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ORIGINAL ARTICLE

Does Supplementation with Calcium During Pregnancy Affect the Mineral Concentration in Mature Breast-Milk?

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Although the relationship between calcium intake during lactation and breast milk calcium concentration has been studied by many investigators, evidence available on the effects of calcium supplementation during pregnancy on the calcium content of breast-milk is limited. The aim of this double-blind placebo-controlled trial was to determine the effect of calcium supplementation during the 3rd trimester of pregnancy on the calcium concentration of mature breast-milk.

Materials and Methods: Sixty-eight pregnant women in Ahwaz city, southwest Iran were randomly assigned to the "Calcium" (1 gr/d) or "Placebo" groups from the 28th-30th week of gestation until delivery. Breast-milk samples were taken 1.5 to 3 months after delivery. Calcium concentrations were measured using atomic absorption spectrometry. There were no significant differences between the two groups with respect to the demographic characteristics, anthropometric indices, and dietary energy and calcium intakes, at baseline.

Results: No differences were found in calcium concentrations in mature breast-milk between the two groups [mean±SD were 228±38 and 235±42 mg/L in the calcium and placebo groups, respectively, (P=0.49)].

Conclusion: Our study did not support the hypothesis that calcium supplementation during pregnancy affects the calcium concentration later in mature breast-milk.

Key Words: Calcium, Pregnancy, Breast-milk, Calcium supplement

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Introduction

Calcium requirements for skeletal mineralization in early life are supplied by the mother across the placenta during fetal life and through breast milk during infancy.[1] Both pregnancy and lactation place significant stress on maternal calcium homeostasis, potentially resulting in substantial changes in bone mineral content[2]. At birth,

an infant's body contains 20-30 g calcium, most of which is deposited during the second half of pregnancy.[1,3]

The total intake of calcium in an exclusively breastfed infant depends on breast milk composition; it is therefore important to identify those determinants which may affect calcium concentration in the mother's milk.[4] Many investigators have examined either the effects of calcium intake during lactation on the mineral concentration in breast-milk,[5-7] or maternal calcium intake on the pregnancy outcome;[8-12] however results from studies focusing on the effects of calcium intake during pregnancy on later breast-milk calcium are insufficient. This possible association was proposed for the first time by Prentice et al.,[4] who noticed a difference in breast-milk calcium concentration between Gambian and Cambridge lactating mothers. In the early 80s, a supplementation trial with a high-calcium food supplement among Gambian pregnant women in a village showed a significant increase in breast-milk calcium compared to the adjacent control villages. The effect disappeared after replacement of the high calcium food supplement with a local low-calcium food supplement. Ortega et al. also suggested the same association in their observational study on Spanish mothers[13] in which they assessed daily calcium intake in a group of women during the third trimester of pregnancy and followed them up to 40 days after delivery. Mean breast-milk calcium concentration in mothers who had consumed $\geq$ 1100 mg/d calcium was significantly higher than those who consumed <1100 mg/d (273 versus 239 mg/L).

Not enough literature on the possible effects of calcium supplementation during pregnancy on calcium content of breast milk is available. To our knowledge, the only published controlled trial so far is that of Jarjou et al. on Gambian women accustomed to a very low dietary calcium intake ($\approx$350 mg/d),[1] justifying further investigations in different populations. The present double blind placebo-controlled trial was therefore, designed to determine the effects of maternal calcium supplementation during the third trimester of pregnancy on mature breast-milk calcium concentration 1.5 to 3 months after delivery in healthy mothers from Ahwaz city, south west of Iran.

Materials and Methods

A total of seventy-seven urban pregnant women before the 28th week of gestation, attending the two selected "maternal care clinics" of Ahwaz, were invited to participate in this double-blind randomized placebo controlled trial. Inclusion criteria were age 18-35 years, parity $\leq$ 2, singleton pregnancy, lack of any chronic or metabolic diseases, taking no nutrient supplements except iron and folate, and intention to breast-feed. Between the 28th and the 30th weeks of gestation, pregnant women were randomly divided into two groups, one receiving a "calcium supplement" (1000 mg calcium/d in the form of calcium carbonate capsules, Darou Pakhsh Co., Tehran) and the other "Placebo" (lactose, same company). Supplementation continued up to time of delivery. Participants were assigned randomly by numbers. The code numbers were held by a member of the study team (AD) who had no direct contact with participants, nor was he involved in field or laboratory work. Flow chart of recruitment is shown in Fig. 1. Subjects were advised to take the capsules with a meal when iron and folate tablet was not taken. The study team leader, principal investigators, health staff and participants were blinded to the interventions.

At the beginning of the study, anthropometric indices were measured. Demographic data were collected using a questionnaire. Weight and height were measured to the nearest 100 g (Detecto Scales Inc., USA) and 0.1cm (fixed tape), respectively according to the WHO protocols.[14] Body mass index (BMI) was calculated using the equation Wt (kg) / Ht $(m)^2$.

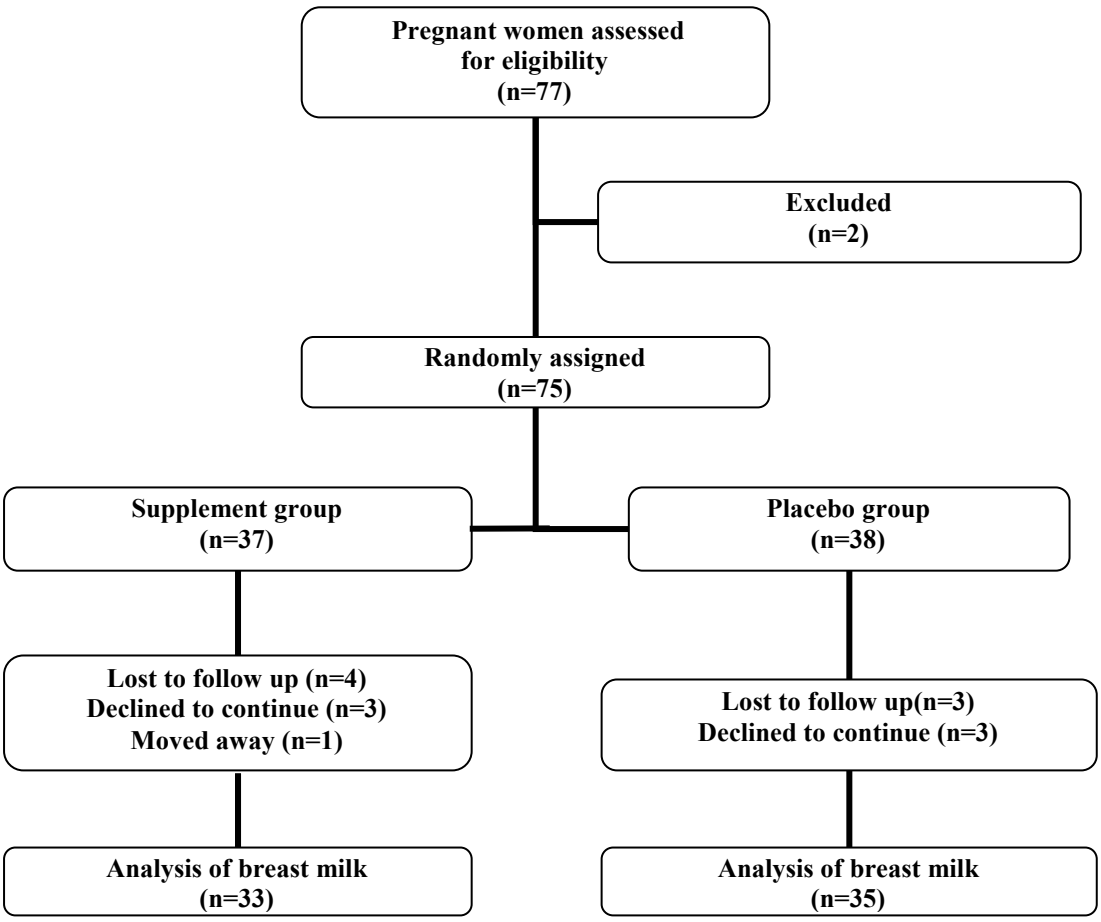

Fig. 1. Flow chart of recruitment for the randomized, controlled trial of calcium supplementation in pregnant Iranian women.

Pre-pregnancy weights were self-reported by participants. Adherence to the study protocol was repeatedly emphasized for all women several times, and compliance determined for each by dividing the number of capsules taken by the total number of assigned capsules.

Mothers were regularly contacted after delivery and asked to visit the clinics 1.5 to 3 months postpartum for giving milk samples; due to the standard national vaccination program, they were required to refer to the health centre 45 days after delivery. Thus, almost all breast milk samples were taken on that day before vaccination. About 10 ml of fore milk was collected manually from both breasts between 08:00-10:00 and analyzed separately. Values presented here are the mean of calcium concentrations of both breasts. Sampled milks were stored at –20°C, until further analysis.

Milk sample preparation for ashing and acid hydrolysis was done according to the method suggested by Laskey et al.[15] Breast-

milk calcium was measured using atomic absorption spectrometry (Unicam 919, England) at 422.7nm. Mean recovery and CV of intra-assay were 101% and 3%, respectively.

Random samples of calcium and placebo capsules (21 each) were analyzed by atomic absorption to determine the amount of calcium. Means of calcium content were 485 (450 to 533) and <1 mg in calcium and placebo capsules, respectively. Dietary calcium intake was estimated using a Food Frequency Questionnaire (FFQ) containing 43 food and beverage items. The FFQ had been used before in pregnant mothers in the same population[16] and in a large study in 40-60 year old women in Tehran, Iran[17]. The FFQ has been published[16] and copies are available from the author (MK). The FFQs were filled at the beginning of the study as well as at the time of milk sampling. Energy intake during the first four weeks of study was calculated using a three-day 24-hour dietary recall method (2 non-consecutive days plus 1 weekend). Iranian food composition table was used to calculate dietary energy and calcium intakes.[18]

Data were analyzed using SPSS software (version 9.00). Chi-square and t-tests were employed, when applicable, for statistical analyses. Differences were considered as "significant" whenever P values were <0.05.

Informed written consents were taken from all participants. The protocol was approved by "The Research Committee" and "The Ethics Committee", Tehran University of Medical Sciences and Health Services, Tehran.

Results

Subjects' characteristics at 28-30th week of pregnancy for the 68 women who completed the study are shown in Table 1.

Mean±SD of age and pre-pregnancy weight were 25.8±5.5 years and 61.3±11.4 kg, respectively. Participants were mostly housewives (94 and 89% in calcium and placebo groups, respectively). All of the infants were being exclusively breastfed at the time of milk sampling. Subjects were not cigarette smokers. There were no significant differences between the study groups regarding possible effective factors on human milk composition including age, mother's weight, and parity.[19]

Table 1. Subjects' characteristics at 28-30th wk of pregnancy*

	Calcium supplement (n= 33)	Placebo (n=35)
Age (yr) †	26.2±5.8†	25.4±5.3
Pre-pregnancy weight (kg)	60.2±10.9	62.3±11.9
Height (cm)	163±6	162±6
Pre-pregnancy BMI (kg/m2)	22.6±3.9	23.7±3.8
Pregnancy weight gain (kg)	12.8±3.8	11.7±3.1
Parity [n (%)]:		
Nulliparity	18 (55)	18 (52)
Primiparity	10 (30)	12 (34)
Parity 2	5 (15)	5 (14)
Education [n (%)]:		
Illiterate	5 (15)	2 (6)
Elementary	7 (21)	8 (23)
Secondary/High school	20 (61)	22 (63)
Academic	1 (3)	3 (8)

* No significant differences between the intervention groups; † Mean±SD (all such variables)

Dietary calcium intake during pregnancy and lactation, energy intake during pregnancy, and adherence to protocol are shown in Table 2.

In both groups, during pregnancy and lactation, mean of the percentage of calcium provided by dairy products was about 48%, without any significant difference between the groups. Moreover, dietary calcium intakes were similar during pregnancy and lactation in both groups. In the calcium and placebo groups, 72.7 and 77.1% had compliance rates of >80%, respectively. Mean±SD of total daily calcium intake was 1382±178 mg

in the calcium supplement group, based on calculated compliance rates. Calcium supplementation increased the daily intake 2.7 fold. None of the subjects continued to take calcium supplement after delivery.

Table 2. Mean±SD of daily dietary energy (Mj) and calcium (mg) intakes among pregnant and lactating women, and protocol adherence in Calcium and Placebo groups*

	Calcium supplement (n=33)	Placebo (n=35)
Dietary intake in pregnancy:		
Energy (Mj/d)	7.4±1.5	7.7±0.9
Calcium (mg/d)†	539±172	579±176
Dietary intake in lactation:		
Calcium (mg/d)	537±153	561±149
Adherence to the protocol (%)‡	84.4±5.9	83.7±5.6

* No significant differences between the intervention groups; † The amount of calcium from supplements is not included; ‡ Determined by dividing the number of capsules taken to the total number of assigned capsules.

Calcium concentration for each woman was calculated as the mean of calcium concentration in the two breast samples. Mean±SD of calcium concentration in mature breast milk were 228±38 and 235±42 mg/L in calcium and placebo groups, respectively (p=0.49).

Discussion

Results of the present trial showed that daily consumption of 1g calcium supplement during the third trimester of pregnancy did not affect calcium concentration of mature breast milk after delivery among Ahwazi mothers.

To estimate dietary calcium intake, we employed FFQ, which assesses calcium more accurately than other nutrients.[20] Dietary calcium intake during pregnancy was almost half of the "Adequate Intake (AI)", i.e. 1000 mg.[21] This figure is close to the intakes reported by other investigators, especially in developing countries;[22-24] however, it was less than the amount reported among women living in the Karaj and Shemiran districts, closer to Tehran (627±248 mg/d).[25] The reported calcium intakes among pregnant mothers in developed countries[8,26] are higher than the amount estimated in this study. Calcium intakes of pregnant women in India[27] and Gambia[6] were lower than the intakes in our study.

The majority of supplementation trials with calcium during pregnancy have been carried out in pregnant women at an increased risk of hypertension/pre-eclampcia, using 2 g calcium supplement daily;[26,28] however in this study, we asked healthy pregnant women to take a moderate amount of calcium as a supplement in order to prevent a total calcium intake above 2.5 g, which has been considered as "Tolerable Upper Intake Level (UL)" during pregnancy[21]. Generally, calcium supplementation trials during pregnancy fall into two categories; in the first category, pharmacological and preventive effects of calcium supplements are assessed in pregnant women whose calcium intakes have already been adequate, while in the second, the effects of calcium repletion in those pregnant women who have less than adequate intakes are investigated.[29] The present study falls into the latter group. No complaint or side effect was reported by the participants, which might be because of the lower dose of calcium supplement taken with a meal (i.e. 1 g/d), as compared to other studies (mostly 2g/d). Jarjou *et al*. also reported that the supplement of 1500 mg/d calcium in Gambian pregnant women was well accepted and no adverse effects were noted.[1] Dietary calcium intake during lactation was not significantly different between the two groups. Moreover, we found that dietary calcium intakes during pregnancy and lactation were similar in both groups, suggesting

a dietary consistency in terms of calcium content over the whole study period. Such an assessment, not reported in similar studies,[6,13,30-31] removes any doubt about the possibility of dietary changes over time (i.e. from pregnancy to lactation), which obviously could confound the results.

A wide range of calcium concentration in breast milk has been reported by different investigators[32-34]. A review of literature available shows calcium concentrations of 84 to 462 mg/L, with the median of 252 mg/L in mature breast milk.[32] Calcium concentration in mature breast milk in this study (≈ 230mg/L) was close to that median. The most important finding of the present study was that daily supplementation with 1g calcium during the third trimester of pregnancy did not affect calcium concentration in mature breast-milk. As mentioned earlier, limited literature regarding this relationship is available. Prentice et al. proposed that there was an association between calcium status during pregnancy and later milk calcium content during lactation,[4] a suggestion based on few observational studies. Ortega *et al.* also suggested the same relation between calcium intake in the third trimester of pregnancy and calcium concentration later in breast milk[13]. They categorized the participants (57 women during their third trimester of pregnancy) into two groups according to dietary calcium intakes (< or ≥ 1100 mg/d). The cut-off point was approximately 92% of RDA at the time of the study. Findings of this study did not confirm the hypothesis. The biologic basis of such categorization has not been explained clearly by the authors. It seems that the cut-off point had been selected arbitrarily, and is therefore seriously prone to bias. In a few papers which have been published by analyzing the same dataset (measurement of dietary intakes of 57 pregnant women) different cut-off points have been selected for evaluating the relationship between nutrient intakes during the third trimester of pregnancy and the nutrients concentrations in breast milk obtained on the 40th day postpartum. In the papers, different cut-off points have been used for categorizing the participants and significant statistical associations were reported in all of them.[13, 35-39]

Our finding that increasing calcium intakes during pregnancy do not affect breast milk calcium concentration in the subsequent lactation is similar to the results of a study in Gambian women accustomed to a very low dietary calcium intake,[1] which to our knowledge is the only published randomized controlled trial so far, documented on this subject; These results are similar to the results of calcium supplementation trials during lactation period.[6,40] Therefore, it can be suggested that breast milk calcium concentrations are mainly determined by factors that are not related to maternal calcium intakes during pregnancy and lactation. Parity >2 was an exclusion criterion in our study, but in the study of Jarjou et al. women with parity up to 10 were among the participants.[1] Although there is some evidence that parity does not affect calcium concentrations in breast milk, it should be noted that the sample sizes of such studies were small.[41] Larger studies with adequate numbers of participants in different categories of parity are required.

In summary, it is concluded that a 1 g/d calcium supplement during the third trimester of pregnancy does not affect calcium concentration of mature breast milk.

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