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Vanitha Janakiram, MD—Award Winner

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Calcium Supplements and Bone Resorption in Pregnancy

A Randomized Crossover Trial

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Background: Pregnancy is a time of increased need for calcium. The role of calcium supplements in altering maternal responses to fetal demand for calcium is not fully understood. This article describes the results of a randomized, crossover trial of calcium supplementation on bone resorption among pregnant women.

Design Setting/ Participants: Thirty-one Mexican women at 25–35 weeks gestation participated in the study for 20 days. Each woman received a 1200 mg calcium supplement on 10 consecutive days and a multivitamin without calcium for 10 days. Urine samples were collected daily. Two pooled specimens from each subject (representing urine from multivitamin days and from calcium days) were preserved, and levels of cross-linked, N-telopeptides of type I collagen (NTX), a biomarker of bone resorption, were measured. Dietary calcium intake was assessed using a food-frequency questionnaire.

Results: Of the 31 participants, 27 (87.1%) showed reductions in urinary NTX levels while ingesting calcium supplements. When not ingesting calcium, NTX levels for the 31 subjects had a mean of 96.8 nM BCE/mM creatinine; this was significantly higher ($p<0.001$) than the mean urinary NTX levels of 83.2 nM BCE/mM creatinine during ingestion of the calcium supplements. Neither age nor dietary calcium intake was a significant predictor of treatment effect.

Conclusion: A bedtime, 1200-mg calcium supplement during the third trimester of pregnancy reduces maternal bone resorption by an average of 13.6 nM BCE/mM creatinine (14%), as reflected by urinary NTX levels. These results suggest that calcium supplements reduce maternal skeletal-bone turnover during the third trimester of pregnancy. (Am J Prev Med 2003;24(3):260–264)

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Introduction

Pregnancy requires increased calcium requirement, with 20 g to 30 g of calcium needed for fetal skeletal mineralization.[1] Maximal calcium accretion occurs during the third trimester. The maternal response to this demand for calcium is complex and may involve multiple adjustments in the maternal intestines, kidneys, and bones.

The role of the maternal skeleton in providing calcium to the fetus is not completely understood. There is clear histologic[2] and biochemical[3,4] evidence that the maternal skeletal system undergoes increased bone resorption in pregnancy. Biochemical markers of bone resorption rise gradually throughout pregnancy, reaching 100%–200% of nonpregnant values by the third trimester in all women.[4,5] However, evidence of net maternal bone loss during pregnancy has been inconsistent. Several studies have failed to find a relationship between the number of previous pregnancies and bone-mineral density or fracture risk.[6,7] These inconsistencies may be related to design limitations of the study, since small changes in bone density are difficult to detect using ultrasound.[8] Alternatively, the maternal skeletal response to the fetal demand for calcium may be highly individualized, depending on factors such as maternal diet, age, parity, and body mass.[9]

Calcium supplements during pregnancy have long been suggested as a means of reducing maternal skeletal remodeling during pregnancy.[10] In the past, increased dietary calcium, often taken in the form of supplements, was recommended routinely during pregnancy. However, a recent advisory panel concluded that dietary calcium requirements are not increased in pregnancy.[11] Several studies have documented bone mineral and hormonal changes during pregnancy but did not investigate the effect of calcium supplements.[3,12,13] Another study demonstrated that an oral calcium load led to an exaggerated calcemic response in pregnant women, compared to controls, and no difference in markers of bone turnover between the two groups within 12 hours,[14] but the study did not address the long-term effects of supplementation.

A study by Hillman et al.[15] of 19 pregnant women with a calcium intake of 1300 mg/day followed participants from the first trimester through the third trimester and randomized participants to either supplementation with 1 g calcium carbonate per day or a placebo. The calcium supplemented group showed significantly increased total serum calcium as well as significantly decreased PTH and 1,25 dihydroxyvitamin D. There was no difference in levels of markers of bone formation between the two groups. No markers of bone resorption were measured.

Recently, a new marker for bone resorption was developed to measure cross-linked N-telopeptides (NTX) of type I collagen.[16] Because the NTX crosslink is unique to bone type I collagen, the level of urinary NTX is a specific marker of bone breakdown. Recent studies demonstrate that the percentage change in NTX provides the most sensitive measure of gain and loss of bone-mineral density compared with other bone markers,[17] even in pregnant women.[5] Here we report the first systematic study of the effects of calcium supplementation on a marker of bone resorption in pregnant women.

Methods

Study Design

We conducted a randomized crossover trial of calcium supplementation among pregnant women in Mexico City. A marker for bone resorption (NTX), which has been demonstrated to be sensitive and specific in the context of pregnant women and calcium supplementation, was used as our outcome. The current recommended daily calcium intake for pregnant women is 1300 mg for women aged 14–18 years and 1000 mg for pregnant women ≥18 years.[11] Previous studies suggested that women in Mexico City often had low levels of dietary calcium.[18] Further, the traditional Mexican diet contains phytates and oxalates that may inhibit calcium absorption.[19] To assure adequate calcium intake for study participants of all ages, despite differences in absorption and metabolism, we provided a daily 1200-mg calcium supplement. Endocrine studies demonstrate that calcium supplements taken in the evening are more effective than supplements taken at other times in suppressing bone resorption.[20] Thus, we instructed participants to take the supplement at bedtime.

The study duration was 20 days. Women were assigned to Group 1 or Group 2 sequentially according to order of enrollment at the onset of the study. The first group (n=16) received a 1200 mg calcium supplement during Period 1 (days 1–10) of the study, then a multivitamin without calcium (placebo) during Period 2 (days 11–20). The second group (n=15) received a multivitamin without calcium during Period 1 and a 1200 mg calcium supplement during Period 2.

Subjects

Study nurses who visited prenatal clinics associated with the IMSS (Instituto Mexicano del Seguro Social), a large governmental HMO in Mexico City that serves primarily low-to-moderate income women, recruited the participants. Prospective candidates were screened by interview with a study nurse and completion of a health questionnaire by their physicians. Exclusion criteria were as follows: <25 weeks or >36 weeks of gestation, use of calcium supplements or multivitamins (including calcium during current pregnancy), hypertension, preeclampsia, history of renal disease, history of premature delivery, or a high-risk pregnancy as defined by their medical provider. The women were then provided transportation to the research facility at the American British Cowdray (ABC) Hospital, where the baseline nutritional survey was administered and informed consent for participation in the study was obtained. Thirty-four women met inclusion criteria and agreed to participate in the study, but

Table 1. Baseline subject characteristics, by group

Variable	Group 1	Group 2	*p* value[a]
	Mean(SD)(Range)	**Mean(SD)(Range)**	
Age (years)	25.8 (6.8) (15–36)	28.7 (6.0) (18–43)	0.6722
Age group (years)	***n* (%)**	***n* (%)**	
14–18	2 (12.5%)	1 (7%)	
19–30	9 (56.25%)	9 (60%)	
31–50	5 (31.25%)	5 (33%)	
	Mean(SD)(Range)	**Mean(SD)(Range)**	
Estimated dietary calcium intake	1031 (334) (603–1559)	959 (260) (543–1575)	0.3574
Calcium group	***n* (%)**	***n* (%)**	
<1000 mg	8 (50%)	9 (60%)	
1000–1300 mg	4 (25%)	5 (33%)	
>1300 mg	4 (25%)	1 (7%)	
	Mean(SD)(Range)	**Mean(SD)(Range)**	
Weeks of pregnancy	30.8 (2.5) (27–35)	30.1 (2.7) (25–36)	0.7059
Blood lead (μg/dL)	7.7 (6.1) (2–25)	9.3 (4.4) (5–22)	0.2354
Blood lead group	***n* (%)**	***n* (%)**	
<10 μg/dL	13 (81.25%)	10 (67%)	
≥10 μg/dL	3 (18.75%)	5 (33%)	

[a]*p* value from *t*-test of equality of sample means.
SD, standard deviation.

three were eventually excluded owing to delivery before completion of the study. The 31 remaining women were included in the final analysis. The Institutional Review Boards of the National Institute of Public Health of Mexico and the Harvard School of Public Health approved this study.

Nutrient Intake

Nutritional status of all women was assessed at the onset. To assess dietary calcium intake, a food frequency questionnaire (based on the frequency of consumption of 128 food items in the past 12 months) was used. This questionnaire has been validated in the Mexican population and previously used with women of reproductive age.[18]

Follow-Up

The participants were instructed to take supplements before bedtime and to collect their second morning urine in the sterile flasks provided. Study nurses visited participants daily, collecting urine samples and performing pill counts. Compliance, assessed by the nurse's daily pill counts, was 100%. Participants and data collectors were blinded to group assignment.

Urine sample volumes were measured and recorded. All sample volumes were >20 cc to assure accuracy of NTX measurement. A 10-cc sample was labeled, frozen, and preserved. In this analysis, equal aliquots of urine were pooled for each subject from every day that the subject took the multivitamin without calcium; as a second specimen for each subject, equal aliquots of urine were pooled from every day that the subject took the calcium supplement. Thus, two pooled urine specimens (representing urine from multivitamin days and urine from calcium days) were collected from each of the 31 subjects. NTX levels of each pooled specimen were then measured using a commercially available, competitive-inhibition enzyme-linked immunosorbent assay (Osteomark, Ostex International, Seattle WA). NTX concentrations are expressed as nanomoles of bone collagen equivalents (nM BCE) per liter corrected for creatinine in units of nM BCE/mM creatinine. The BCE unit is the most readily interpretable in terms of mass of bone resorbed.[16]

Statistical Analysis

Descriptive statistics by treatment group and time period were calculated. Age and dietary calcium intake were categorized based on the recommended dietary calcium intake by age groups during pregnancy and lactation.[11] Statistical significance (*p*-values) were estimated from the means and standard errors, assuming a Student *t*–distribution. Standard methods for the analysis of a 2×2 crossover trial, using ordinary least squares regression (PROC GLM), were used.[21] Carryover effects were tested by inclusion of an indicator variable in the model. All statistical analyses were performed using SAS (Release 8.01, SAS Institute, Inc., Cary NC, 1999–2000).

Results

Summary statistics for baseline subject characteristics by group are shown in Table 1. There were no statistically significant differences by group assignment for the variables available at baseline. Subjects ranged in age from 15 to 43 years and were, on average, in their 30th week of pregnancy upon enrollment. Based on the recommended dietary allowance (RDA) for calcium during pregnancy by age, 17 (55%) of participants fell below the RDA for their age group.

Of the 31 participants, 27 showed reduction in NTX level with calcium ingestion (see Figure 1). Urinary NTX levels for the 31 subjects not ingesting calcium had a mean of 96.8 (range: 43–191) nM BCE/mM creatinine, which was significantly higher ($p<0.001$; paired *t*-test) than the mean urinary NTX levels during ingestion of the calcium supplements of 83.2 (range:

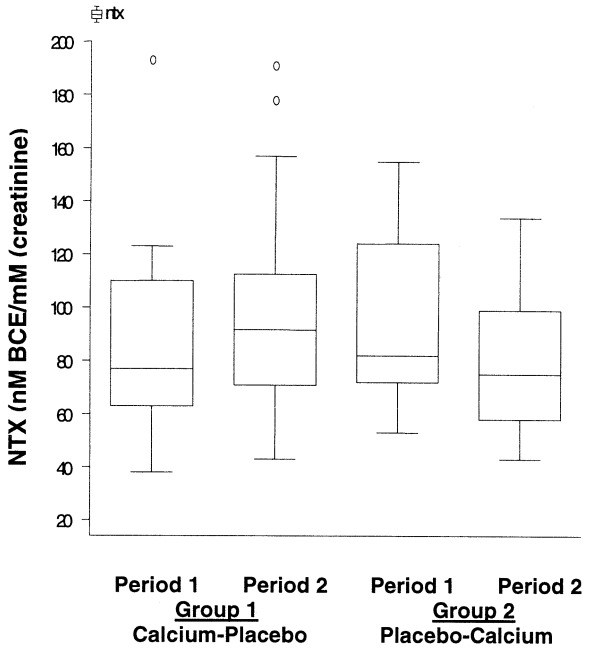

Figure 1. Boxplot of N-telopeptides of type I collagen (NTX) by treatment Group 1 (calcium-placebo) or Group 2 (placebo-calcium) and Period 1 (days 1–10) or Period 2 (days 11–20). Boxplot indicates the minimum, 25th, 50th (median), 75th percentile, and maximum NTX values for each group and time period.

38–193) nM BCE/mM creatinine. The difference in urinary NTX levels was fairly consistent across individuals. Table 2 shows the average change in NTX by group and treatment period. Both groups showed statistically significant differences in NTX levels between calcium and placebo periods.

In the first model, an indicator variable for carryover effect was not significant. For additional models, it was assumed that there was no carryover of treatment effect from Period 1 (days 1–10) to Period 2 (days 11–20). However, persistence of a treatment effect cannot be ruled out.

In multivariate models including age and dietary calcium intake, both separately and combined, neither covariate was a significant predictor of treatment effect. The pooled estimate of treatment effect, combining the responses from Period 1 and Period 2, was 13.7 (p=0.0001). In other words, levels of NTX were, on average, 13.7 nM BCE/mM creatinine higher (indicating higher bone mobilization) during the placebo period than when women were taking calcium supplements. There was no significant interaction between treatment and period, thus providing additional evidence of no carryover effect.

Discussion

The results of this study show that a 1200 mg calcium supplement taken at bedtime during the third trimester of pregnancy reduces maternal bone resorption by an average of 13.6nM BCE/mM creatinine. This amounts to an average decrease in NTX of 14%. This is the first study (to our knowledge) to measure the effects of calcium supplementation on suppressing bone resorption using NTX as a measure of bone resorption. Our study confirms earlier findings of approximately 99nM BCE/mM creatinine NTX excretion during the third trimester without calcium supplements.[3,5] The average increase in NTX excretion for women at 27 weeks of pregnancy and nonpregnant women is 25nM BCE/mM creatinine.[17] Thus, the average reduction in NTX of 13.5nM BCE/mM creatinine shown in this study represents a large return towards nonpregnant levels of NTX excretion.

In our study, Group 2 showed a lower NTX level during supplementation and a slightly greater reduction in NTX levels between calcium and placebo time periods than Group 1. This is consistent with the hypothesis that calcium supplementation continues to suppress NTX levels even after supplementation is stopped, and that the relatively low placebo levels of NTX for Group 1 represent a biological "washout effect" of calcium supplementation. However, since we do not have baseline levels of NTX for both groups, we cannot rule out the possibility that differences in baseline NTX levels were responsible for this observation.

Increases in bone turnover during pregnancy produce higher lead levels in maternal blood.[22] Concerns about fetal exposure to lead have sparked recent interest in calcium supplementation in pregnancy. Bone accumulates lead over time and accounts for over 95% of lead burden in adults.[23] Lead crosses the placenta readily. Maternal bone lead has been shown to predict adverse fetal outcomes in terms of birth weight[23] and infant mental development.[24] The findings of this study

Table 2. NTX (mean and SD) by group and treatment period

Group	*n*	Period	Treatment	Mean (SD)	*p* value[a]
1: Calcium–placebo	16	1	Calcium	87.5 (37.9)	0.0391
		2	Placebo	99.5 (42.9)	
2: Placebo–calcium	15	1	Placebo	93.9 (32.1)	0.0002
		2	Calcium	78.5 (24.2)	

[a]*p* value from *t*-test of equality of sample means.
NTX, N-telopeptides of type I collagen; SD, standard deviation.

suggest that there is a decrease in bone turnover during pregnancy with calcium supplementation. Taken together, these findings suggest that calcium supplements may reduce fetal exposure to lead during pregnancy. Further studies measuring lead levels and fetal outcomes during calcium supplementation in pregnancy are needed to test this hypothesis.

The maternal skeletal contribution to fetal calcium needs is poorly understood. Direct investigation of the maternal skeleton is limited, since the most sensitive techniques require ionizing radiation and are unsuitable during pregnancy. Conflicting evidence in the field and lack of correlation between fracture risk and parity led the Institute of Medicine's panel to conclude that dietary calcium needs are not increased during pregnancy.[11] However, there is evidence that the maternal response to calcium intake during pregnancy is highly individualized, making a maternal response difficult to detect. While the ultimate effect on the maternal skeleton remains unclear, our findings further attempt to elucidate the role that maternal calcium supplementation might play in altering the maternal response to calcium needs.

There are several limitations to this study. No significant difference was observed in the effect of calcium supplements on NTX levels between women with high dietary calcium levels and women with low dietary levels. However, the nutritional survey used in this study had been validated in the general population and not in pregnant women; therefore, it may not have accurately reflected participant calcium intake. Further, dietary calcium habits often change significantly in the course of pregnancy, and a recall survey may not have accurately reflected participants' actual calcium intake during the study. The small number of participants enrolled and the lack of information on parity and bone mass also limited the conclusions of this study.

Despite these limitations, our study showed that women in the third trimester of pregnancy experience a modest decrease in NTX with a 1200 mg calcium supplement taken at bedtime. This decrease was fairly consistent across individuals. These results suggest that calcium supplementation does alter the skeletal response to the increased demand for calcium experienced during pregnancy. Further research simultaneously measuring the effect of calcium supplements on biochemical markers and ultrasound measurements of bone mineral density during pregnancy may elucidate the impact of calcium supplements on the maternal skeleton. In addition, future studies involving more women and measuring the effect of body mass index, parity, age, and nutrition on the change in NTX levels during calcium supplementation are needed to elucidate individual differences in the maternal skeletal response to calcium supplementation.

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