Calcium Plus Vitamin D Supplementation During the Third Trimester of Pregnancy in Adolescents Accustomed to Low Calcium Diets Does Not Affect Infant Bone Mass at Early Lactation in a Randomized Controlled Trial\textsuperscript{1,2}

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Abstract

Background: Pregnancy and lactation in adolescents with low calcium intake may impair fetal growth and infant bone mass. Objective: We investigated the effects of calcium plus vitamin D supplementation during pregnancy in Brazilian adolescent mothers consuming low calcium diets (~600mg/d) on fetal biometry and infant bone mass, and the relation between infant and maternal bone mass during early lactation.

Methods: Infants of mothers who received calcium (600 mg/d) plus cholecalciferol (200 IU/d) supplementation (n = 30) or placebo (n = 26) from 26 wk of gestation until parturition were studied. Fetal biometric measurements at 23 and 36 wk of gestation were obtained from medical records. Infant anthropometric and total body bone measurements [bone mineral content (BMC), bone area (BA), and bone mineral density (BMD)] at 5 wk postpartum were assessed by dual-energy X-ray absorptiometry. Maternal BMD \textit{z} scores for total body, lumbar spine, total hip, and femoral neck at 5 wk postpartum were obtained. Group comparisons were adjusted for significant covariates.

Results: Maternal mean serum 25-hydroxyvitamin D was 59 nmol/L at baseline in both groups. No differences in fetal measurements at 36 wk of gestation were observed between the groups, except for body weight and its increment from 23 to 36 wk, which were higher in the supplemented group (6.8%, \textit{P} = 0.014 and 10.5%, \textit{P} = 0.07, respectively). Infant BMC (61.1 ± 21.7 g), BA (167 ± 79 cm\textsuperscript{2}), and BMD (0.385 ± 0.069 g/cm\textsuperscript{2}) did not significantly differ between the groups. In the placebo group, infant BMC and BA were negatively correlated with maternal BMD \textit{z} scores for total body (r = -0.40 and r = -0.47; \textit{P} < 0.05) and hip (r = -0.41 and r = -0.46; \textit{P} < 0.05). In contrast, no correlations were observed in the supplemented group.

Conclusions: Calcium and vitamin D supplementation of the adolescents studied resulted in higher fetal body weight at 36 wk of gestation and had no effect on infant bone mass at 5 wk postpartum. Because correlations between maternal and infant bone mass were evident only in the placebo group, infant bone mass appeared to be more dependent on maternal skeletal mass when calcium intake was low. This trial was registered at clinicaltrials.gov as NCT01732328. \textit{J Nutr} 2015;145:1515–23.

Keywords: calcium plus vitamin D supplementation, fetal skeletal growth, infant bone mass, adolescent mothers, pregnancy, lactation

Introduction

Fetal growth and skeletal development are dependent on the continuous maternal transfer of essential minerals through the placenta. Fetal accretion of calcium increases progressively from 50 mg/d at 20 wk of gestation to 330 mg/d at 35 wk of gestation (1). After delivery, the rate of calcium accretion in the infant is highest during the first months and slows down subsequently. Whole-body calcium accretion is 140 mg/d on average during the first year of life (2).

In adult mothers with habitual adequate calcium intake, metabolic adaptation during pregnancy appears to ensure adequate transfer of calcium to the fetus without requiring an increase in maternal mineral intake (1). However, in adolescent mothers with habitual low calcium intake, the metabolic adaptation appears to be impaired, leading to inadequate transfer of calcium to the fetus. This situation may impair fetal growth and bone mass and increase the risk of osteoporosis later in life (2).
mothers, physiologic adaptation during pregnancy may not be sufficient for optimal fetal bone growth (3–5). Moreover, a low calcium intake by pregnant adolescents may limit the amount of calcium transferred to the fetus because of simultaneous maternal calcium need for bone mass consolidation (4). In theory, calcium supplementation during adolescent pregnancy could benefit fetal bone development.

The results of studies on the effects of maternal calcium supplementation during pregnancy on fetal growth and infant bone mass are controversial (3, 6–9), and most of these studies were conducted in groups of mothers who were predominantly of adult age (6–9). Studies conducted in Egypt (7) and Argentina (6), as subsets of the multicenter WHO calcium supplementation trial with 8325 pregnant women from different countries who had habitually low calcium intake (median, 600 mg/d) (10), showed that maternal supplementation with 1500 mg Ca/d during the second half of pregnancy did not affect sequential biometric measurements of fetal skeletal and somatic growth, or infant body weight and length after delivery. Similarly, calcium intervention studies conducted in women in The Gambia who had very low dietary calcium intake (~350 mg/d) showed no significant effect from calcium supplementation (1500 mg/d from 20 wk of gestation until delivery) on infant birth weight or growth and bone mineral status during the first year of life (8, 11). Moreover, in the Gambian studies, calcium supplementation during pregnancy had adverse effects on maternal bone health, with lower bone mineral content (BMC) in the mothers who were receiving calcium supplementation (12), that persisted over a long period (13). By contrast, a study in American pregnant women found that in those with a dietary calcium intake lower than 600 mg/d, calcium supplementation (2000 mg/d from 22 wk of gestation until delivery) was associated with higher BMC in the newborns (9). In addition, in a study of pregnant adolescents with low dietary calcium intake (~800 mg/d), higher birth weight and higher total body calcium were found in the newborns of those supplemented with dairy products fortified with vitamin D during the second half of pregnancy than in controls (3). The inconsistent results among studies of fetal and infant skeletal growth responses to maternal calcium supplementation during pregnancy may reflect complex interactions between environmental factors, genetics, maternal age (variation in biological maturity), composition and period of exposure to the supplement, nutritional status, and dietary intake of other nutrients besides calcium.

Evidence supports the fact that the limiting effect of low calcium intake on fetal bone growth and neonatal bone mass in adolescent pregnancy could be exacerbated if combined with a suboptimal vitamin D status (5). Maternal insufficient vitamin D status during pregnancy may limit the transfer of calcium for fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium in fetal bone growth and mineralization (5) and affect the vitamin D status of infants (2), affecting their ability to absorb calcium. In a randomized controlled trial (NCT01732328) of Brazilian adolescent mothers with habitually low calcium intake, we observed that supplementation with calcium (600 mg/d) plus cholecalciferol (200 IU/d) from 26 wk of gestation until parturition resulted in higher maternal lumbar spine bone mass and reduced rate of maternal femoral neck bone loss during the first 20 wk of lactation than with placebo (16). We herein report the results of the same trial but this time focusing on the effect of calcium plus vitamin D supplementation on fetal biometric measurements and infant bone mass, and the relations between infant and maternal bone status at early lactation.

Methods

Subjects and study design. Detailed descriptions of the inclusion and exclusion criteria, supplementation protocol, and results of the trial on maternal bone outcomes were previously published (16). Briefly, pregnant adolescents who attended the Maternity School at the Federal University of Rio de Janeiro (UFRJ) in Brazil for prenatal care between September 2009 and June 2011 were recruited to participate in a trial to test the effect of calcium plus vitamin D supplementation during pregnancy on the fetal biometric measurements and bone health of the mothers and their infants. The Maternity School at the UFRJ is a national reference clinic for adolescent pregnancy prenatal care in Brazil. The trial was approved by the UFRJ Maternity School’s ethics committee. Volunteers were eligible if they were between 13 and 19 y of age, pregnant for the first time, carrying a single fetus, and between 21 and 29 wk of gestation, had no chronic health problems or pregnancy complications, were nonsmokers, and intended to breastfeed exclusively or predominantly. All of the participants provided written informed consent after the study was explained to them and their parents or legal guardians.

Details of screening, enrollment, dropouts, and final number analyzed for fetal, neonatal, and infant outcomes are shown in Figure 1. The sample size was calculated based on maternal bone response as the primary outcome of treatment, as previously described (16). Fifty-six pregnant adolescents were longitudinally followed from midpregnancy to 5 wk postpartum. For the analyses of fetal biometric measurements, 52 adolescent mothers were included in the study. Four women were excluded because information on ultrasonographic fetal measurements was not available from their medical records at 23 or 36 wk of gestation. For the analyses of neonatal and infant outcomes, 56 mother–infant pairs were included. With this sample size and based on previous studies (6, 8), the estimated minimum detectable differences in fetal measurements at 36 wk of gestation between the groups were 0.11 cm in femur length, 0.16 cm in humeral length, 0.71 cm in head circumference (HC), 0.87 cm in abdominal circumference (AC), and 100 g in body weight, and 6.0 g in infant BMC, assuming a 95% CI and 80% statistical power.

The pregnant adolescents were randomly assigned in a single-blind fashion to receive during monthly prenatal visits a commercially available supplement containing calcium (600 mg/d) plus cholecalciferol (200 IU/d; Calcium Plus D, Rexall Sundown) or placebo for daily use for 13 wk on average, from 26 wk of gestation (baseline) until parturition. Randomization was performed by a member of the research team in a 1:1 ratio within permuted blocks of size 10. All of the participants were oriented to take one capsule of calcium plus vitamin D supplement or placebo daily during breakfast. No supplements were consumed after 13 wk on average, from 26 wk of gestation (baseline) until parturition. Compliance was controlled by counting the remaining capsules at each visit and by telephone reminders. Adherence to the supplementation was satisfactory considering that, on average, 83% of calcium plus vitamin D capsules and 87% of placebo capsules offered were taken, with no significant differences between the groups (P = 0.24, independent-samples t test). Compliance to the supplementation suggested that the pregnant adolescents studied also followed satisfactorily the health-care recommendations received during prenatal care.

Fetal biometric measurements were obtained on average at 23 ± 1 wk and 36 ± 3 wk of gestation. Anthropometric data at birth were recorded. Infant anthropometric and bone measurements were assessed at 5 wk postpartum. Maternal dietary calcium intake information was assessed by a food frequency questionnaire.

7 Abbreviations used: AC, abdominal circumference; BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BPD, biparietal diameter; HC, head circumference; UFRJ, Federal University of Rio de Janeiro; 25(OH)D, 25-hydroxyvitamin D.
assessed by at least 3 24-h dietary recall questionnaires applied by a
trained nutritionist monthly during prenatal care. Morning blood
samples (20 mL) were collected from each adolescent mother after an
overnight fast at baseline (26 wk of pregnancy), immediately before
random assignment, and at lactation (5 wk postpartum). Information on
pregnancy BMI in kilograms per meter squared, gynecologic age, and
maternal bone mass adequacy measurements and information on breastfeeding were collected. Breastfeeding practice was
classified according to the WHO indicators (21).

Maternal measurements. The gestational week at baseline was assessed
by ultrasonographic examination. Dietary calcium intake was analyzed
based on a Brazilian food database (22) by using the Avanutri program
(Version Revolution 4.0). Serum 25-hydroxyvitamin D [25(OH)D]
concentration was analyzed by using a chemiluminescent enzyme-
labeled immunometric assay (Liaison). Changes in serum 25(OH)D
concentrations were calculated as differences between measurements
obtained at 5 wk postpartum and those at 26 wk of pregnancy. Bone
mineral density (BMD) of the total body, lumbar spine (L1-L4, LS), and
hip (total and femoral neck) were assessed by using DXA with a Lunar
diXA densitometer and the enCore 2008 version 12.20 software (GE Healthcare). Maternal bone mass adequacy (BMD
a maximum division of 15 kg and 5 g. The increment (Δ) in length
and body weight from birth to 5 wk postpartum was calculated.

Infant BMC, BMD, and bone area (BA) of the total body were assessed by using DXA with a Lunar iDXA densitometer and enCore
2008 version 12.20 software (GE Healthcare). Infants were placed in a
supine position, wrapped in cotton fabric without clothes or diapers, and
placed in the center of the equipment with a protective cotton roll to
ensure the comfort of the babies. The cotton fabric used did not
substantially attenuate the X-ray beam. Mothers were invited to
breastfeed their infants before the scan to encourage the infant to sleep
during the measurements. If any movement by the infant occurred during
DXA scanning, the measurement was repeated just one more time. Infant
bone measurements were analyzed after exclusion of the infant’s head
in order to favor reproducibility, as recommended for children (26), and
expressed as total body BMC, BMD, and BA (minus head). All DXA
scanning and calibration was performed by the same operator. Quality
control of the DXA measurements followed standard procedures.
Measurements on the calibration block (daily) and on the calibration
spine phantom (weekly) supplied by the manufacturer had coefficients of
variation <0.7%.

Statistical analyses. Differences in maternal general characteristics at
a given time point between the groups were assessed by using the
independent-samples t test, except for serum 25(OH)D concentrations,
which were examined by using ANCOVA with adjustment for season.
Differences in percentile distribution of ponderal index between groups
were assessed by using the chi-square test.

The effects of calcium plus vitamin D supplementation on fetal
biometric measurements at 36 wk of gestation and on changes of fetal
biometry measurements from 23 to 36 wk of gestation were examined by
using ANCOVA, with adjustment for significant covariates. Potential
confounders (covariates) that could affect fetal measures at 36 wk of
gestation and changes in fetal measurements over time were identified in
the overall group by using multiple linear regression models with
backward elimination of those that were nonsignificant. Models were
constructed for a given fetal biometric measurement at 36 wk of gestation
or for a given change in fetal biometric measure as the dependent variable
and potential covariates as independent variables. Covariates remained in
the model at P < 0.05. Independent variables tested as potential covariates
were maternal chronologic age and height, prepregnancy BMI, gynecologic
age, serum 25(OH)D concentration at 26 wk of pregnancy, dietary
calcium intake during pregnancy, gestational week, BPD at 36 wk
of gestation, and time elapsed since start of supplementation. When testing
for fetal biometric changes, the corresponding fetal measurement at 23 wk
of gestation, changes in BPD from 23 to 36 wk of gestation, and the actual
time elapsed between the 2 ultrasonographic measurements were also
included as independent variables.

The effect of calcium plus vitamin D supplementation on neonatal
and infant anthropometric measurements at birth and at 5 wk
postpartum, respectively, and on infant bone measurements at 5 wk
postpartum were also examined by using ANCOVA, with adjustment
for significant covariates. Significant covariates for a given neonatal or
infant measurement were identified in the whole group by using
multiple linear regression models with backward elimination of those
that were nonsignificant. Potential factors tested in the overall group by
multiple regression for all neonatal measurements evaluated at birth
and infant measurements at 5 wk postpartum were dietary calcium
intake during pregnancy, gynecologic age, prepregnancy BMI, and
Calcium, vitamin D, and fetal and infant growth 1517

FIGURE 1 Flowchart of study recruitment, random assignment, and
participants lost to follow-up.
compliance rate. When testing for anthropometric measurements at birth, maternal gestational week at birth, chronologic age, height, and serum 25(OH)D concentration at 26 wk of pregnancy were also included in the regression model. Moreover, when testing for anthropometric measurements at 5 wk postpartum and anthropometric changes from birth, other potential factors that were included in the regression model were maternal chronologic age, height, and serum 25(OH)D concentration at 5 wk postpartum, changes in serum 25(OH)D concentration from 26 wk of pregnancy to 5 wk postpartum, weight and length at birth, infant chronologic age, and breastfeeding practice (2 categories; namely, exclusive and predominant). Finally, for infant bone measurements and maternal bone mass at 5 wk postpartum were evaluated by using the Pearson partial correlation analysis with adjustment for infant chronologic age. Statistical analyses were performed with SPSS 12.0 software for Windows. Results are reported as means ± SDs. Values at P < 0.05 were considered significant. P values between 0.05 and 0.10 were considered trends.

Results

Maternal characteristics according to groups (calcium plus vitamin D and placebo) are shown in Table 1. Information on general characteristics of the adolescent mothers during the study was previously described in more detail (16). No significant differences at baseline were found between the groups, except for calcium intake, which was lower in the calcium plus vitamin D group than in the placebo group (P < 0.05) (Table 1). Taking all of the adolescent mothers into account, mean calcium and vitamin D dietary intake from midpregnancy to parturition (613 mg/d and 34 IU/d, respectively) represented ~47% and 17% of the international recommended intake of calcium and vitamin D for pregnant adolescents (20). Before the intervention (26 ± 1 wk of pregnancy), 46% of the mothers in the placebo group and 43% of the mothers in the supplemented group had serum 25(OH)D concentrations ≥50 nmol/L. No differences in serum 25(OH)D concentrations were found between the groups at 26 wk of pregnancy. However, at 5 wk postpartum, serum 25(OH)D concentrations tended to be 19% higher in the calcium plus vitamin D group than in the placebo group (P = 0.075) (Table 1). At that time, 33% and 23% of the mothers in the placebo and supplemented groups, respectively, had serum 25(OH)D concentrations ≥50 nmol/L.

The effects of calcium plus vitamin D supplementation during pregnancy on fetal biometric measurements at 36 wk of gestation and on fetal biometric changes from 23 to 36 wk of gestation were evaluated after adjustment for significant covariates (Table 2). The factors in the multiple linear regression models that significantly affected at least one fetal measurement and/or changes in fetal measurements were maternal chronologic age and height, prepregnancy BMI, gynecologic age, serum 25(OH)D concentration at 26 wk of pregnancy, gestational week, BPD at 36 wk of gestation, and time elapsed since the start of supplementation. For changes in fetal measurements, the change in BPD from 23 to 36 wk of gestation was an additional significant factor.

At 36 wk of gestation, no significant differences in fetal measurements were observed between the groups, except for estimated body weight, which was 6.8% higher in the calcium plus vitamin D group (P = 0.014) (Table 2). The mean increment in estimated fetal body weight from 23 to 36 wk of gestation was 2.2 ± 0.8 kg in the overall group, and it tended to be 10.5% higher in the fetuses of supplemented mothers (P = 0.07). The mean increases in the fetal femoral and humeral lengths were 2.9 ± 0.9 cm and 2.2 ± 0.7 cm, respectively, from 23 to 36 wk of gestation. These increments were similar between the 2 groups.

All of the babies except one were born at term, with no significant differences in gestational week at birth between the groups (supplemented, 39.5 ± 1.7 wk vs. placebo, 39.0 ± 1.6 wk). The mean birth weight was adequate (3.3 ± 0.5 kg), with only 3 babies having low birth weights (~3 ≤ z score < −2; calcium plus vitamin D group, n = 2 and placebo group, n = 1). The mean birth body length was adequate (48.6 ± 2.7 cm), although 8 babies were born with low length (z score < −2; calcium plus vitamin D group, n = 4 and placebo group, n = 4). Most of the neonates had a ponderal index at birth between the 10th and 90th percentiles (placebo, 80%; calcium plus vitamin D group, 60%). In the supplemented group, 13% of the neonates had a ponderal index below the 10th percentile (n = 4) and 27% above the 90th percentile (n = 8). In the placebo group, there were no neonates with a ponderal index below the 10th percentile, and 20% of the neonates had a ponderal index above the 90th percentile (n = 5). The percentile distribution in ponderal index at birth was not significantly different between the 2 groups (P = 0.65). According to the WHO child growth assessment (27), as adopted by the Brazilian Ministry of Health (28), the weight-for-length z score at birth was adequate, with the exception of 3 babies classified as wasted (~3 ≤ z score < −2) in the calcium plus vitamin D group and 5 babies classified as overweight (~3 ≤ z score < 2; calcium plus vitamin D group, n = 4 and placebo group, n = 1).

| TABLE 1 | Maternal characteristics according to intervention group1 |
|----------|------------------|----------------|-----------|
|          | Placebo | Calcium plus vitamin D |
|          | n  | Value | n  | Value | p  |
| Chronologic age, y | 26 | 17.2 ± 1.0 | 30 | 16.8 ± 1.5 | 0.31 |
| Gynecologic age, y | 25 | 5.0 ± 1.7 | 30 | 5.0 ± 2.1 | 0.91 |
| Height, m | 22 | 1.61 ± 0.05 | 29 | 1.60 ± 0.06 | 0.44 |
| Prepregnancy BMI, kg/m² | 23 | 20.9 ± 4.5 | 28 | 22.2 ± 3.4 | 0.26 |
| Dietary calcium intake, mg/d | 26 | 743 ± 457 | 30 | 550 ± 276 | 0.02 |
| Serum 25(OH)D at 26 wk of pregnancy, nmol/L | 26 | 57.9 ± 20.7 | 30 | 59.5 ± 20.6 | 0.78 |
| Serum 25(OH)D at 5 wk postpartum, nmol/L | 26 | 59.5 ± 29.6 | 30 | 73.9 ± 29.6 | 0.08 |

1 Values are means ± SDs. Comparisons between the groups by using the independent-samples t test, except for serum 25(OH)D concentrations, which were examined by using ANCOVA with adjustment for season. 25(OH)D, 25-hydroxyvitamin D.

2 At baseline.

3 Average of 3 24-h dietary records obtained between 26 wk of pregnancy and parturition.
At 5 wk postpartum, when infant bone measurements were performed, all of the babies were breastfed, most of them exclusively (placebo group, 85%; calcium plus vitamin D group, 77%). At that time, the mean weight-for-age $z$ score was adequate (mean weight: $4.2 \pm 0.7$ kg), with 4 babies having low weight-for-age $z$ scores ($\leq -2$; calcium plus vitamin D group, $n = 3$ and placebo group, $n = 1$) and one baby (calcium plus vitamin D group) with a high weight-for-age $z$ score ($\geq 2$). The mean length-for-age $z$ score was adequate (mean length: $54.1 \pm 2.7$ cm) in the overall group ($z$ score $\geq -2$), with only 4 babies having low length-for-age $z$ scores ($z$ score $< -2$; calcium plus vitamin D group, $n = 1$ and placebo group, $n = 3$).

The effects of calcium plus vitamin D supplementation during pregnancy on the neonatal and infant anthropometric measurements at birth and at 5 wk postpartum, respectively, and on infant anthropometric changes over time and bone mass at 5 wk postpartum, were evaluated after adjustment for significant covariates (Table 3). At birth, the factors in the multiple linear regression models that affected at least one neonatal anthropometric measurement were gestational week and compliance rate, and maternal prepregnancy BMI, height, and dietary calcium intake during pregnancy. At 5 wk postpartum, the factors that affected infant anthropometric measurements were weight and length at birth, infant age, compliance rate, maternal age, and prepregnancy BMI. The factors that affected bone measurements were infant weight, length, and exact age (in days) at 5 wk postpartum, and maternal age and time elapsed since menarche. No significant differences in birth body weight, body length, and HC were observed between the calcium plus vitamin D and placebo groups (Table 3). Similarly, infant weight and length at 5 wk postpartum and their increments from birth to 5 wk postpartum were not significantly different between the 2 groups (Table 3). At 5 wk postpartum, the mean infant BMC, BA, and BMD were $61.1 \pm 21.7$ g, $167 \pm 79$ cm$^2$, and $0.385 \pm 0.069$ g/cm$^2$, respectively, with no significant differences between the groups (Table 3).

Associations between infant bone measurements and maternal BMD $z$ scores for total body, lumbar spine, total hip, and femoral neck were examined according to groups at 5 wk postpartum (Table 4). In the placebo group, infant total body BMC and BA were negatively correlated with maternal BMD $z$ scores for total body ($P < 0.05$) and total hip ($P < 0.05$). The negative correlation between maternal lumbar spine BMD $z$ score and infant total body BMD ($P = 0.08$) and BA ($P = 0.05$) approached significance. Furthermore, significant positive correlations were observed between infant total body BMD and maternal BMD $z$ scores for total body, lumbar spine, total hip, and femoral neck ($P \leq 0.05$) (Table 4). In contrast, in the supplemented group, no correlations were found between infant bone measurements and maternal BMD $z$ scores for all the sites evaluated (Table 4).

**Discussion**

This study showed that calcium plus vitamin D supplementation during late pregnancy in Brazilian adolescent mothers who were accustomed to a low calcium intake did not affect skeletal fetal measurements and infant bone mass. However, with supplementation, we found an indication of subtle changes in the relation between maternal and neonatal bone mass, consistent with the previously reported degree of maternal bone preservation during the postpartum period in the supplemented group (16). These findings are in agreement with physiologic adaptations described in adult mothers during pregnancy and lactation in...
TABLE 3  Effect of maternal calcium plus vitamin D supplementation during pregnancy on neonate anthropometric measurements at birth and on infant anthropometric and bone measurements at 5 wk postpartum1

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo</th>
<th>Calcium plus vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Value</td>
</tr>
<tr>
<td>At birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>21</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Length, cm</td>
<td>21</td>
<td>48.6 ± 2.5</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>25</td>
<td>34.6 ± 1.4</td>
</tr>
<tr>
<td>At 5 wk postpartum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>26</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Weight gain since birth, kg</td>
<td>23</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>Length, cm</td>
<td>26</td>
<td>54.1 ± 1.7</td>
</tr>
<tr>
<td>Length increase since birth, cm</td>
<td>26</td>
<td>5.7 ± 1.7</td>
</tr>
<tr>
<td>Total body BMC, g</td>
<td>26</td>
<td>59.6 ± 20.8</td>
</tr>
<tr>
<td>Total body BA, cm2</td>
<td>26</td>
<td>160 ± 80</td>
</tr>
<tr>
<td>Total body BMD, g/cm2</td>
<td>26</td>
<td>0.393 ± 0.056</td>
</tr>
</tbody>
</table>

1 Values are adjusted means ± SDs. Total BA, BMC, and BMD were measured minus head. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BW, birth weight; GA, gynecologic age; GW, maternal gestational week at birth; L, length; ppBMI, prepregnancy BMI; Wt, weight; 25(OH)D, 25 hydroxyvitamin D.

2 P values refer to the comparison between the calcium plus vitamin D and placebo groups by using ANCOVA after adjusting for significant covariates.

3 Factors tested in the overall group by using multiple linear regression models for measurements at birth were ppBMI, dietary calcium intake during pregnancy, maternal chronological age at 26 wk of pregnancy, GW and height at 26 wk of pregnancy, compliance rate, serum 25(OH)D concentration at 26 wk of pregnancy, and GA. When testing for anthropometric measurements at 5 wk postpartum and anthropometric changes from birth, the factors tested in the whole group by multiple regression were ppBMI, breastfeeding practice, Ca intake, changes in serum 25(OH)D concentration from 26 wk of pregnancy to 5 wk postpartum, infant chronological age, BW, L at birth, maternal chronological age and Ht at 5 wk postpartum, compliance rate, GA, and 25(OH)D concentration at 5 wk postpartum. For bone measurements at 5 wk postpartum, Wt and L at 5 wk postpartum were also included in the regression model.

order to provide calcium for fetal growth and breast-milk production, and consequently for neonatal bone mass acquisition, even in conditions of habitually low calcium intake (6–8).

Calcium plus vitamin D supplementation during the third trimester of gestation did not affect femoral and humeral lengths in the fetuses of the adolescent mothers studied, after adjustment for significant covariates. Consistent with our findings, Abalos et al. (6) showed that calcium supplementation from ~13 wk of gestation until delivery in young adult women with habitually low calcium intake (600 mg/d) did not affect fetal growth measurements from 20 to 36 wk of gestation, including femoral and humeral lengths. By contrast, in a retrospective cohort study in pregnant adolescents, Chang et al. (4) found significantly longer femurs in fetuses whose mothers had higher dairy intake (>3 servings/d) at entry into prenatal care (20–34 wk) than those with low dairy intake (<2 servings/d). These controversial results may be explained in part by the fact that the effect on femoral length in the study by Chang et al. (4) was observed with dairy products that provide other nutrients besides calcium and vitamin D that might also have contributed to femoral growth.

Irrespective of supplementation, mean femoral length, BPD, HC, AC, and estimated body weight at 36 wk of gestation in our study population were adequate (percentile values: 10th–90th) in the 2 groups, according to reference charts of fetal biometric variables in Brazilian pregnancies (29). In addition, the increases in fetal biometric measurements from 23 to 36 wk of gestation were similar in the 2 groups, except for estimated body weight. Fetuses whose mothers received the calcium plus vitamin D supplement had a mean body weight of 180 g higher at 36 wk of gestation than those taking the placebo (P < 0.015) (Table 2). Moreover, the increment in fetal estimated body weight from 23 to 36 wk of gestation tended to be 220 g higher in the supplemented group than in the placebo group (P < 0.07), suggesting that calcium plus vitamin D supplementation accelerated fetal somatic growth during the second half of pregnancy. However, these results should be interpreted with caution considering that the higher estimated fetal body weight at 36 wk of gestation and the higher rate of body weight increase from 23 to 36 wk of gestation in the supplemented group did not persist over time, because neonatal and infant growth anthropometric measurements did not differ significantly between the groups.

Systematic reviews of studies on the effect of maternal calcium supplementation on several pregnancy and infant outcomes concluded that calcium supplementation has no significant effect on infant length and HC but resulted in a small increase (65–80 g) in birth weight (30–32), supporting the hypothesis that calcium

TABLE 4  Correlations between infant bone measurements and maternal bone mass at 5 wk postpartum according to intervention group1

<table>
<thead>
<tr>
<th>Maternal variable</th>
<th>Total BMC, g</th>
<th>Total BA, cm2</th>
<th>Total BMD, g/cm2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMC z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.40*</td>
<td>−0.47*</td>
<td>0.51*</td>
</tr>
<tr>
<td>Calcium plus vitamin D</td>
<td>−0.03</td>
<td>−0.14</td>
<td>−0.06</td>
</tr>
<tr>
<td>Lumbar spine BMD z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.35</td>
<td>−0.39*</td>
<td>0.39*</td>
</tr>
<tr>
<td>Calcium plus vitamin D</td>
<td>−0.03</td>
<td>−0.13</td>
<td>−0.21</td>
</tr>
<tr>
<td>Total hip BMD z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
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<tr>
<td>Calcium plus vitamin D</td>
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<td>−0.01</td>
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<tr>
<td>Femoral neck BMD z score</td>
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<td></td>
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<tr>
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<tr>
<td>Calcium plus vitamin D</td>
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<td>−0.06</td>
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</table>

1 Pearson partial correlation adjusted for infant chronologic age. Supplemented group, n = 30, and placebo group, n = 26. Total BA, BMC, and BMD were measured minus head. *Significant correlations, P ≤ 0.05. BA, bone area; BMC, bone mineral content; BMD, bone mineral density.
supplementation may increase somatic fetal growth. However, the clinical significance of this small effect on birth weight was considered uncertain (30, 31). In fact, no effect on infant birth weight, length, and HC were observed in randomized controlled maternal calcium supplementation trials in different populations (6–8). Consistently, in our study, the body weight, length, and HC of the neonates at birth did not differ between the groups. Moreover, irrespective of supplementation, the mean anthropometric birth measurements were adequate in the 2 groups when compared to the international standards (24).

Irrespective of supplementation, at 5 wk postpartum, weight-for-age and length-for-age indices were adequate for most infants (24). Calcium plus vitamin D supplementation during pregnancy did not affect growth and bone measurements in infants at 5 wk of age. The similar increments in weight and length from birth to 5 wk postpartum in the 2 groups and the lack of significant differences in BMC, density, and area at 5 wk postpartum suggest that low maternal calcium intake during pregnancy was not the primary determining factor of infant growth and bone mass acquisition in the group studied. Our findings are in agreement with the study in Egypt (7), in which maternal calcium supplementation (1500 mg/d) during pregnancy did not significantly affect weight, length, HC, and midarm circumference of infants during the first year of life, compared with placebo. Moreover, our results agree with those from the study in The Gambia in which maternal calcium supplementation (1500 mg/d) during pregnancy did not affect infant growth and infant bone mass at 2, 13, and 52 wk postpartum (8). In contrast, a study in American pregnant young women showed that in those mothers consuming 600 mg Ca/d or less, total body BMC at the first week after birth was ~15% higher in infants born to mothers supplemented with calcium (2 g/d) during pregnancy than in those who received placebo (9). Controversial results on infant bone mass reflect in part differences in the timing of the neonatal DXA scan, the dose, composition of, and period of exposure to the supplement, and the time after cessation of supplementation (3, 8).

At 5 wk postpartum, all of the mothers studied were breastfeeding their infants similarly in both groups. Infant bone mass at this moment did not differ between the 2 groups, suggesting that irrespective of maternal calcium intake, physiologic adaptations such as increased calcium intestinal absorption during pregnancy and bone calcium mobilization during lactation supplied the calcium demand for fetal growth and infant bone mass acquisition in the adolescent mothers studied. However, the negative correlations between maternal total body and total hip BMD z scores and infant BMC and area suggest increased maternal bone loss in support of fetal/infant demands in the placebo group. Meanwhile, the absence of these correlations in the supplemented group is consistent with an apparent maternal bone calcium economy during pregnancy and/or early lactation with the use of calcium plus vitamin D supplement, as suggested by our previous findings on maternal bone status (16). The mothers who received the calcium plus vitamin D supplement had reduced rates of maternal femoral neck bone loss from 5 to 20 wk of lactation compared with those who received the placebo (16).

Infant BMD is known to decrease ~30% in the first 6 mo of life (14) as a consequence of a faster increase in bone marrow than in cortical BA, a process described as “physiologic osteoporosis of infancy” (33). Therefore, a higher infant BMD during the first postpartum weeks is not necessarily indicative of more adequate infant bone growth and, conversely, a higher infant BMD may indicate a slower rate of infant bone develop-
group. Although this variable was entered as a covariate in the statistical analyses, it may have masked other uncontrolled differences between the study groups. Finally, the sample size was not powered to detect group differences in fetal skeletal growth lower than 3% and differences in BMC lower than 10%.

In conclusion, this study indicated that supplementation with 600 mg/d Ca and 200 IU/d cholecalciferol from 26 wk of gestation until parturition did not affect the fetal skeletal growth and infant bone mass of babies born to Brazilian adolescent mothers who habitually had low calcium intake (~600 mg/d). However, infant bone mass at 5 wk of lactation seemed to be more dependent on maternal bone calcium mobilization when maternal calcium intake was low. This is consistent with our previous findings (16) that providing extra calcium combined with vitamin D by supplementation, aiming at the recommended 1300 mg Ca/d (20) during adolescent pregnancy, contributes to maternal bone calcium economy during pregnancy and/or early lactation. Our results suggest that regardless of maternal calcium intake, the physiologic adaptations that occur in the adolescent mother during pregnancy and lactation supply the calcium demand of the fetus and infant.

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References


