltimore and California groups, but the difference was significant only in relation to the Baltimore group ( $P < 0.05$ ).

Serum PTH concentrations did not significantly differ across the 3 periods in the California cohort, and no significant cor-

relations were noted between PTH and other variables measured in the California group. Among the Brazil women, PTH was significantly higher at postpartum compared with the first trimester ( $1.7 \pm 0.6$  compared with  $1.2 \pm 0.6$  pmol/L;  $P < 0.02$ ). Simple correlations between biochemical measures and maternal characteristics were examined in each physiologic state within each cohort and in the group as a whole. Of interest, among Brazil women, PTH was positively associated with dietary calcium at the first trimester ( $r = 0.779$ ,  $P = 0.008$ ) and postpartum ( $r = 0.733$ ,  $P = 0.016$ ) and negatively associated with 1,25(OH)<sub>2</sub>D at postpartum ( $r = -0.722$ ,  $P = 0.018$ ). Serum 1,25(OH)<sub>2</sub>D was negatively associated with dietary calcium intake at postpartum when all groups were combined ( $r = -0.325$ ,  $P = 0.05$ ).

Among the adult subjects at trimester 3, VO+ was not significantly different between California and Brazil women despite the variable calcium intake between cohorts (Table 1). In the Baltimore adolescents, VO+ was significantly higher ( $P < 0.05$ ) than that observed among the California or Brazil cohorts. In all groups, VO+ was significantly higher at trimester 3 than postpartum ( $P < 0.05$ ).

In the combined adult cohorts (California and Brazil), mean VO+ increased by 119% from prepregnancy/trimester 1 to trimester 3 ( $P < 0.01$ ). Despite the differing physiologic stage at which the first study measure was obtained, the magnitude of increase in VO+ observed during trimester 3 was similar in the California and Brazil groups. Moreover, in the combined adult groups, the relative fraction of TEP used for bone deposition (VO+/TEP) increased by 86% during pregnancy ( $P < 0.05$ ).

In the combined adult cohorts, VO+ decreased by 29% from trimester 3 to the postpartum period ( $P < 0.05$ ), and similarly among the Baltimore teens VO+ decreased by 18% from the third trimester to the postpartum period ( $P < 0.008$ ). In the adult women (California and Brazil), VO+/TEP values decreased by 21% from the third trimester to the postpartum period ( $P < 0.05$ ), but this did not significantly differ from the third trimester to the postpartum period among the adolescent Baltimore cohort.

### Multiple regression analysis of determinants of calcium kinetics

Potential relations between calcium kinetic variables and maternal variables within each cohort and in the group as a whole were examined (Table 2). In the Baltimore cohort at trimester 3, both age (across the age range of 13.5–18.3 y;  $P = 0.096$ ) and 1,25(OH)<sub>2</sub>D ( $P = 0.059$ ) exhibited nonsignificant trends for inverse associations with VO+. Similarly, VO+/TEP was inversely associated with 1,25(OH)<sub>2</sub>D ( $P = 0.003$ ) and positively associated with dietary calcium ( $P = 0.004$ ). During the postpartum period, VO+ was positively related to dietary calcium intake ( $P = 0.002$ ) and 25(OH)D ( $P = 0.047$ ), and it was higher among African Americans ( $P = 0.004$ ), as was also seen with African ethnicity in the Brazil cohort. In addition, VO+ was negatively associated with breastfeeding ( $P = 0.002$ ) (Table 2). In these adolescent women, the combined factors of dietary calcium, 25(OH)D, African ethnicity, prepregnancy BMI, and breastfeeding explained  $\sim 84\%$  of the variability of VO+ during the postpartum period. In the California cohort at trimester 3, there was a trend ( $P = 0.055$ ) for an inverse relation between serum 25(OH)D and TEP. At postpartum, 1,25(OH)<sub>2</sub>D was positively related to TEP ( $P = 0.044$ ), and there

was a nonsignificant trend for a positive association between 25(OH)D and TEP ( $P = 0.096$ ) (Table 2).

In the Brazil women at the first trimester, significant positive relations between dietary calcium and VO+, and between 1,25(OH)<sub>2</sub>D and TEP, were observed. There was a nonsignificant trend for African ethnicity to be positively associated

with TEP ( $P = 0.089$ ). By the third trimester, VO+ was significantly negatively associated with 1,25(OH)<sub>2</sub>D ( $P = 0.008$ ) and positively associated with PTH ( $P = 0.003$ ). African ethnicity was significantly negatively associated with TEP at trimester 3 ( $P = 0.021$ ). At postpartum, VO+ was significantly positively related to dietary calcium ( $P = 0.0001$ ) and African

**TABLE 1**  
Calcium kinetic measurements during pregnancy and postpartum by study cohort<sup>1</sup>

Variable and study cohort	Prepregnancy/first trimester	Third trimester	Postpartum
Age at entry in study (y)			
BA	—	16.5 ± 1.4	—
CA	29.5 ± 2.6 <sup>a</sup>	—	—
BR	30.4 ± 4.0 <sup>a</sup>	—	—
BMI (kg/m <sup>2</sup> )			
BA	23.8 ± 2.9 <sup>b,A</sup>	—	26.4 ± 6.1 <sup>b,B</sup>
CA	22.5 ± 2.7 <sup>a,A</sup>	—	24.3 ± 3.7 <sup>a,B</sup>
BR	22.3 ± 2.7 <sup>a,A</sup>	—	24.6 ± 2.5 <sup>a,B</sup>
Urinary calcium (mg/d)			
BA	—	257 ± 85 <sup>a,B</sup>	62 ± 33 <sup>a,A</sup>
CA	173 ± 88 <sup>b,B</sup>	249 ± 149 <sup>a,C</sup>	75 ± 49 <sup>a,A</sup>
BR	296 ± 136 <sup>a,C</sup>	216 ± 124 <sup>a,B</sup>	94 ± 44 <sup>a,A</sup>
Fractional calcium absorption (%)			
BA	—	52.6 ± 15.2 <sup>a,A</sup>	29.6 ± 10.8 <sup>a,B</sup>
CA	32.5 ± 9.4 <sup>a,A</sup>	53.4 ± 11.6 <sup>a,B</sup>	35.5 ± 10.1 <sup>a,A</sup>
BR	70.9 ± 15.7 <sup>b,A</sup>	87.6 ± 13.4 <sup>b,B</sup>	67.5 ± 19.1 <sup>b,A</sup>
Endogenous fecal calcium (mg/d)			
BA	—	114 ± 28 <sup>a,A</sup>	106 ± 25 <sup>a,A</sup>
CA	91 ± 11 <sup>a,A</sup>	113 ± 13 <sup>a,A</sup>	98 ± 12 <sup>a,A</sup>
BR	89 ± 13 <sup>a,A</sup>	105 ± 14 <sup>a,A</sup>	94 ± 14 <sup>a,A</sup>
25(OH)D (nmol/L)			
BA	—	50.5 ± 22.2 <sup>a,A</sup>	45.8 ± 26.2 <sup>a,A</sup>
CA	62.8 ± 17.5 <sup>A</sup>	52.0 ± 24.5 <sup>a,A</sup>	52.5 ± 25.5 <sup>a,A</sup>
BR	—	—	—
1,25(OH) <sub>2</sub> D (pmol/L)			
BA	—	252 ± 104 <sup>a,B</sup>	95 ± 22 <sup>b,A</sup>
CA	108 ± 35 <sup>a,A</sup>	230 ± 90 <sup>a,B</sup>	106 ± 41 <sup>a,b,A</sup>
BR	160 ± 60 <sup>a,A</sup>	225 ± 48 <sup>a,B</sup>	134 ± 44 <sup>a,A</sup>
PTH (pmol/L)			
BA	—	—	—
CA	3.4 ± 1.4	2.6 ± 1.5	3.1 ± 1.9
BR	1.2 ± 0.6 <sup>A</sup>	1.3 ± 0.6 <sup>A,B</sup>	1.7 ± 0.6 <sup>B</sup>
VO+ (mg/d)			
BA	—	1422 ± 364 <sup>a,B</sup>	1162 ± 353 <sup>a,A</sup>
CA	428 ± 141 <sup>a,A</sup>	929 ± 274 <sup>b,C</sup>	644 ± 83 <sup>b,B</sup>
BR	461 ± 170 <sup>a,A</sup>	1015 ± 305 <sup>b,C</sup>	737 ± 139 <sup>a,B</sup>
TEP (mg)			
BA	—	5314 ± 1085 <sup>b,B</sup>	4705 ± 1170 <sup>a,A</sup>
CA	4531 ± 1914 <sup>a,A</sup>	5209 ± 1009 <sup>a,b,B</sup>	4470 ± 1026 <sup>a,A</sup>
BR	3918 ± 600 <sup>a,A</sup>	4296 ± 796 <sup>a,A</sup>	3943 ± 841 <sup>a,A</sup>
VO+/TEP			
BA	—	0.27 ± 0.06 <sup>a,A</sup>	0.26 ± 0.08 <sup>a,A</sup>
CA	0.11 ± 0.04 <sup>a,A</sup>	0.19 ± 0.07 <sup>b,C</sup>	0.15 ± 0.03 <sup>b,B</sup>
BR	0.12 ± 0.05 <sup>a,A</sup>	0.24 ± 0.06 <sup>a,b,B</sup>	0.19 ± 0.04 <sup>b,B</sup>
Breast-milk calcium (mg/d)			
BA	—	—	158 ± 69 <sup>a</sup>
CA	—	—	237 ± 85 <sup>a</sup>
BR	—	—	—

<sup>1</sup> All values are means ± SDs. Values within a column with different superscript lowercase letters are significantly different,  $P < 0.05$  (ANOVA followed by Tukey-Kramer test). Values within a row with different superscript uppercase letters are significantly different,  $P < 0.05$  (repeated-measures ANOVA). BA, Baltimore ( $n = 23$ ; the sample size for the BA cohort during the postpartum period was  $n = 15$ ); BR, Brazil ( $n = 10$ ); CA, California ( $n = 13$ ); PTH, parathyroid hormone; TEP, total exchangeable pool; VO+, bone calcium deposition rate; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

**TABLE 2**  
Multiple regression analysis of calcium kinetic measures<sup>1</sup>

	Prepregnancy/first trimester	Third trimester	Postpartum
<b>VO+ (mg/d)</b>			
BA	—	$R^2 = 0.25$ 1,25(OH) <sub>2</sub> D [0.059]* Age [0.096]*	$R^2 = 0.84$ Calcium [0.002] Breastfeeding [0.002]* Ethnicity [0.004] Prepregnancy BMI [0.035]* 25(OH)D [0.047]
CA	$R^2 = 0.59$ Calcium [0.016]	None	None
BR	$R^2 = 0.59$ Calcium [0.019] Prepregnancy BMI [0.06]	$R^2 = 0.96$ Ethnicity [0.074]* Prepregnancy BMI [0.062] 1,25(OH) <sub>2</sub> D [0.008]* PTH [0.003]	$R^2 = 0.99$ Ethnicity [0.001] Prepregnancy BMI [0.016] 1,25(OH) <sub>2</sub> D [0.038]* Calcium [0.0001] PTH [0.0004]*
Combined groups	—	$R^2 = 0.37$ Age [ $<0.0001$ ]* 1,25(OH) <sub>2</sub> D [0.029]*	$R^2 = 0.69$ Ethnicity [ $<0.0001$ ] Calcium [0.0003] Breastfeeding [0.0012]*
<b>TEP (mg)</b>			
BA	—	$R^2 = 0.16$ Prepregnancy BMI [0.064]	$R^2 = 0.46$ Ethnicity [0.022]* 1,25(OH) <sub>2</sub> D [0.090]*
CA	None	$R^2 = 0.39$ 25(OH)D [0.055]*	$R^2 = 0.58$ 1,25(OH) <sub>2</sub> D [0.044] 25(OH)D [0.096]
BR	$R^2 = 0.59$ 1,25(OH) <sub>2</sub> D [0.048] Prepregnancy BMI [0.063]* Ethnicity [0.089]	$R^2 = 0.62$ Ethnicity [0.021]* Prepregnancy BMI [0.055]	$R^2 = 0.55$ Prepregnancy BMI [0.023] Ethnicity [0.079]*
Combined groups	—	$R^2 = 0.24$ Age [0.010]* Ethnicity [0.056]* Prepregnancy BMI [0.027]	$R^2 = 0.25$ Calcium [0.032] Breastfeeding [0.036]*
<b>VO+/TEP</b>			
BA	—	$R^2 = 0.59$ 1,25(OH) <sub>2</sub> D [0.003]* Calcium [0.004] Prepregnancy BMI [0.002]*	$R^2 = 0.31$ Ethnicity [0.048]
CA	$R^2 = 0.36$ Calcium [0.089]	$R^2 = 0.45$ 25(OH)D [0.035]	None
BR	None	$R^2 = 0.64$ 1,25(OH) <sub>2</sub> D [0.043]* PTH [0.052]	$R^2 = 0.62$ Ethnicity [0.015] Prepregnancy BMI [0.020]*
Combined groups	—	$R^2 = 0.32$ Ethnicity [0.002] 1,25(OH) <sub>2</sub> D [0.018]* Prepregnancy BMI [0.060]*	$R^2 = 0.42$ Ethnicity [ $<0.0001$ ]

<sup>1</sup> *P* values are shown in brackets. Each bone calcium kinetic measurement (dependent variable) was initially related to the following maternal independent variables: age, ethnicity (African compared with non-African), breastfeeding (yes or no), prepregnancy BMI, serum 1,25(OH)<sub>2</sub>D, serum 25(OH)D, serum PTH, and dietary calcium intake. Independent variables were sequentially removed by backward elimination based on *P* values. Variables left in the model were significant at the *P* < 0.10 level. \*Denotes a significant inverse association between variables. BA, Baltimore (*n* = 23; the sample size for the BA cohort during the postpartum period was *n* = 15); BR, Brazil (*n* = 10); CA, California (*n* = 13); PTH, parathyroid hormone; TEP, total exchangeable pool; VO+, bone calcium deposition rate; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

ethnicity (*P* = 0.001) and negatively related to PTH (*P* < 0.001) and 1,25(OH)<sub>2</sub>D (*P* = 0.038) (Table 2). In these adult women, the combined factors of 1,25(OH)<sub>2</sub>D, PTH, African ethnicity, and prepregnancy BMI explained ~96% of the variability of VO+ at the third trimester, and with the addition of dietary calcium explained ~99% of the variability of VO+ at postpartum.

Pooled analysis of all trimester 3 cohorts found VO+ to be significantly higher among adolescents (1422 ± 364 mg/d; *n* = 23) compared with adults (966 ± 287 mg/d; *n* = 23) (*P* < 0.001) (Table 2). In the pooled data at trimester 3, serum 1,25(OH)<sub>2</sub>D was significantly inversely associated with both VO+ (*P* < 0.03) and with VO+/TEP (*P* < 0.02).

A pooled analysis at postpartum showed that serum  $1,25(\text{OH})_2\text{D}$  was no longer significantly associated with  $\text{VO}+$  or  $\text{VO}+/\text{TEP}$ ; instead,  $\text{VO}+$  was significantly positively associated with dietary calcium intake ( $P < 0.0001$ ). African ethnicity was significantly positively related and breastfeeding was significantly negatively related to  $\text{VO}+$  at postpartum ( $P < 0.001$ ).

## DISCUSSION

The heterogeneity of the 3 pregnant and postpartum cohorts studied provided an opportunity to assess common relations while also identifying notable differences in determinants of  $\text{VO}+$  across pregnancy and the early postpartum period. In the adult cohorts studied,  $1,25(\text{OH})_2\text{D}$  increased significantly by 40–50% from early/prepregnancy to the third trimester. In all pregnant cohorts,  $1,25(\text{OH})_2\text{D}$  concentrations during the third trimester were significantly higher than those observed during the early postpartum period. Comparable pregnancy-related increases in  $1,25(\text{OH})_2\text{D}$  over the course of gestation and similar concentrations during the third trimester have been noted in several other studies in adult pregnant women (13–17). At present, functional outcomes associated with these increases remain poorly characterized.

Although recent longitudinal human studies have noted elevated concentrations of  $1,25(\text{OH})_2\text{D}$  during pregnancy (17, 20), few data on determinants of these increases and their relation to  $\text{VO}+$  are available. Synthesis of  $1,25(\text{OH})_2\text{D}$  is known to increase in response to suboptimal calcium intakes. Both the adolescents and Brazil women had physiologic challenges that may have affected calcium homeostasis across gestation and lactation. The Brazil women went through pregnancy with calcium intakes of 450 mg/d; <5% of US pregnant women have similarly low calcium intakes (43). The Baltimore teens ingested more than twice the calcium but needed to provide 20–30 g Ca for fetal skeletal mineralization before the age at which peak bone mass is achieved. Despite the highly variable calcium intakes, ages studied, and variable analytic methods used, mean  $1,25(\text{OH})_2\text{D}$  concentrations during pregnancy did not significantly differ between cohorts and in the group as a whole at late pregnancy, and there was no significant relation between dietary calcium and  $1,25(\text{OH})_2\text{D}$ . The mean concentrations of  $1,25(\text{OH})_2\text{D}$  observed in these women were comparable to those recently reported in pregnant women receiving 400 IU vitamin  $\text{D}_3$  (17). In our study population, serum  $1,25(\text{OH})_2\text{D}$  was negatively associated with dietary calcium intake in the group as a whole only during the postpartum period.

The systemic and cellular effects of elevated  $1,25(\text{OH})_2\text{D}$  concentrations on calcium homeostasis during pregnancy have yet to be fully elucidated as a function of age, race, and habitual calcium intake. Recent data by Hollis et al (17) found that supplementation with vitamin  $\text{D}_3$  resulted in significant increases in  $1,25(\text{OH})_2\text{D}$  throughout pregnancy, but no data on maternal or fetal skeletal outcomes were reported. Among our group as a whole, elevated  $1,25(\text{OH})_2\text{D}$  during late pregnancy was associated with significantly lower  $\text{VO}+$  and a significantly lower fraction of TEP being used for  $\text{VO}+$ . However, without concurrent data on bone calcium resorption, we were unable to determine whether the change in  $\text{VO}+$  was associated with net differences in maternal or fetal calcium retention. In this study population, we previously reported a significantly positive association between  $1,25(\text{OH})_2\text{D}$  and calcium absorption in both the California (25) and Brazil women (31), but similar serum

$1,25(\text{OH})_2\text{D}$  concentrations among the Baltimore adolescents were not significantly associated with calcium absorption in this age group (30). In our group as a whole, elevated  $1,25(\text{OH})_2\text{D}$  was associated with significantly increased calcium absorption only during the postpartum period. We did not have information on vitamin D binding protein in all women and were therefore unable to assess relative contributions of free compared with bound  $1,25(\text{OH})_2\text{D}$  to observed outcomes. Genetic polymorphisms in genes involved in the vitamin D pathway are also known to affect  $25(\text{OH})\text{D}$  among African Americans (44) and others (45); their potential impact on variable responses to the physiologic challenges of pregnancy and lactation merits further study.

Dietary calcium intake had differential effects on  $\text{VO}+$  between late pregnancy and lactation. During the third trimester,  $1,25(\text{OH})_2\text{D}$  was a significant determinant of  $\text{VO}+$ , and dietary calcium intake at the third trimester was not significantly related to  $\text{VO}+$ , maternal  $1,25(\text{OH})_2\text{D}$ , or PTH. A similar lack of a relation between dietary calcium intake and either  $1,25(\text{OH})_2\text{D}$  (20) or bone turnover markers at either mid- or late gestation (46) has recently been reported in a cohort of 171 pregnant adolescents. In contrast, in each of our study groups at postpartum and in the entire cohort, dietary calcium intake was significantly positively associated with increased  $\text{VO}+$ . Differences in study designs and oral tracer administration between sites precluded us from analyzing bone calcium resorption and the degree to which the measured changes in bone calcium deposition were associated with skeletal calcium retention. Dietary calcium intake appears to have a differential impact on skeletal turnover between late pregnancy and early lactation. More data on these associations are needed as a function of habitual diet, race, and vitamin D status. Recent Gambian data showed that calcium supplementation of women with habitually low calcium intakes did not improve skeletal calcium retention across pregnancy but was instead associated with a significant decrease in postpartum bone mass at the hip (47).

In both adult groups of our study, and in the adolescents,  $\text{VO}+$  was significantly lower during the postpartum period than during pregnancy, but this decrease was less pronounced in the adolescents. In the postpartum period, adolescents had significantly higher  $\text{VO}+$  than did adult California women with similar calcium intakes, but  $\text{VO}+$  was not significantly higher than that observed among the Brazil women postpartum. In the postpartum group as a whole,  $\text{VO}+$  was significantly inversely associated with dietary calcium intake. Previous kinetic data on  $\text{VO}+$  among lactating women consuming low- ( $n = 4$ ) or high- ( $n = 4$ ) calcium diets found that  $\text{VO}+$  tended to be lower in the group with a low calcium intake, but this difference was not significant ( $P = 0.12$ ) (48). In the present study, all of the adult women were breastfeeding so the impact of lactation alone on  $\text{VO}+$  could not be assessed among these cohorts. However, teens who breastfed partially or exclusively had significantly lower  $\text{VO}+$  when compared with those who formula fed their infants.

Both higher bone calcium retention and increased  $\text{VO}+$  have been noted in both African American children and adults in comparison with other ethnic groups (49). A positive effect of African ethnicity on  $\text{VO}+$  was evident during the postpartum period but not during pregnancy in our study group. At postpartum, African ethnicity was associated with significantly higher  $\text{VO}+$ , independent of age, calcium intake, and breastfeeding status. Racial differences in  $\text{VO}+$  were less evident during pregnancy,

which may suggest that during gestation physiologic mechanisms of calcium and bone homeostasis prevail over genetic/lifestyle factors. Whether the racial differences noted at postpartum translate into differences in bone mass across lactation in women of African ethnicity is not known.

The TEP represents the readily exchangeable fraction of the miscible calcium pool. As expected, TEP was significantly higher during pregnancy than during the postpartum period in the California and Brazil groups. It was also significantly higher among the adolescents (Baltimore) compared with the adult Brazil women at the third trimester. In all pregnant women combined, the size of this exchangeable calcium compartment was not significantly affected by 1,25(OH)<sub>2</sub>D or calcium intake. There were notable differences in VO<sub>+</sub> as a fraction of TEP. During pregnancy in the group as a whole, a significant inverse association was evident between 1,25(OH)<sub>2</sub>D and VO<sub>+</sub>/TEP, which suggested that less miscible calcium was being used for VO<sub>+</sub> in those with elevated 1,25(OH)<sub>2</sub>D.

During pregnancy, VO<sub>+</sub> reflects calcium deposition into maternal and/or fetal bone; relative partitioning between the two cannot be determined. Another study limitation is the lack of standard reference materials to allow for standardization of biochemical measures between sites. Standard reference materials are now available for 25(OH)D but not for calcitriol or PTH. Despite these analytic limitations, remarkably similar trends and associations with 1,25(OH)<sub>2</sub>D were noted between sites.

In summary, higher serum 1,25(OH)<sub>2</sub>D concentrations were associated with lower VO<sub>+</sub> during pregnancy; additional work is needed to identify determinants and functional maternal or fetal outcomes associated with 1,25(OH)<sub>2</sub>D concentrations across pregnancy. During pregnancy, VO<sub>+</sub> was largely independent of calcium intake but was influenced by maternal age. In contrast, during the early postpartum period, higher dietary calcium intakes were significantly associated with higher VO<sub>+</sub>, and VO<sub>+</sub> varied as a function of ethnicity and lactation. These findings highlight the need for future research that assesses the determinants of whole-body calcium dynamics and their impact on net skeletal balance across the reproductive period.

We thank the study participants for volunteering to participate in these longitudinal studies and the nursing staff at each of the clinical centers for their support of the work. We also thank Stephanie Atkinson for helpful feedback and advice on the interpretation of these data.

The authors' responsibilities were as follows—KOO, CMD, LDR, SAA, and JCK: designed the research and performed the experiments; and GG and CMD: were responsible for the statistical analyses. All of the authors assisted in the interpretation of the data and in the writing of the manuscript. None of the authors had any conflicts of interest to disclose.

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