Interrelationship between bone turnover markers and dietary calcium intake in pregnant women: a longitudinal study

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Abstract

This longitudinal study evaluated bone turnover and the interrelationship between changes in bone biomarkers and habitual dietary calcium intake during pregnancy in a group of women ranging widely with regard to dietary calcium intake. Thirty-nine healthy pregnant and 30 nonpregnant women were studied. Calcium, phosphorus, $1\alpha,25$-dihydroxyvitamin D (1,25diHOD), bone alkaline phosphatase (bALP), carboxyterminal propeptides of type I procollagen (PICP) and carboxyterminal telopeptides of type I collagen (βCTX and ICTP) were measured in serum and calcium, and creatinine and aminoterminal telopeptide (NTX) were determined in urine. Serum calcium and phosphorus did not change but the urinary Ca/Creat ratio and 1,25diHOD increased throughout pregnancy ($P < 0.001$ and $P < 0.0001$, respectively). Serum b-ALP and PICP increased during the last two trimesters ($P < 0.0001$ and $P < 0.001$, respectively). All studied bone resorption markers increased compared to nonpregnant values throughout pregnancy. The highest increment was observed in the third trimester. The level of significance decreased as follows: βCTX > NTX >ICTP. Serum 1,25 diHOD versus calcium intake showed a positive and significant correlation ($r = 0.51$, $P < 0.02$). A negative correlation between the absolute change in βCTX, NTX, and b-ALP between the third and second trimester and calcium intake at the end of pregnancy was observed in pregnant women who did not cover adequately calcium intake requirements ($r = -0.47$, $P < 0.03$; $r = -0.41$, $P < 0.05$; and $r = -0.43$, $P < 0.05$, respectively). These results suggest that skeletal response to pregnancy may not be entirely independent of maternal calcium intake, especially in women with usually low calcium intake. In summary, not only hormonal changes in calcium metabolism that occur during pregnancy but also other considerations, such as low dietary calcium intake, may lead to an increment in the biological activity of the skeleton. Additional studies must be conducted to confirm our findings and to gain a better understanding of skeletal response to a low calcium intake during pregnancy.
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Keywords: Pregnancy; Women; Bone turnover markers; Customary low calcium intake

Introduction

Postmenopausal estrogen deficiency and age-related bone loss are the two main factors related to osteoporosis in women. However, there are a great number of individuals affected by osteoporosis with no identifiable cause, which is termed "idiopathic osteoporosis." Both pregnancy and lactation induce several hormonal changes, most of which are closely related to bone and mineral metabolism. To date, the effects of these two transitory states in a woman's life on the maternal skeleton are not fully understood, and their possible involvement in the pathogenesis of osteoporosis remains unknown. In this regard, as early as 1948 Albright and Refenstein reported two cases of idiopathic osteoporosis aggravated by pregnancy [1].

Normal total fetal skeleton accretion during pregnancy is about 30 g of calcium [2], and about 80% (250–300 mg/

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day) occurs during the third trimester. Several adaptive changes in maternal calcium homeostasis take place to meet the calcium demands of the growing fetus. Serum ionized calcium levels remain within normal range and there is no renal calcium conservation. However, calcium intestinal absorption is increased and there is some contribution of calcium from the maternal skeleton [3]. In this regard, a high normal range value of 24-h calcium excretion or physiological hypercalciuria is observed. In addition, dietary calcium intestinal absorption is enhanced early in pregnancy because of the increment in both $1\alpha25$-dihydroxyvitamin D (1,25diHOD) and the intestinal expression of vitamin D-dependent Ca-binding protein calbindin 9k-D [4].

The contribution of maternal skeletal calcium metabolism is evidenced in changes in markers of bone formation and bone resorption [5]. Indeed, bone remodeling increases early in gestation suggesting bone activity and rises to twice normal range at the end of pregnancy, which corresponds to the peak rate of maternal-fetal calcium transfer. It is important to take into account that maternal skeletal activity may depend on calcium intake. If maternal bone mineral were the main source to cover the calcium demands of the fetus, the maternal skeleton could lose about 3% of its mineral content per pregnancy [6]. Whether maternal bone mass decreases due to calcium demand during pregnancy or increases because of estrogen levels and increased bone loading with weight gain in the last trimester is still a matter of controversy [6].

There are few longitudinal studies during pregnancy and these reports have shown marked biological bone activity. However, to our knowledge, they do not address the issue of bone remodeling changes occurring in a calcium-deficient maternal environment in which responsiveness to skeletal demand of pregnancy may be different and the calcium transfer from the maternal skeleton may become the most important factor in fetal growth.

Based on the above, the first objective of the present longitudinal study in pregnant women was to evaluate bone turnover during pregnancy in women ranging widely in dietary calcium intake, using several sensitive bone formation and resorption markers. In addition, and given that the impact of calcium intake on maternal bone metabolism during pregnancy remains unknown to date, the second objective of this report was to study the interrelationship between changes in bone turnover markers and usual dietary calcium intake in a subgroup of pregnant women whose customary dietary calcium intake is below adequate levels.

Subjects and methods

Volunteers and study design

Between March 1999 and March 2000, 100 healthy women were recruited at the Obstetrics Section of a suburban hospital located in the outskirts of Buenos Aires (Diego Paroissien Hospital, La Matanza, Buenos Aires Province)

where they received prenatal medical care. At their first visit, all patients were subjected to a routine clinical interview and the physicians completed a questionnaire recording age, socioeconomic status, medical history, dietary habits, and obstetric features. In compliance with the inclusion criteria (Caucasian, healthy, first pregnancy, with no history of bone disease nor medication that could affect bone and calcium metabolism) 69 women were enrolled in the present study and 39 of them completed the study.

The study was approved by the Human Ethics Committee of the Hospital and all the women who agreed to participate were informed verbally about the study and gave written consent. As mentioned in the following paragraph, these pregnant women were of low or middle socioeconomic level, and characteristically they do not seek medical assistance until pregnancy is quite advanced. Because of this, only 20 pregnant women went in for their first obstetric visit at the end of the first trimester and the remaining women did during the second trimester. All women delivered healthy full-term infants of adequate body weight.

In order to provide adequate controls, we studied a group of 30 young nonpregnant healthy women of similar age who volunteered for this purpose. Potential volunteers were not included if they had a history of bone disease or were taking medication known to affect bone. All the subjects were of low- to middle-income socioeconomic strata. Demographic and anthropometric characteristics at the time of enrollment were not statistically different between pregnant and nonpregnant women. The pregnant women ranged in age from 17 to 30 years (21.4 ± 4.6) and the nonpregnant controls from 18 to 27 years (22.7 ± 3.9). There was no significant difference between the mean age of the two groups.

Usual calcium intake was determined by a food-frequency questionnaire (FFQ) [7]. Nutrient-intake data were collected by three-day records and frequency consumption of dairy products (FCDP), calcium-enriched foods and vegetables [8]. Calcium intake was calculated with a computer program containing food composition data from The National Food Composition Tables [9]. Foods that were not listed on these tables were obtained from the CENEXA tables [10] or the product label. After completing the questionnaire at their first visit, the subjects were informed of the beneficial effect of consuming dairy products. The women completed the questionnaire a second time at the end of the third trimester.

Laboratory

The pregnant women were examined at three time points during this longitudinal study: in the first trimester (T1: 12–15 weeks), second trimester (T2: 18–22 weeks), and third trimester (T3: 36–38 weeks). Only one point was taken for the nonpregnant women as a normal prepregnancy value. At each point blood samples (between 8 and 9 A.M. after an overnight fast) and urine samples (the second voiding discarding the first morning urination) were obtained. Serum and urine were stored at $-20°C$ until analyzed. The
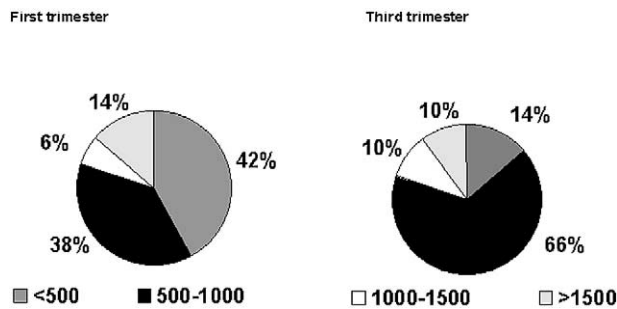
Fig. 1. Distribution of calcium intake in the studied pregnant women during the first and third trimesters (mg/day).

values corresponding to the first trimester were obtained in the 20 women who attended their first obstetric visit during weeks 12 to 15 of pregnancy (T1).

Serum and urinary calcium samples were measured by atomic absorption spectrophotometry using lanthanum chloride as an interference suppressor [11]. Serum phosphorus and urinary creatinine were measured by colorimetric methods [11]. Serum 1,25diHOD was measured using a radioreceptor immunoassay (Incstar, Stillwater, MN). The intraassay coefficient of variation (CV) was 10% [12].

Markers of bone formation included bone-specific alkaline phosphatase (b-AL) and the carboxyterminal propeptide of type I procollagen (PICP). The b-AL was measured using a colorimetric method (Boehringer Mannheim, Germany) after bone isoenzyme precipitation with wheat-germ lectin [11]. Intraassay CV was 6%. PICP was measured by radioimmunoassay (RIE) (Orion Diagnostica, Oulu, Finland). The intraassay CV was 4%.

Markers of bone resorption included the two serum carboxyterminal C-telopeptide cross-linked of type I collagen: βCTX and ICTP and the urinary aminoterminal cross-linking region of type I collagen telopeptide (NTX). The βCTX was measured by immunoassay (ELISA) (CrossLaps one step, Osteometer BioTech, Herlev, Denmark) with an in-

traassay CV of 6%. ICTP was measured by radioimmunoassay (Orion Diagnostica, Oulu, Finland) with an intraassay CV of 6%. Urinary NTX was measured by ELISA using a commercial kit (Osteomark, Ostex International, Inc., Seattle, WA, USA). The intraassay CV was 6%. Urinary NTX and Ca values were expressed as creatinine ratio (NTX/Creat and Ca/Creat, respectively).

All biochemical analyses were processed at the same time to avoid interassay errors.

Data analysis

Data were expressed as absolute values using mean ± standard deviation. As calcium intake was not normally distributed, the Wilcoxon rank-sum test was used to test differences in calcium intake between the first and the second questionnaire. The difference between pregnant and nonpregnant women was calculated using a nonpaired *t* test. Longitudinal comparisons in pregnant women were performed using repeated measures analysis of variance (ANOVA). Pairwise significant differences were assessed using Scheffé range test. Statistical analysis was performed using the SPSS program (SPSS for Windows version 8.0). Results were considered to be significant if $P < 0.05$.

Results

Calcium intake

Calcium intake for the control nonpregnant women was not significantly different from that of pregnant women at their first visit (median: 526; range between 25th and 75th quartiles: 356–717, $n = 30$). There were no significant differences in dietary calcium intake between the first and second recall periods. Median dietary calcium intake of pregnant women ($n = 39$) at their first visit was 524 mg/day

Table 1
Levels of calcium, phosphate, 1,25dihydroxyvitamin D and biochemical markers of bone turnover in non-pregnant women and during the three trimesters of pregnancy (mean ± SD)

Bone marker	Calcium (mg/dl)	Phosphate (mg/dl)	1,25diHOD (pg/ml)	b-ALP (U/L)	PICP (ug/L)	β-CTX (nM)	NTX nmolBCE/mMcreat	ICTP (ug/L)
Non-pregnant women	9.5 ± 0.2a	4.3 ± 0.2a	23 ± 3a	52 ± 13a	97 ± 46a	2.23 ± 1.03a	98 ± 55a	3.7 ± 1.2a
1st Trimester (12 ± 2 weeks)	9.3 ± 0.3a	4.4 ± 0.2a	109 ± 31b	60 ± 12a,b	118 ± 59a,b	3.44 ± 1.78a,b	129 ± 76a,b	4.7 ± 2.1a,b
2nd Trimester (25 ± 2 weeks)	9.5 ± 0.2a	4.3 ± 0.3a	133 ± 3c	63 ± 12b	140 ± 74b	4.27 ± 1.65b	166 ± 81b	6.1 ± 2.0b
3rd Trimester (35 ± 3 weeks)	9.4 ± 0.3a	4.2 ± 0.3a	143 ± 4c	91 ± 29c	226 ± 102c	6.27 ± 2.86c	234 ± 105c	7.2 ± 2.0c
Non-pregnant vs. 1st trimester	Ns.	Ns.	P < 0.0001	Ns.	Ns.	Ns.	Ns.	Ns.
Non-pregnant vs. 2nd trimester	Ns.	Ns.	P < 0.0001	P < 0.001	P < 0.001	P < 0.0001	P < 0.0001	P < 0.002
Non-pregnant vs. 3rd trimester	Ns.	Ns.	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.00001	P < 0.0001	P < 0.001
2nd vs. 3rd trimester	Ns.	Ns.	Ns.	P < 0.0001	P < 0.0001	0.00025	P < 0.006	P < 0.02

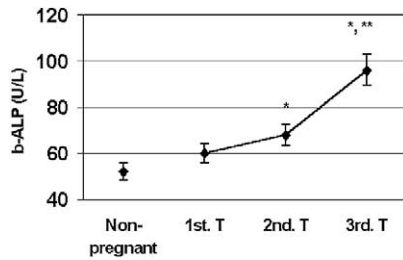[a,b,c] Indicates statistical significance.

NS.: not significant

Fig. 2. Bone alkaline phosphatase levels (U/liter). *$P < 0.0001$ compared to nonpregnant levels; **$P < 0.0001$ compared to the other trimesters of pregnancy.

(interquartile range: 327–755). Only 8 pregnant women had a calcium intake above the adequate intake (AI) (13) of 1000 mg/day and 16 had a Ca intake below 500 mg/day (Fig. 1). During the third trimester, and after explaining the beneficial effect of consuming dairy products, calcium intakes ($n = 39$) raised to 792 mg/day (interquartile range: 457–1021). However, 31 pregnant women still presented calcium intake values below 1000 mg/day, similar to those observed at the first recording (Fig. 1).

Serum and urinary calcium, serum phosphorus, and 1α,25-hydroxyvitamin D levels

Concentration of calcium and phosphorus were within normal range in both pregnant and nonpregnant control women, and no differences were observed among the studied trimesters of pregnancy (Table 1). Urinary calcium/Creat ratio increased during pregnancy. Although values remained within normal range, urinary calcium/Creat ratio was significantly higher in the second and third trimesters compared to the first trimester (0.116 ± 0.056 and 0.142 ± 0.013 vs 0.078 ± 0.039 mg/mg; $P < 0.004$ and $P < 0.001$, respectively). The increment between the second and third trimesters was not significant.

As expected, 1,25diHOD levels were significantly higher in the first trimester compared to nonpregnant values ($P < 0.0001$) and continued to increase significantly during the second and third trimesters of pregnancy ($P < 0.0001$) (Table 1). Although the differences between the last two trimesters did not reach statistical significance, these two levels were significantly higher compared to both nonpregnant controls and to the first trimester of pregnancy ($P < 0.0001$ and $P < 0.001$, respectively) (Table 1).

Biochemical markers of bone turnover

As expected, all the studied biochemical markers increased throughout pregnancy.

During the first trimester, serum b-ALP was not found to be significantly increased compared to nonpregnant control women (60 ± 12 vs 52 ± 13 U/L) (Table 1 and Fig. 2). However, b-ALP levels were significantly higher during the second and third trimesters compared to nonpregnant values

(63 ± 12 and 91 ± 29 vs 52 ± 13 U/L, respectively; $P < 0.0001$). Expressed as percentage of change regarding nonpregnant values, the increase in each trimester was 21 and 25% respectively. A significant increase in b-ALP was observed between the second and third trimesters ($P < 0.0001$) showing a percentage of change of 44% (Table 1 and Fig. 2).

Serum PICP levels did not increase significantly in the first trimester compared to nonpregnant values (118 ± 61 vs 97 ± 46 μg/l) (Table 1). However, a significant increase compared to nonpregnant values was observed during the second and third trimesters showing a percentage of change of 44% ($P < 0.001$) and 133% ($P < 0.0001$), respectively. The increment between the third trimester and second trimester was 62% ($P < 0.0001$) (Table 1).

The levels of the three studied bone resorption markers: serum βCTX and ICTP and urinary NTX/Creat ratio exhibited the same pattern of change, although the level of significance differed. During the first trimester, resorption marker levels increased compared to nonpregnant values, without reaching significance. In contrast, the increment was significantly higher compared to nonpregnant values during the second trimester. The highest increment was observed in the third trimester and it was significantly different from the level of the second trimester (Table 1 and Fig. 3).

It is noteworthy that in the present report the level of significance decreased as follows: βCTX > NTX > ICTP, showing that ICTP was the least sensitive resorption marker. The percentage of increase during the second trimester compared to nonpregnant values was βCTX 91% ($P < 0.0001$), NTX 69% ($P < 0.0001$), and ICTP 64% ($P < 0.02$) and the percentage of change observed between the third trimester and nonpregnant values was βCTX 181% ($P < 0.0001$), NTX 139% ($P < 0.0001$), and ICTP 94% ($P < 0.001$). Finally, the percentage of change between the second and third trimesters was βCTX 47% ($P < 0.00025$), NTX 40% ($P < 0.006$), and ICTP 18% ($P < 0.02$).

Taking into account that according to the present results, βCTX was the most sensitive bone resorption marker, the mean values for all the studied periods were plotted in Fig. 3.
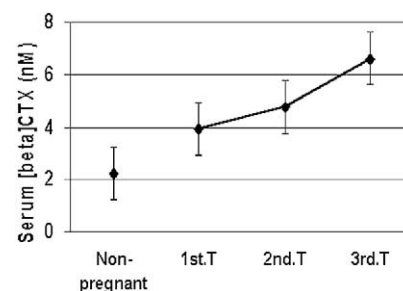

Fig. 3. Levels of βCTX (nM). *$P < 0.0001$ compared to nonpregnant control women; **$P < 0.00025$ compared to the other two trimesters of pregnancy.
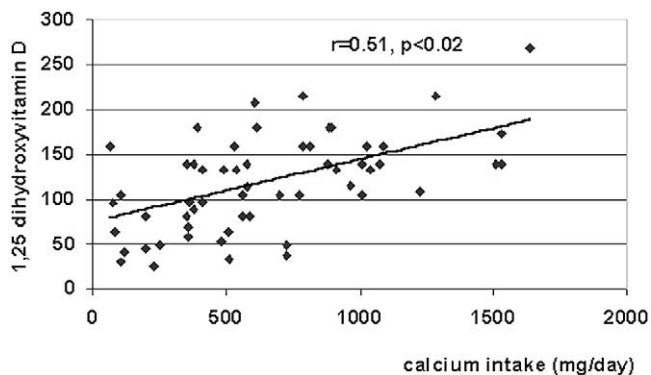
Fig. 4. Correlation between 1,25-dihydroxyvitamin D and calcium intake.

Correlation with calcium intake

For the pregnant women, in the first and third trimester (*n* = 59) levels of 1,25diHOD presented a positive and significant correlation with dietary calcium intake (*r* = 0.51, *P* < 0.02) (Fig. 4).

The absolute change in bone markers between the third and second trimesters of pregnancy versus dietary calcium intake at the end of pregnancy was evaluated in the 24 women presenting dietary calcium intake below AI at the first visit. The increment in serum βCTX correlated negatively with dietary calcium intake (*r* = −0.47, *P* < 0.03) (Fig. 5) as did NTX (*r* = −0.41, *P* < 0.05) and b-ALP (*r* = −0.43, *P* < 0.05). The other studied biochemical markers did not reach a significant correlation.

Discussion

Calcium demand is increased during pregnancy; however, the increment in calcium absorption rate could cover the increased requirements of the mother [14]. For this reason, there is widespread concern that pregnancy does not require extra calcium above the AI of 1000 mg Ca/day recommended for nonpregnant healthy women [13]. This amount is easily achieved by consuming dairy products. In the present study, differences among calcium intake of mothers, assessed by FFQ and FCDP, which estimates calcium intake over a defined period and in the recent past, were more than fourfold. Moreover, 80% of pregnant women had calcium intakes below AI. This finding was not unexpected because previous partial food intake records showed calcium intake of an elevated percentage of our population to be below IA, due to dietary habits common to all socioeconomic levels [15–16].

In agreement with other authors, the present results showed that calcium homeostasis is greatly affected by pregnancy. Indeed, intestinal calcium absorption, urinary calcium excretion, and bone activity are modified during pregnancy. Previous studies showed that enhanced calcium absorption, from 20–25% to about 50% during gestation, is observed in the mother [17]. This increment is probably the major adaptation of pregnant women in order to satisfy the normal mineralization of the growing fetus. The exact mechanism implied is still unclear, but may involve different pregnancy-associated hormones such as estrogen, prolactin, and placental lactogen, which can stimulate 1α-hydroxylase activity [5]. This enzyme increases the production rate of 1,25diHOD, which, in turn, stimulates calcium absorption from the gut. Indeed, changes in the absorption of calcium have been positively associated with 1,25-dyhidroxyvitamin D levels [18] and the increment observed in our healthy pregnant women, above those of the nonpregnant controls from the first trimester until term, suggests that calcium absorption is enhanced. Similarly, in another period of maximal requirement and retention of calcium as puber-
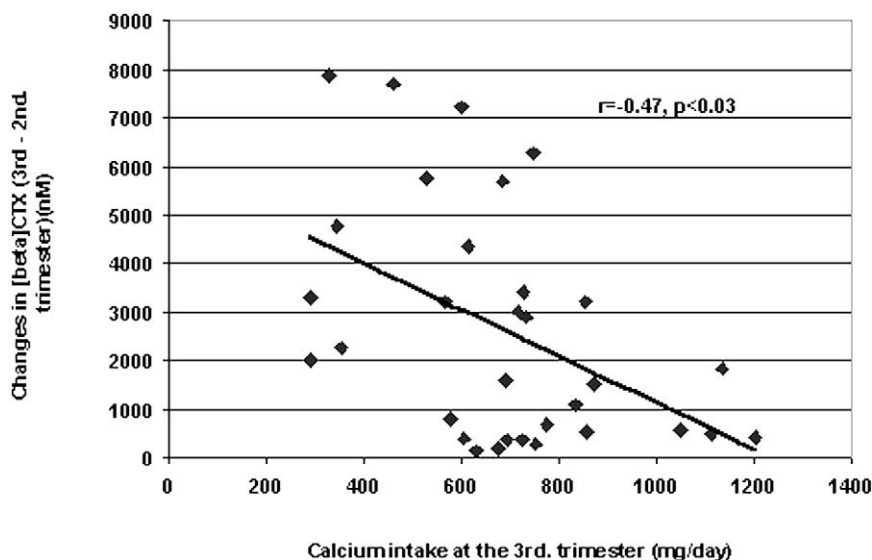

Fig. 5. Correlation between changes in βCTX levels (third minus second trimesters) versus calcium intake during the third trimester of pregnancy.

tal growth spurt also calcitriol levels are highest [19] and calcium absorption efficiency peaks correlating with bone mass accretion [20].

Heaney and Skillman [17] demonstrated an increase in calcium balance and calcium absorption and found both to rise progressively throughout pregnancy. In this regard, they showed that accretion increased steadily throughout pregnancy reaching a peak of more than twice usual nonpregnant levels during the last 10 weeks. In agreement, others showed that pregnancy results in increased intestinal absorption, and measurement of calcium balance during the later stage of pregnancy is generally positive [21], evidencing the existence of a mechanism to induce a positive balance to store calcium in advance for fetal growth and the subsequent production of breastmilk [22]. It is well known that balance and intestinal absorption of calcium is closely related to calcium intake and it was reported that some individuals can adapt to a prolonged low intake of calcium by increasing the efficiency of absorption [18]. However, the effect of different calcium intakes during pregnancy is uncertain because few studies have addressed this issue, and most were conducted in groups of subjects who exceeded current dietary recommendations. In this regard, the threshold behavior of calcium intake shows that bone accumulation, below a certain value of calcium intake, is a linear function of intake and, above this threshold, bone retention is limited and a plateau is achieved [23]. Under normal conditions, calcium absorption is closely related to the apparent retention of calcium because the amount of urinary calcium excretion account for a low percentage of calcium intake. Although urinary calcium excretion increased with the gestational age in the pregnant women included in this study, it remained within the normal range. Balance techniques were not applied in this study; however, the finding of increased calcitriol levels and calcium excretion within normal range indirectly suggests an increase in both calcium absorption and accretion. Based on these relations, it is likely that the behavior of calcitriol with regard to calcium intake would be similar to that of calcium retention. Therefore, a plateau should be reached at values above adequate calcium intake, but probably not observed in the present study because only six of the pregnant women had calcium intake values clearly above the adequate intake.

The amount of urinary calcium/Creat excretion increased during pregnancy, although it remained within normal range. The higher urinary calcium excretion during pregnancy [4,5] is probably secondary to an increment in glomerular filtration rate (GFR) and hyperabsorption of calcium [24]. Fasting calcium after correcting for creatinine excretion avoids the component of calcium absorption. However, even when dietary calcium is deficient, there is a relatively high calcium loss in the fasting urine probably caused by the raised GFR during pregnancy, which exceeds the reabsorptive capacity of the kidney [25].

Studies on changes in skeletal calcium content during pregnancy are limited due to concerns about fetal exposure to radiation, and they differ as to changes in bone mineral density (BMD). Indeed, BMD has been reported to increase, remain unchanged, and decrease during pregnancy [26] and other reports have suggested a redistribution of mineralization from trabecular to cortical bone [27]. Ritchie et al. concluded that pregnancy does not appear to negatively affect maternal bone among well-nourished women [26]. Possibly, confounding factors such as modification in body weight could influence changes in bone density and affect interpretation of data [28].

Nevertheless, it is agreed that bone turnover increases during pregnancy, suggesting marked bone activity [27,29]. Heaney and Skillman [17] were the first researchers to show the anticipatory change in maternal bone remodeling by using gold standard methods as the stable isotope of calcium, Ca^{48}. They showed that resorption pattern was similar to that of changes in accretion but was initially depressed and only rose significantly in the 30th–34th weeks of pregnancy. Similarly, in the studied population of healthy pregnant women, significant changes in bone turnover markers were observed during pregnancy compared to nonpregnant values: they rose without reaching significance in the first trimester and continued to increase throughout pregnancy. This pattern is foreseeable given the slight maternal-fetal calcium transfer in early pregnancy compared with the peak rate that takes place in the third trimester. The mean percentage of change in bone formation and in resorption did not differ greatly between the first and third trimesters and ranged between 152 and 192% for markers of bone formation (b-ALP and PICP, respectively) and 183, 181, and 153% for bone resorption markers (βCTX, NTX, and ICTP, respectively). One might expect differences between bone formation and resorption markers; however, these two processes are normally coupled and occur in multiple bone remodeling units. Moreover, the different specificity, peripheral metabolism, and stage of bone cell differentiation may render interpretation of data on bone markers difficult. However, considered together they reflect an increase in bone activity during pregnancy.

Although response of the two studied bone formation markers was similar, percentage of change in PICP was greater than b-ALP. This could be explained by the fact that they reflect different stages of osteoblast function that may be stimulated differently by hormones or certain local factors released during pregnancy [27]. Besides, the three studied bone resorption markers, which are indicators of organic matrix breakdown, also presented different amplitude of change. A similar response was observed in serum βCTX and NTX, and both were higher than ICTP. According to previous studies, the increment in serum βCTX indicates an increase in bone resorption in the mother that is independent of the growing fetal skeleton because of the small amount of βCTX from the fetus [27].

During pregnancy, changes in hormone secretion, several growth factors, and cytokines occur physiologically and may influence bone turnover. Among them, there is evi-

dence of increased PTH-related protein (PTH-rp) production during pregnancy [30]. This hormone plays an important role in placental calcium transport, fetal growth, and development, as well as in milk production. During pregnancy, however, results regarding PTHrp levels appear to be controversial because of the immunoassay employed (N-terminal, C-terminal, midmolecule region-IRMA, or RIA methods). PTHrp determination by RIA (PTHrp1-86, DSL) was performed in a small group of subjects in the present study. A weak increasing trend was observed during pregnancy (data not shown), suggesting the existence of a possible mechanism that may be responsible for the increment in bone turnover.

Nutritional status prior or during pregnancy may influence maternal skeletal response; however, there are few studies on the impact of calcium intake on bone mineral changes. Some studies concluded that calcium intake had no influence on changes in bone mineral in pregnant women [31,32], whereas others concluded it did [33]. In this regard, it must be kept in mind that the maximum transfer of calcium from the mother to the fetus takes place during the third trimester of pregnancy. At this moment, the maternal skeleton undergoes great stress. If maternal dietary supply to meet the fetal demands for calcium is inadequate, calcium release from the maternal skeleton may become an important mechanism to meet fetal growth. A number of the studied pregnant women improved their calcium intake after detailed information on the benefits of dairy consumption; however, 31 continued with a calcium intake lower than the AI in the third trimester. Although BMD was not measured in the present study, the influence of maternal calcium intake on bone activity was studied by evaluating the changes in biochemical markers of bone turnover between the third and second trimesters of pregnancy versus the estimated maternal calcium intake during this period of pregnancy. The levels of βCTX, NTX, and b-ALP correlated negatively with calcium intake, suggesting greater bone turnover at low intakes. Indeed, even though the studied population was small, our results evidenced that βCTX levels of women with calcium intake above the AI (1000 mg/day) increased twofold in the third trimester compared to the second trimester. Serum βCTX increased more than twofold in a large percentage of women whose calcium intake was close to 50% of AI (500 mg/day) and was found to be as much as eight times higher in the third trimester compared to the second trimester in pregnant women with very low calcium intake. Similarly, b-ALP levels increased up to three times in pregnant women with a very low calcium intake. These findings may suggest that skeletal response to pregnancy might not be entirely independent of maternal calcium intake, especially in women with customarily low calcium intake. To what extent this increment in bone turnover could affect bone mass status is, as yet, unknown and needs further detailed investigations. However, these results suggest some evidence that mothers with a customarily low calcium intake appear to have a higher

activity of bone and may benefit from higher calcium intake during pregnancy.

In summary, further longitudinal studies with a larger number of subjects are necessary to define the impact of calcium intake on bone remodeling during pregnancy. Nevertheless, not only hormonal changes in calcium metabolism that occur during pregnancy but also other considerations, such as low dietary calcium intake, could lead to an increment in the biological activity of the skeleton. Additional studies must be conducted to confirm our findings and to gain a better understanding of skeletal response to a low calcium intake during pregnancy.

Acknowledgments

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