Effect of calcium plus vitamin D supplementation during pregnancy in Brazilian adolescent mothers: a randomized, placebo-controlled trial[1–3]

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ABSTRACT

Background: Pregnancy and lactation in adolescents with habitually low calcium intake may adversely affect maternal bone mass.

Objective: We investigated the effect of calcium plus vitamin D supplementation during pregnancy on bone mass during lactation in Brazilian adolescent mothers with low-calcium diets (~600 mg/d).

Design: Pregnant adolescents (14–19 y) randomly received daily calcium (600 mg) plus vitamin D_3 (200 IU) (n = 30) or a placebo (n = 26) from 26 wk of pregnancy (baseline) until parturition. The bone mineral content (BMC), bone area (BA), and bone mineral density (BMD) at the total body, lumbar spine, and hip (total and femoral neck) were evaluated by using dual-energy X-ray absorptiometry at 5 and 20 wk postpartum. Serum hormones and 25-hydroxyvitamin D [25(OH)D] were measured. Group comparisons were adjusted for significant covariates.

Results: The mean serum 25(OH)D concentration was 59 nmol/L at baseline. In comparison with the placebo, 25(OH)D tended to be 14–15 nmol/L higher postpartum in the supplemented group (P = 0.08). Total body and hip BMC and BMD decreased over time ($P \leq$ 0.005) in both groups with a group × time interaction at the femoral neck ($P < 0.04$). Supplemented mothers had higher lumbar spine BA (6.7%; P = 0.002) and lumbar spine BMC (7.9%, P = 0.08) than did mothers who consumed the placebo at 5 wk postpartum. At 20 wk postpartum, differences between groups were more evident, with higher lumbar spine BMC (13.9%), lumbar spine BA (6.2%), and lumbar spine BMD (10.6%) in the supplemented group ($P \leq$ 0.008).

Conclusions: Calcium plus vitamin D supplementation during pregnancy of adolescents with low calcium intake results in higher lumbar spine bone mass and a reduced rate of femoral neck bone loss during lactation. Additional studies are required to determine whether bone effects are temporary or long-lasting. This trial was registered at clinicaltrials.gov as NCT01732328. *Am J Clin Nutr* 2013;98:82–91.

INTRODUCTION

Pregnancy and lactation are periods of additional calcium demand with the maternal necessity to transfer ≤330 mg Ca/d to the growing fetal skeleton and ≤400 mg Ca/d for breast-milk production (1). In adolescent mothers who have not achieved peak bone mass, this high demand needs to be coupled to the calcium needed for maternal bone mass accumulation. Bone calcium retention in adolescent girls is, on average, 300 mg/d during the period of peak bone accretion (2). Consequently, maternal bone mass could be adversely affected in these young mothers especially when calcium intake is low.

Few studies evaluated the effect of pregnancy and lactation on the bone mass of adolescent mothers (3–10). Compared with adult mothers, greater bone losses were seen in adolescents from early pregnancy to 6 wk postpartum (5) and from 2 to 16 wk lactation (3). In lactating adolescents, the total bone mineral content (BMC)[4] was reduced 15% (3), and the total bone mineral density (BMD) was reduced 3.6% (7). As in adult lactating mothers (11), the axial skeleton is the bone region more highly mobilized in adolescent lactating mothers. BMD was shown to be 5% reduced at the hip at 6 mo (10) and 9.7% reduced at the lumbar spine at 3 mo (7) after delivery. Although a reduction in bone mass at the lumbar spine during lactation was shown in adolescent mothers both with low (500 mg/d) (7) and high (1200 mg/d) (6) calcium intakes, the magnitude of reduction appears to be higher in those with lower calcium intakes. Lumbar spine BMD z scores were −1.16 and −0.49 with low (7) and high (6) calcium intakes, respectively. In the latter study, lumbar spine scores after delivery were significantly associated with calcium intake during the third trimester of pregnancy (6). Therefore, a low calcium intake during pregnancy might exacerbate bone loss during lactation in adolescent mothers. An increase in calcium intake by using calcium supplements especially during pregnancy could be protective against excessive loss of bone

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[4] Abbreviations used: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; IGF-I, insulin-like growth factor I; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

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mass during lactation in these mothers. To our knowledge, no studies have been done to test this hypothesis.

Studies in adult mothers showed that, irrespective of habitual calcium intake from the diet, calcium supplementation did not prevent bone loss during pregnancy (12, 13) and lactation (14–18). In contrast to adult mothers, a dietary intervention study in lactating adolescents from 2 to 16 wk postpartum showed a 10% decrease in BMC in adolescents who consumed their habitual diet (900 mg Ca/d), whereas no significant bone loss was observed in those supplemented with calcium-rich foods and calcium supplements (4). Therefore, differently than adults, it is plausible that adolescent mothers may have maternal bone mass benefits through calcium supplementation during pregnancy and lactation.

Because vitamin D is essential for calcium and bone metabolism, a poor vitamin D status during pregnancy may impair calcium use and exacerbate maternal bone loss (1). Moreover, there is evidence that vitamin D insufficiency is highly prevalent in adolescent mothers (19, 20). Therefore, testing the effect of calcium supplementation during adolescent pregnancy on maternal bone mass also requires the provision of supplemental vitamin D to ensure that vitamin D status is not a limiting factor.

The aim of this study was to evaluate longitudinally, in a placebo-controlled trial, the influence of calcium plus vitamin D supplementation during the last trimester of pregnancy on bone mass and bone- and calcium-related hormones during lactation in Brazilian adolescent mothers who habitually consumed low-calcium diets.

SUBJECTS AND METHODS

Subjects and study design

Pregnant adolescents were recruited during prenatal care at the Maternidade Escola, Universidade Federal do Rio de Janeiro, from September 2009 to June 2011 to participate in a trial on the effect of calcium plus vitamin D supplementation during pregnancy on adolescent mother and infant bone health. The trial was approved by the Ethical Committee of Maternidade Escola, Universidade Federal do Rio de Janeiro (www.clinicaltrials.gov; NCT01732328). Volunteers were screened by interview and included in the study if they were between 13 and 19 y of age, pregnant for the first time, carrying a single fetus, between 23 and 29 wk of gestation, and intending to exclusively or predominantly breastfeed. Exclusion criteria were as follows: individuals with chronic health problems, pregnancy complications, smokers, users of nutritional supplements besides iron plus folate supplements provided during standard prenatal care, and mothers who decided not to breastfeed. A full explanation of all the study procedures was given, and the participation of pregnant adolescents occurred after receipt of informed written consent from the adolescent and her parent or legal guardian. Participants were randomly and single-blinded assigned to receive a commercially available supplement (Rexall Sundown) that contained 600 mg Ca (as calcium carbonate) plus 200 IU vitamin D (as cholecalciferol, vitamin D_3) or a placebo (capsules of microcrystalline cellulose and corn starch; Quintessência) from 26 wk of pregnancy (baseline) until parturition. Random assignment was done by a member of the research team in a 1:1 ratio within permuted blocks of size 10. No supplements were consumed after delivery. Primary outcomes were bone measurements at 5 and 20 wk postpartum. Hormones and serum 25-hydroxyvitamin D [25(OH)D] were measured at baseline and postpartum.

On the basis of published data of Brazilian lactating adolescents (7, 21), it was estimated that 25 subjects per group would be sufficient to detect a significant difference of 0.5 in the lumbar spine BMD z score with assumption of a 95% CI and 80% power. Details on screening, enrollment, dropouts, and the final number analyzed are shown in **Figure 1**. During the 21-mo recruitment period, 103 pregnant adolescents were interviewed, 84 pregnant adolescents were eligible and randomly assigned for treatment, and 56 pregnant adolescents were analyzed. All analyzed subjects completed the longitudinal study from mid-pregnancy to 5 wk postpartum, and of these adolescents, 47 subjects completed the study at 20 wk postpartum.

All participants were oriented to take one capsule of calcium plus vitamin D supplement or the placebo daily during breakfast from 26 wk of pregnancy until parturition. Breakfast was chosen as the meal time to ingest the capsules to avoid the concomitant intake of the prenatal iron supplements prescribed for use at lunch or dinner. Capsules of calcium plus vitamin D or placebo were provided monthly to subjects by a member of the research team during prenatal visits. Compliance was controlled by counting the remaining capsules at each visit and by telephone reminders. The dose of 600 mg Ca/d was chosen to bring the total daily calcium intake close to 1300 mg/d (22), with consideration that Brazilian adolescent mothers habitually consume a low-calcium diet ($\sim$500 mg/d) (7, 23). The dose of 200 IU vitamin D/d was chosen to provide the current Adequate Intake of vitamin D for pregnant adolescents at the time when our study recruitment started (September 2009) (22). Furthermore, as already justified, we chose a calcium supplement that contained vitamin D to ensure that use of the calcium provided would not be limited by vitamin D deficiency, which was likely to occur in the adolescent mothers studied (19). The capsules were well accepted by the pregnant adolescents with no adverse effects reported. Compliance was not different between groups, with an average of 83 $\pm$ 15% and 87 $\pm$ 11% of capsules offered being consumed by supplemented and placebo groups, respectively (P = 0.24; t test).

Information on calcium and vitamin D dietary intake was collected during prenatal care visits at least monthly. Morning blood samples (20 mL) were collected from each participant after an overnight fast at baseline (26 wk of pregnancy), immediately before random assignment, and during lactation at 5 and 20 wk postpartum. Serum was separated, and aliquots were stored, at –80°C until laboratory analysis. All biochemical indexes (*see* Laboratory analysis) were analyzed at 26 wk of pregnancy and at 5 and 20 wk postpartum, except for serum estradiol, which was analyzed at 26 wk of pregnancy and 5 wk postpartum. Information on prepregnancy BMI (in kg/m^2) and other general characteristics were collected at the first prenatal care visit, and body weight, height, and bone measurements were evaluated at 5 and 20 wk postpartum. Information on breastfeeding was collected at each postpartum visit. The breastfeeding practice was classified as exclusive, predominant, complementary, or no breastfeeding according to the WHO (24). Information on the season (spring, summer, autumn, and winter) was registered at each time point. The latitude of Rio de Janeiro is 22° 57′ S. This part of Brazil is mostly sunny all year round, with air temperatures rarely <15°C or >38°C. Information on self-reported

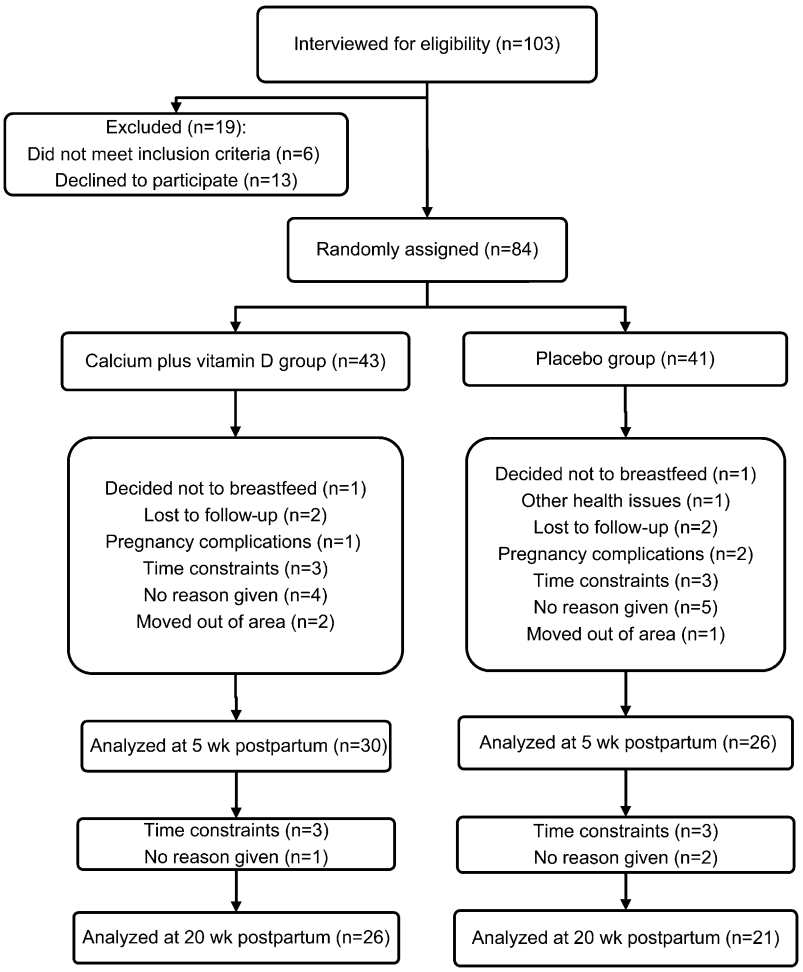

FIGURE 1. Flow diagram of recruitment, random assignment, losses, and follow-up of study participants.

ethnicity (race) was registered as of African descent (black), white (white), or mixed ethnicity (black and white).

Dietary intake analysis

Calcium and vitamin D dietary intake was assessed by at least three 24-h dietary recall questionnaires applied by a trained nutritionist. Calcium intake was analyzed on the basis of a Brazilian food database (25) with the use of the AVANUTRI program (Version Revolution 4.0; Avanutri & Nutrição Serviços e Informática Ltda ME). Vitamin D intake was analyzed on the basis of the USDA database (26) for nonprocessed foods and on the basis of label information for industrialized foods.

Laboratory analysis

Serum 25(OH)D, intact parathyroid hormone (PTH), and insulin-like growth factor I (IGF-I) were analyzed by using a chemiluminescent enzyme-labeled immunometric assay (Liaison; Diasorin). Serum prolactin and estradiol were measured by using an immunoenzymetric assay (DiAsource ImmunoAssays). Serum calcium was measured by using a colorimetric assay (Bioclink Quibasa Química Básica). All samples were analyzed in duplicate, and if duplicates were different by >10%, the sample was reanalyzed. Mean intraassay CVs were as follows: 5.4% for intact PTH, 4.3% for 25(OH)D, 3.0% for IGF-I, 3.9% for prolactin, and 2.4% for estradiol. Assay performances were monitored by using control sample sets that contained serum-based material provided by the manufacturer. Low and high controls for all biochemical analysis were always within the expected range. With the use of the midrange of the manufacturer's specifications for low and high controls, accuracy was 86% for 25(OH)D, 98% for intact PTH, and 95% for IGF-I. External quality control was not available.

Anthropometric and bone measurements

Standing height and body weight were measured by using a stadiometer (Seca) and a calibrated electronic scale (Filizola), respectively. BMC and BMD of the total body, lumbar spine (L1–L4), and hip (total and femoral neck) were assessed by using dual-energy X-ray absorptiometry (DXA) by using the Lunar iDXA densitometer with software enCore 2008 version 12.20 (GE Healthcare). The same operator performed all scanning and calibration. The performance of the DXA equipment was evaluated daily for the calibration block and weekly for the calibration spine phantom and had CVs ≤0.7%. CVs for the lumbar spine, total hip, and femoral neck were 0.83%, 0.49%, and 1.28%, respectively. z scores for total-body BMD and lumbar spine

BMD were obtained by comparison with an age-, sex-, and race-matched reference according to the manufacturer's database. Total-body calcium content was calculated as 32.2% of the total BMC (27). Changes on bone measurements at the total body, lumbar spine, total hip, and femoral neck were calculated as differences between measurements obtained at 20 and 5 wk postpartum.

Statistical analysis

Differences between groups on general characteristics (continuous variables) at a given time point were assessed by using the independent-samples *t* test. Categorical variables season, breastfeeding practice, and return of menstruation were compared by group by using the chi-square test.

Effects of intervention group (calcium plus vitamin D supplement compared with the placebo), time, and group × time interactions on biochemical indexes and bone measurements were examined without adjustment for potential confounders by using repeated-measures ANOVA in the subset of adolescent mothers who had paired measures at all time points (placebo: $n = 21$; calcium plus vitamin D: $n = 26$). For serum 25(OH)D concentrations, these effects were tested with adjustment for season.

Potential confounders (covariates) that could affect bone measures at 5 and 20 wk postpartum and bone changes from 5 to 20 wk postpartum were examined in the whole group. Significant covariates were identified by using multiple linear regression models with backward elimination of those that were nonsignificant. Models were constructed for a given bone measurement

TABLE 1
General characteristics of the adolescent mothers during the study[1]

	Groups		
Characteristic	Placebo	Calcium plus vitamin D	*P*
At 26 wk of pregnancy (baseline)			
Chronologic age (y)	17.2 ± 1.0 [26]	16.8 ± 1.5 [30]	0.31
Time elapsed since menarche (y)	5.3 ± 1.7 [26]	5.3 ± 2.1 [30]	NS
BMI before pregnancy (kg/m^2)	20.9 ± 4.5 [21]	21.9 ± 3.4 [26]	0.39
Dietary calcium intake (mg/d)[2]	743 ± 457 [26]	500 ± 276 [30]	0.02
Dietary vitamin D intake (IU/d)[2]	32 ± 40 [23]	35 ± 38 [30]	NS
Season			0.29
Spring	9 (34.6)	18 (60.0)	
Summer	5 (19.2)	3 (10.0)	
Autumn	6 (23.1)	5 (16.7)	
Winter	6 (23.1)	4 (13.3)	
Ethnicity			NS
Black	6 (23.1)	9 (30.0)	
White	10 (38.5)	10 (33.3)	
Mixed black and white	10 (38.5)	11 (36.7)	
At 5 wk postpartum			
Body weight (kg)	59.7 ± 13.3	61.8 ± 11.8	NS
Height (m)	1.61 ± 0.06	1.59 ± 0.06	0.35
Breastfeeding practice			0.23
Exclusively	22 (84.6)	23 (76.7)	
Predominantly	4 (15.4)	4 (13.3)	
Complementary	0	3 (10.0)	
No breastfeeding	0	0	
Total duration of breastfeeding (d)	33 ± 11	32 ± 10	NS
Return of menstruation	1 (3.8)	3 (10)	0.37
Time elapsed since return of menstruation (d)[3]	1	11 ± 4	NS
At 20 wk postpartum			
Body weight (kg)	56.1 ± 10.3	57.9 ± 9.8	NS
Height (m)	1.60 ± 0.06	1.59 ± 0.07	0.44
Breastfeeding practice			0.23
Exclusively	7 (33.3)	3 (11.57)	
Predominantly	9 (42.9)	11 (42.3)	
Complementary	2 (9.5)	6 (23.1)	
No breastfeeding	3 (14.3)	6 (23.1)	
Total duration of breastfeeding (d)	127 ± 32	127 ± 39	NS
Return of menstruation	8 (38.1)	19 (73.1)	0.04
Time elapsed since return of menstruation (d)[3]	65 ± 45	61 ± 39	NS

[1] For continuous variables, all values are means ± SDs; *n* in brackets. For categorical variables, all values are *n* values; percentages of the total in parentheses. Comparisons between groups were determined by using the independent-samples *t* test (continuous variables) or chi-square test (categorical variables). NS for $P > 0.50$.

[2] Average of three 24-h dietary records obtained between 26 wk of pregnancy and parturition.

[3] Time elapsed from the day of return of menstruation until the day of the postpartum study.

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TABLE 2
Group and time effects on biochemical indexes of the adolescent mothers during the study[1]

	Placebo (n = 21)			Calcium plus vitamin D (n = 26)			P		
	26 wk of pregnancy	5 wk postpartum	20 wk postpartum	26 wk of pregnancy	5 wk postpartum	20 wk postpartum	Group	Time	Group × time interaction
Serum estradiol (nmol/L)	13.4 ± 10.2	0.14 ± 0.07	—	13.3 ± 8.7	0.12 ± 0.04	—	NS	<0.001	NS
Serum prolactin (μg/L)	106.6 ± 55.2	97.1 ± 121.7	49.7 ± 49.8	130.4 ± 99.9	81.4 ± 99.5	30.0 ± 18.8	NS	<0.001	0.12
Serum IGF-I (ng/mL)	280 ± 89	225 ± 54	271 ± 59	258 ± 88	234 ± 65	302 ± 59	NS	0.20	0.06
Serum iPTH (pg/dL)	37.2 ± 19.6	46.6 ± 40.0	57.9 ± 29.4	39.7 ± 15.9	52.1 ± 37.8	59.9 ± 29.5	NS	<0.001	NS
Serum calcium (mmol/L)	2.03 ± 0.20	2.15 ± 0.21	2.15 ± 0.20	2.03 ± 0.30	2.07 ± 0.20	2.17 ± 0.22	0.48	0.009	NS
Serum 25(OH)D (nmol/L)[2]	57.1 ± 20.6	62.3 ± 31.2	53.0 ± 21.1	61.0 ± 20.4	77.5 ± 31.1	66.7 ± 20.9	0.08	<0.001	0.12

[1] All values are means ± SDs. P values were obtained from repeated-measures ANOVA. NS for $P > 0.50$. IGF-I, insulin-like growth factor I; iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
[2] Season was entered as a covariate.

at a time point or for a given postpartum bone change as the dependent variable and potential covariates as independent variables. Covariates remained in the model at $P < 0.05$. Independent variables tested were chronologic age, body weight, body height, season (4 categories), years since menarche, percentage of total capsules offered consumed (percentage of compliance), days postpartum, dietary calcium intake during pregnancy, intervention group, breastfeeding practice (4 categories), and return of menstruation (yes or no). When testing for bone changes, the bone variable at 5 wk postpartum and change in body weight from 5 to 20 wk postpartum were also included as independent variables. The effect of calcium plus vitamin D supplementation on bone measurements at 5 and 20 wk postpartum and bone changes over time was examined by using ANCOVA with adjustment for significant covariates. Associations between variables were evaluated by using Pearson's correlation analysis.

Statistical analyses were performed with SPSS 12.0 for Windows software (SPSS Inc). Results are reported as means ± SDs. Values at $P \leq 0.05$ were considered significant. P values between 0.05 and 0.10 were considered trends.

RESULTS

At entry in the study (26 ± 1 wk of pregnancy), chronologic age and time elapsed since the onset of menarche were, on average, 17.0 and 5.3 y, respectively, with no significant differences between groups (**Table 1**). Prepregnancy BMI was within the normal range for age in both groups at, on average, 21. The dietary intake of calcium from midpregnancy to parturition was, on average, 613 mg/d in the whole group, 47% of that recommended for adolescents (22, 28), and was lower in the calcium plus vitamin D group ($P < 0.02$) (Table 1). The dietary intake of vitamin D from midpregnancy to parturition was, on average, 34 IU/d in the whole group, ~6% of that currently recommended for pregnant and lactating women (28).

All participants delivered at term, and there was no significant difference in the length of gestation between supplemented and placebo groups (39 ± 2 wk in the whole group). Postpartum measurements were done at 5 ± 1 and 20 ± 3 wk after parturition. At 5 wk postpartum, all adolescent mothers (n = 56) were breastfeeding their infants, most of them exclusively (placebo: 85%; calcium plus vitamin D: 77%) (Table 1). At 20 wk postpartum, 81% of mothers (n = 38) were breastfeeding (placebo: 86%; calcium plus vitamin D: 77%), mostly as predominant and some complementary (Table 1). There were no significant differences in body weight and height between the 2 groups at 5 and 20 wk postpartum or in changes of body weight during the postpartum period (−0.6 ± 2.5 and −1.1 ± 2.5 kg for placebo and calcium plus vitamin D groups, respectively).

Serum estradiol and serum prolactin decreased ($P < 0.001$), serum IGF-I did not change, and serum PTH and serum calcium increased ($P \leq 0.009$) from 26 wk pregnancy to postpartum, with no difference between groups. Serum IGF-I showed a tendency toward a group × time interaction (P = 0.06) (**Table 2**).

With consideration of all adolescent mothers (n = 56) at entry in the study, the serum 25(OH)D concentration was, on average, 59 nmol/L, and 43% of mothers had concentrations <50 nmol/L (placebo: n = 12; calcium plus vitamin D: n = 12) and 5% of

mothers had concentrations <27.5 nmol/L (placebo: $n = 3$) (28). There was no significant difference in serum 25(OH)D between the 2 groups at 26 wk of pregnancy. Serum 25(OH)D (adjusted for season) was influenced by time during the study with higher values at 5 wk postpartum ($P < 0.001$) (Table 2). Serum 25(OH)D in the supplemented group tended to be 15.2 nmol/L higher than in the placebo group at 5 wk postpartum and 13.7 nmol/L higher at 20 wk postpartum (group effect, $P = 0.08$). At 20 wk postpartum, serum 25(OH)D concentrations <50 nmol/L were present in 43% of the placebo group and 35% of the calcium plus vitamin D group. Only 2 adolescent mothers (one mother in each group) had serum 25(OH)D concentrations <27.5 nmol/L at this time.

With consideration of adolescent mothers who had paired samples at 5 and 20 wk postpartum ($n = 47$), BMC, BMD, and BMD z scores at the total body, total hip, and femoral neck decreased ($P \leq 0.005$), whereas those at the lumbar spine bone area (BA) increased ($P = 0.04$) over time postpartum (**Table 3**). The group designation tended to influence total-body and lumbar spine BMD z scores, with higher values in the supplemented group ($P \leq 0.06$). There was a group × time interaction for BMC and BMD at the femoral neck ($P < 0.04$) (Table 3).

In the whole group, there were negative correlations between BMC at the total body ($r = -0.32$), lumbar spine ($r = -0.30$), total hip ($r = -0.31$), and femoral neck ($r = -0.35$) and serum PTH at 20 wk postpartum ($P < 0.05$). Moreover, BMD z scores at the total body ($r = -0.32$), lumbar spine ($r = -0.31$), and femoral neck ($r = -0.29$) correlated negatively with serum prolactin at 20 wk postpartum ($P < 0.05$).

Factors in the multiple-regression models that significantly affected at least one bone measurement at 5 and/or 20 wk postpartum in the whole group were maternal age, body weight, height, intervention group, percentage of compliance, dietary calcium intake during pregnancy, time postpartum, breastfeeding, return of menstruation, time since the onset of menarche, and season. For changes in bone measurements from 5 to 20 wk postpartum, the change in body weight was an additional significant factor.

Effects of calcium plus vitamin D supplementation during pregnancy on bone measurements at 5 and 20 wk postpartum and on bone changes over postpartum time, adjusted for significant covariates, are examined in **Tables 4**, **5**, and **6**, respectively.

At 5 wk postpartum, the lumbar spine BA was, on average, 6.7% higher in the calcium plus vitamin D group than in the placebo group ($P < 0.01$), and the lumbar spine BMC tended to be 8.5% higher in the calcium plus vitamin D group ($P = 0.08$) (Table 4). At 20 wk postpartum, differences between groups at the lumbar spine were more evident (Table 5). Compared with the placebo group, the BMC, BA, and BMD at lumbar spine were, on average, 13.9%, 6.2%, and 10.6% higher, respectively, in the calcium plus vitamin D group ($P \leq 0.008$). As a consequence, adolescent mothers who received the calcium plus vitamin D supplement during pregnancy had higher lumbar spine BMD z scores ($P = 0.007$).

There were no significant differences between groups in bone changes adjusted for significant covariates from 5 to 20 wk postpartum at the total body, lumbar spine, and total hip (Table 6). However, there was a significant group difference at the femoral neck, with a less-negative change in BMD at this bone site in the supplemented group ($P = 0.02$) (Table 6). With consideration of all mothers, the reduction in the BMD z score from 5 to 20 wk postpartum at the total body ($r = 0.340$), lumbar spine ($r = 0.364$), and total hip ($r = 0.291$) correlated positively with serum PTH at 5 wk postpartum ($P < 0.05$).

TABLE 3
Group and time effects on bone measurements of the adolescent mothers during lactation[1]

	Placebo ($n = 21$)		Calcium plus vitamin D ($n = 26$)		P		
	5 wk	20 wk	5 wk	20 wk	Group	Time	Group × time interaction
Total body							
BMC (g)	2030 ± 212	2008 ± 214	2112 ± 322	2101 ± 315	0.29	<0.001	0.18
BA (cm^2)	1971 ± 94	1962 ± 99	1954 ± 114	1956 ± 109	NS	0.27	0.08
BMD (g/cm^2)	1.029 ± 0.080	1.022 ± 0.077	1.078 ± 0.129	1.072 ± 0.130	0.13	0.005	NS
BMD z score	−0.65 ± 0.94	−0.75 ± 0.93	0.01 ± 1.28	−0.10 ± 1.35	0.06	<0.001	NS
Lumbar spine							
BMC (g)	47.78 ± 7.32	48.01 ± 7.17	53.03 ± 11.74	53.37 ± 10.80	0.07	0.33	NS
BA (cm^2)	46.30 ± 4.26	46.65 ± 4.19	48.09 ± 4.99	48.48 ± 5.02	0.19	0.04	NS
BMD (g/cm^2)	1.030 ± 0.114	1.027 ± 0.103	1.098 ± 0.172	1.098 ± 0.164	0.11	NS	NS
BMD z score	−1.30 ± 0.86	−1.33 ± 0.80	−0.69 ± 1.22	−0.74 ± 1.19	0.06	0.28	NS
Total hip							
BMC (g)	26.81 ± 3.22	25.99 ± 3.19	28.20 ± 5.81	27.63 ± 5.83	0.29	<0.001	0.24
BA (cm^2)	27.48 ± 1.74	27.52 ± 1.69	26.74 ± 1.89	26.85 ± 1.86	0.19	0.14	NS
BMD (g/cm^2)	0.977 ± 0.115	0.946 ± 0.115	1.052 ± 0.189	1.027 ± 0.194	0.11	<0.001	0.29
BMD z score	−0.31 ± 0.96	−0.56 ± 0.96	0.29 ± 1.45	0.06 ± 1.50	0.11	<0.001	NS
Femoral neck							
BMC (g)	4.27 ± 0.58	4.08 ± 0.56	4.44 ± 0.74	4.32 ± 0.77	0.30	<0.001	0.04
BA (cm^2)	4.21 ± 0.27	4.21 ± 0.29	4.19 ± 0.31	4.20 ± 0.30	NS	0.49	NS
BMD (g/cm^2)	1.014 ± 0.125	0.968 ± 0.119	1.061 ± 0.166	1.029 ± 0.174	0.23	<0.001	0.03
BMD z score	0.03 ± 1.03	−0.30 ± 0.99	0.42 ± 1.26	0.15 ± 1.31	0.23	<0.001	0.30

[1] All values are means ± SDs. P values were obtained from repeated-measures ANOVA. NS for $P > 0.50$. BA, bone area; BMC, bone mineral content; BMD, bone mineral density.

TABLE 4
Effects of calcium plus vitamin D supplementation during pregnancy on bone measurements of the adolescent mothers at 5 wk postpartum[1]

	Groups			
	Placebo (n = 26)	Calcium plus vitamin D (n = 30)	P	Significant adjusted covariates[2]
Total body				
BMC (g)	2091 ± 206	2126 ± 205	NS	Age, Wt, season, TPP
BA (cm^2)	1985 ± 67	1978 ± 67	NS	Wt, Ht, TPP
BMD (g/cm^2)	1.050 ± 0.092	1.072 ± 0.093	0.39	Age, Wt, season, percentage of compliance
BMD z score	−0.42 ± 0.98	−0.07 ± 0.97	0.18	Wt, season, TPP
Lumbar spine				
BMC (g)	49.37 ± 8.57	53.58 ± 8.55	0.08	Wt, Ht, TEM
BA (cm^2)	46.22 ± 3.50	49.30 ± 3.49	0.002	Ht, TEM, TPP, percentage of compliance
BMD (g/cm^2)	1.058 ± 0.138	1.088 ± 0.142	0.43	Wt
BMD z score	−1.09 ± 1.04	−0.76 ± 1.04	0.24	Wt
Total hip				
BMC (g)	28.05 ± 3.90	28.25 ± 3.90	NS	Wt, TPP
BA (cm^2)	27.73 ± 1.20	27.13 ± 1.20	0.07	Wt, Ht, season, TPP, breastfeeding
BMD (g/cm^2)	1.008 ± 0.138	1.041 ± 0.137	0.37	Wt, season
BMD z score	−0.06 ± 1.09	0.21 ± 1.08	0.37	Wt, season
Femoral neck				
BMC (g)	4.42 ± 0.50	4.45 ± 0.50	NS	Age, Wt, TPP
BA (cm^2)	4.24 ± 0.23	4.21 ± 0.24	NS	Age, Wt, Ht, season
BMD (g/cm^2)	1.040 ± 0.127	1.058 ± 0.126	NS	Wt, season
BMD z score	0.24 ± 0.98	0.40 ± 0.98	NS	Wt, season

[1] All values are adjusted means ± SDs. P values refer to the comparison between calcium plus vitamin D and placebo groups by using ANCOVA after adjustment for significant covariates. NS for $P > 0.50$. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; Ht, body height; TEM, time elapsed since the onset of menarche; TPP, time postpartum; Wt, body weight.

[2] Factors tested in the whole group by using multiple regression were chronologic age, Wt, Ht, season at 5 wk postpartum, TEM, TPP, dietary calcium intake during pregnancy, percentage of compliance, and breastfeeding practice at 5 wk postpartum.

DISCUSSION

There is indirect evidence that an increase in calcium intake during pregnancy and lactation may contribute to reduced postpartum bone losses in adolescent mothers, particularly in those who consume low-calcium diets (4–7). In this placebo-controlled study, we investigated the effect of calcium plus vitamin D supplementation during pregnancy on bone mass and related hormones during lactation in adolescent mothers who habitually consumed ~600 mg Ca/d.

At midpregnancy, 43% of mothers had a serum 25(OH)D concentration <50 nmol/L (28), which was consistent with the high prevalence of vitamin D insufficiency during pregnancy and lactation worldwide (29, 30), especially in adolescents (19, 20, 32). Although serum 25(OH)D tended to be higher in the supplemented group than in the placebo group at postpartum measurements (P = 0.08), the prevalence of a serum 25(OH)D concentration <50 nmol/L remained high at 20 wk postpartum in both groups (43% in the placebo group and 35% in the calcium plus vitamin D group). This result was probably expected because the amount of supplemental vitamin D used in our study (200 IU/d) was insufficient to provide the current vitamin D Recommended Dietary Allowance for pregnant and lactating women (600 IU/d) (28). In contrast, because the dietary vitamin D intake of adolescents studied was very low (~35 IU/d), the supplemental vitamin D contributed substantially to an increase in their total vitamin D intake.

The relation between maternal vitamin D status and bone mass during pregnancy and lactation is unclear (29). In adolescent mothers, maternal vitamin D insufficiency was associated with higher serum PTH (19, 20), higher bone resorption during pregnancy (31), and lower maternal bone mineral status during lactation (19). In the current study, at midpregnancy and during lactation, no associations between maternal serum 25(OH)D and serum PTH or bone mineral status were shown.

Calcium plus vitamin D supplementation during pregnancy did not influence serum PTH postpartum in the adolescent mothers studied, which was also observed in adult Gambian mothers supplemented with calcium during pregnancy (18). Moreover, irrespective of supplementation, serum PTH increased from midpregnancy to lactation in the adolescent mothers. Serum PTH does not typically increase from pregnancy to lactation in adult women with an adequate calcium intake (17), but it was shown to be higher in lactation than during pregnancy in adult mothers accustomed to a low-calcium diet (18, 33) and in adolescent mothers (23). Therefore, increased PTH during lactation may have a homeostatic bone role in adolescent mothers. Although PTH was not associated with maternal bone mass during lactation in adults (1, 34, 35), increased serum PTH during lactation was associated with better bone recovery postweaning in adolescent mothers (7). Consistently in the current study, irrespective of supplementation, adolescent mothers with higher postpartum PTH had smaller reductions in BMD z scores at the total body, lumbar spine, and total hip from 5 to 20 wk postpartum.

TABLE 5
Effect of calcium plus vitamin D supplementation during pregnancy on bone measurements of the adolescent mothers at 20 wk postpartum[1]

	Groups			
	Placebo (n = 21)	Calcium plus vitamin D (n = 26)	P	Significant adjusted covariates[2]
Total body				
BMC (g)	2013 ± 212	2097 ± 212	0.19	Wt, Ht
BA (cm^2)	1963 ± 52	1955 ± 52	NS	Wt, Ht, TPP, Ca intake
BMD (g/cm^2)	1.019 ± 0.092	1.074 ± 0.092	0.06	Percentage of compliance, Wt, season, age
BMD z score	−0.80 ± 1.05	−0.05 ± 1.07	0.02	Wt, RM
Lumbar spine				
BMC (g)	47.34 ± 7.88	53.92 ± 7.80	0.008	Wt, Ht, season, TEM, TPP
BA (cm^2)	46.08 ± 3.44	48.93 ± 3.43	0.007	Percentage of compliance, Ht, TEM
BMD (g/cm^2)	1.007 ± 0.128	1.114 ± 0.127	0.008	TEM, season, ΔWt
BMD z score	−1.45 ± 0.96	−0.64 ± 0.97	0.007	Season, ΔWt
Total hip				
BMC (g)	26.28 ± 4.00	27.40 ± 4.00	0.35	Wt
BA (cm^2)	27.37 ± 1.36	26.97 ± 1.36	0.32	Ht
BMD (g/cm^2)	0.954 ± 0.147	1.020 ± 0.143	0.12	Wt
BMD z score	−0.50 ± 1.15	0.01 ± 1.15	0.14	Wt
Femoral neck				
BMC (g)	4.11 ± 0.48	4.29 ± 0.48	0.22	Wt, Ht, percentage of compliance
BA (cm^2)	4.22 ± 0.22	4.20 ± 0.21	NS	Age, Ht, Ca intake
BMD (g/cm^2)	0.981 ± 0.128	1.018 ± 0.127	0.32	Wt, percentage of compliance
BMD z score	−0.20 ± 1.00	0.07 ± 1.00	0.37	Wt, percentage of compliance

[1] All values are adjusted means ± SDs. P values refer to the comparison between calcium plus vitamin D and placebo groups by using ANCOVA after adjusting for significant covariates. NS for $P > 0.50$. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; Ca intake, dietary calcium intake during pregnancy; Ht, body height; RM, return of menstruation; TEM, time elapsed since the onset of menarche; TPP, time postpartum; Wt, body weight; ΔWt, changes in body weight from 5 to 20 wk postpartum.

[2] Factors tested in the whole group by using multiple regression were chronologic age, Wt, Ht, season at 20 wk postpartum, TEM, TPP, Ca intake, breastfeeding practice at 20 wk postpartum, RM, percentage compliance, and ΔWt.

Irrespective of supplementation, BMC, BMD, and BMD z scores at the total body, total hip, and femoral neck decreased from 5 to 20 wk postpartum in adolescent mothers as a whole group, which was consistent with the fact that most of them were lactating during that period. After we controlled for significant covariates typically know to affect bone mass postpartum, such as breastfeeding practice, time postpartum, and return of menstruation (1), calcium plus vitamin D supplementation during pregnancy influenced bone measurements during lactation in the adolescent mothers, especially at 20 wk postpartum and at the lumbar spine, with higher bone status indexes in supplemented mothers. In the placebo group, adjusted BMD z scores at 5 and 20 wk postpartum were, respectively, −0.42 and −0.80, in the total body and −1.09 and −1.45, respectively, in the lumbar spine. z scores were comparable to those previously described in nonsupplemented Brazilian lactating adolescents (7) and lower than previously described in nonpregnant, nonlactating Brazilian adolescents (total-body BMD z score: −0.09; lumbar spine BMD z score: −0.46) (36). In supplemented mothers in the current study, BMDs at 5 and 20 wk postpartum were, respectively, −0.07 and −0.05 in the total body and −0.76 and −0.64 in the lumbar spine. Differences in adjusted BMD z scores between supplemented and placebo groups were significant at 20 wk postpartum ($P \leq 0.02$). Also, at 20 wk postpartum, adjusted BA and BMC at the lumbar spine were 6.2% and 13.9% higher, respectively, in the supplemented group ($P \leq 0.008$), which suggested higher periosteal apposition in this group (1).

Bone measurements at the total hip and femoral neck were not significantly different between groups during the postpartum period. However, the significant decrease in BMD at the femoral neck in the whole group from 5 to 20 wk postpartum ($P < 0.001$) was less pronounced in the supplemented (3.0%) than placebo (4.5%) groups ($P = 0.02$), which suggesting an effect from supplementation. Irrespective of supplementation, no significant decreases were observed in bone mineral at the lumbar spine from 5 to 20 wk postpartum, which was consistent with previously reported results in in postpartum adolescent mothers (10). Moreover, changes from 5 to 20 wk postpartum in all other bone measurements were not different between supplemented and placebo groups. These results suggested that the apparent economy in bone calcium mobilization in adolescent mothers supplemented with calcium and vitamin D during late pregnancy probably occurred during pregnancy or before the fifth week of lactation. Consistent with this hypothesis, in Brazilian adult women with low calcium intakes (~500 mg Ca/d), higher calcium intakes during late pregnancy improved the bone calcium balance at early lactation (33). Moreover, it was reported that adolescents with higher calcium intakes during the last trimester of pregnancy, when the efficiency of calcium absorption is substantially increased, appeared to be protected against loss of trabecular bone at the lumbar spine during the early postpartum period (6).

The strength of this study was that it described detailed longitudinal data of bone mass during 20 wk of lactation in

TABLE 6
Effects of calcium plus vitamin D supplementation during pregnancy on changes in bone measurements of the adolescent mothers from 5 to 20 wk postpartum[1]

	Groups			
	Placebo (n = 21)	Calcium plus vitamin D (n = 26)	P	Significantly adjusted covariates[2]
Δ Total body				
BMC (g)	−17.48 ± 22.14	−14.72 ± 21.94	NS	TEM, RM, ΔWt, breastfeeding
BA (cm^2)	−4.06 ± 19.49	−1.80 ± 19.30	NS	RM, Ca intake
BMD (g/cm^2)	−0.005 ± 0.014	−0.008 ± 0.015	NS	ΔWt, breastfeeding
BMD z score	−0.08 ± 0.15	−0.12 ± 0.14	0.43	ΔWt, breastfeeding
Δ Lumbar spine				
BMC (g)	0.42 ± 1.76	0.19 ± 1.74	NS	RM, breastfeeding, BMC at L1
BA (cm^2)	0.35 ± 1.19	0.39 ± 1.19	NS	—
BMD (g/cm^2)	−0.001 ± 0.027	−0.001 ± 0.031	NS	RM, ΔWt, breastfeeding, BMD at L1
BMD z score	−0.03 ± 0.23	−0.05 ± 0.23	NS	Breastfeeding, z score for BMD at L1
Δ Total hip				
BMC (g)	−0.70 ± 0.60	−0.67 ± 0.60	NS	Wt, Ht, breastfeeding
BA (cm^2)	0.07 ± 0.34	0.09 ± 0.34	NS	Wt, Ht
BMD (g/cm^2)	−0.031 ± 0.018	−0.025 ± 0.020	0.28	TPP, ΔWt, breastfeeding
BMD z score	−0.24 ± 0.18	−0.24 ± 0.18	NS	TPP, breastfeeding
Δ Femoral neck				
BMC (g)	−0.18 ± 0.11	−0.14 ± 0.11	0.20	Wt, Ca intake
BA (cm^2)	0.01 ± 0.07	0.00 ± 0.07	NS	Wt, Ca intake
BMD (g/cm^2)	−0.047 ± 0.018	−0.033 ± 0.020	0.02	TPP, percentage of compliance
BMD z score	−0.33 ± 0.18	−0.28 ± 0.18	0.30	—

[1] All values are adjusted means ± SDs. P values refer to the comparison between calcium plus vitamin D and placebo groups by using ANCOVA after adjustment for significant covariates. NS for $P > 0.50$. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; Ca intake, dietary calcium intake during pregnancy; Ht, body height; L, lumbar spine; RM, return of menstruation; TEM, time elapsed since the onset of menarche; TPP, time postpartum; Wt, body weight; Δ, change; ΔWt, changes in body weight from 5 to 20 wk postpartum.

[2] Factors tested in the whole group by using multiple regression were chronologic age, Wt, Ht, season at 20 wk postpartum, TEM, TPP, Ca intake, breastfeeding practice at 20 wk postpartum, RM, percentage of compliance, ΔWt, and bone measurement at 5 wk postpartum.

adolescent mothers, who are an understudied group, by taking into account nutritional and physiologic factors. The study also had limitations that may hinder public health application. The start of supplementation was at 26 wk of gestation, which covered the last trimester of pregnancy. Other studies have started supplementation earlier in pregnancy at 20 wk (18, 37), which may have possibly affected outcomes. Unfortunately, it was not possible to make DXA measures immediately after delivery. Differences might have been more evident immediately after the supplementation period ended. Also, calcitriol was not measured in the study; therefore, it is not known if the vitamin D provided in the supplement affected the active form of vitamin D.

To our knowledge, there are no published data on the bone mass of Brazilian adult mothers. These mothers might have a similar bone response during lactation as adolescent mother do because of their habitually low calcium intake (33). Also, it is not known what would have been the bone response to the trial of a nonpregnant, nonlactating adolescent control group from the same population. Therefore, it is not known if bone responses to supplementation were related to the habitually low calcium diet of adolescent mothers or because the mothers were adolescents or both.

A difficulty of the study was the follow-up of the 56 adolescent mothers analyzed at baseline and 5 wk postpartum. Nine mothers were lost for the 20-wk measurement, which reduced the effective sample size for the bone change over the postpartum time assessment. Nevertheless, the magnitude of significant differences between groups in bone measures at the lumbar spine at 20 wk postpartum, after adjustment for confounding factors, probably reduced the potential bias because of uncontrolled factors, such as the unknown bone status before pregnancy. In contrast, results may not be generalized because bone responses may be different in populations accustomed to different calcium intakes.

In conclusion, this study indicated that adolescent mothers who received calcium plus vitamin D supplementation during the last trimester of pregnancy had higher bone mass, especially at the lumbar spine, and a reduced rate of bone mass loss at the femoral neck during the first 20 wk of lactation. These findings suggest that Brazilian adolescent mothers accustomed to a dietary intake of 600 mg Ca/d might have higher bone mass during lactation when using calcium and vitamin D supplementation during pregnancy that increases their calcium intake to a concentration close to the recommended 1300 mg/d (22, 28). However, additional studies are needed before the public health application of results. The effects of this trial on fetal and infant bone development are currently being analyzed and will be reported separately. We are also conducting a follow-up study to determine whether the observed bone maternal effects are sustained over time.

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The authors' responsibilities were as follows—MELD: was responsible for subject recruitment, data collection and analysis, and drafting of the manuscript; EPR, MFT, and IP: contributed to subject recruitment and data collection; and CMD and FFB: were the principal investigators and were responsible for the study design and supervision, interpretation of results, and critical review of the manuscript. None of the authors had a conflict of interest.

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