Same nutrient, different hypotheses: disparities in trials of calcium supplementation during pregnancy[1–3]

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ABSTRACT Calcium supplementation during pregnancy has been provided either to increase the intake in those with a deficiency or to obtain a pharmacologic, perhaps nonnutritional, effect in individuals with an adequate calcium intake. A systematic review, including only randomized, double-blind, controlled trials of calcium supplementation during pregnancy was prepared independently for the *Cochrane Library* and updated by us for this paper. In view of the heterogeneity of results included in the meta-analysis, a stratified analysis by baseline dietary calcium intake (mean calcium intake in the population < or ≥900 mg/d) was conducted. On the basis of the results of the 5 randomized, controlled trials available, the risk of high blood pressure was lower in women with low baseline dietary calcium [typical relative risk (TRR): 0.49; 95% CI: 0.38, 0.62]. Of the 4 trials in which subjects had adequate dietary calcium, the TRR of high blood pressure was 0.90 (95% CI: 0.81, 0.99). The risk of preeclampsia was considerably reduced in the 6 trials conducted in populations with low-calcium diets (TRR: 0.32; 95% CI: 0.21, 0.49) but was not reduced as much in women enrolled in the 4 trials with adequate-calcium diets (TRR: 0.86; 95% CI: 0.71, 1.05). On the basis of these results, it seems clear that calcium supplementation during pregnancy for women with deficient calcium intake is a promising preventive strategy for preeclampsia. Calcium supplementation in pregnancy should be evaluated definitively in an adequately sized trial conducted in a population with a low calcium intake because this is the most likely population to benefit from such a nutritional intervention. Long-term health benefits for the offspring are also an attractive possibility. *Am J Clin Nutr* 2000;71(suppl):1375S–9S.

KEY WORDS Calcium supplementation, pregnancy, preeclampsia, high blood pressure, meta-analysis

INTRODUCTION

Our previous work on calcium supplementation during pregnancy is often discussed in the context of the results of a large, methodologically sound trial that was conducted by the National Institutes of Health (NIH) and published in the *New England Journal of Medicine* (1) and has formed the basis for systematic reviews (2, 3). We would like to offer a few important clarifications concerning the interpretation of these findings. Nutrients (as supplements to food) are provided to populations to either increase intakes in those with a deficiency (to prevent or treat functional outcomes related to such a deficit) or to obtain a pharmacologic, perhaps nonnutritional, effect in individuals with an adequate intake of the nutrient in question. Most of our work on calcium supplementation addressed the former, the large *New England Journal* trial the latter.

In the original formal description of calcium–blood pressure hypothesis in 1980 (4), we specifically referred to "the causal association between calcium deficit" and hypertensive disorders of pregnancy and the "causal role of calcium deficiency in the occurrence" of hypertensive diseases of pregnancy. We later concluded that the importance of our observation was that increasing calcium intake in populations with a deficit may reduce the incidence of preeclampsia. We postulated in 1988 a mechanism of action in which "populations with a lower calcium intake than required during pregnancy have an increase in serum parathyroid hormone level" and recommended the implementation of "a large, randomized controlled trial" in a "high risk group of primiparous young women" (5).

Disparities between the 2 largest trials

We conducted the first large trial (6) aimed at reducing the rate of pregnancy-induced hypertension and preeclampsia: 1167 women with a mean baseline calcium intake of 650 mg/d (approximately half of the recommended dietary allowance) were randomly assigned to receive either a supplement providing 2000 mg Ca/d or a placebo. Women took 86% of the supplement on average, increased their urinary excretion of calcium, and had a reduced risk of pregnancy-induced hypertension and preeclampsia although, in the case of the latter, the 95% CI of the odds ratios included unity. In contrast, the trial conducted by the NIH studied the effect of calcium supplementation (2000 mg/d)

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TABLE 1
Indicators of methodologic quality of trials included in the systematic review of calcium supplementation during pregnancy[1]

Reference and year	Allocation sequences	Exclusions after randomization	Concealment of allocation schedule	Double-blinding
		%		
Levine et al (1), 1997	Computer generated	Calcium group: 5.8 Placebo group: 5.3	Yes	Yes[2]
Belizán et al (6), 1991	Computer generated	Calcium group: 2.4 Placebo group: 2.2	Yes	Yes
Purwar et al (9), 1996	Computer generated	Calcium group: 5.8 Placebo group: 5.1	Yes	Yes
Lopez-Jaramillo et al (13), 1997	Random number table	Calcium group: 6.7 Placebo group: 3.6	Yes	Yes
Sanchez-Ramos (14), 1994	Computer generated	Calcium group: 12.1 Placebo group: 0	Yes	Yes
Lopez-Jaramillo et al (15), 1990	Unclear	Large, unexplained discrepancies between groups	Unclear	Yes
Villar and Repke (16), 1990	Computer generated	Calcium group: 5.2 Placebo group: 7.3	Yes	Yes
Lopez-Jaramillo et al (17), 1989	Random number table	Calcium group: 10.9 Placebo group: 15.6	Unclear	Yes
Villar et al (18), 1987	Computer generated	All 52 women randomly assigned were included in the analysis	Yes	Yes
Crowther et al (19), 1999	Computer generated	All 456 women randomly assigned had data on primary outcomes	Yes	Yes

[1] Reference 12.

[2] Tablets could be identified.

in 4336 women without a calcium deficiency (mean baseline intake: 1130 mg/d) to achieve a pharmacologic, preventive effect rather than to correct a nutritional deficit (1). Women took an average of 64% of the supplement and only 20% of them used >90% of the medication (1). Treatment compliance is an issue to be considered in the interpretation of preeclampsia prevention trials (7), particularly in women with a low baseline calcium intake.

Unfortunately, although the treatment tablets used in the NIH trial appeared similar when compared individually, there was a noticeable difference in the intensity of the coloration of the formulation when several tablets were viewed in aggregate (8). To remedy this situation, the researchers packaged the tablets individually in opaque blister packs. Despite the efforts to solve the problem, the possibility of bias exists.

Even in this population with an adequate calcium intake, calcium supplementation reduced the overall relative risk (RR) of hypertensive disorders (RR: 0.90; 95% CI: 0.81, 1.00), pregnancy-induced hypertension (RR: 0.88; 95% CI: 0.78, 1.01), and severe preeclampsia (RR: 0.85; 95% CI: 0.58, 1.23) (1). Interestingly, subgroup analysis [from Figure 1 of reference 1] of the 885 women with a baseline calcium intake between 582 and 846 mg showed a protective effect of calcium supplementation on preeclampsia (RR: 0.70; 95% CI: 0.43, 1.15) similar to the results of our trial (RR: 0.65; 95% CI: 0.35, 1.25) (6). Among the 946 women with high treatment compliance (as in our 1991 trial; 6) the RR of preeclampsia was 0.76 (95% CI: 0.47, 1.22). These RRs are not statistically significant, probably because of a smaller number of events in the subgroup analysis. Because actual numbers were not provided, it was not possible to evaluate further these stratified data (1). A reassuring finding is that, even in a population with such a high total calcium intake (≈3 g/d), there was no significant difference between groups in the rate of urolithiasis during pregnancy or neonatal hypocalcemia (1).

SYSTEMATIC REVIEW

We think that the task at hand is neither to incorporate mechanically the 2 new trials into previous meta-analyses (2, 3), because they were conducted in populations with high (1) or very low (9) calcium intakes and because of the heterogeneity of their results, nor to denigrate the properties or results of the meta-analyses (1). An updated systematic review of calcium supplementation should be undertaken to identify and understand the sources of disparities among trials (subgroup analyses), describing patterns of treatment effect (10, 11).

Fortunately, such an updated, independently conducted systematic review was prepared and further updated in 1999 for the *Cochrane Library* (12). The *Cochrane Library* is an electronic publication, updated quarterly, of systematic reviews of effectiveness of health care interventions. As of 1999, it contained ≈600 systematic reviews of randomized controlled trials and a register of 220 000 clinical trials, (12). The systematic review of calcium supplementation during pregnancy includes all available trials during 1998 with random allocation in which supplements providing ≥1 g Ca/d were given (most trials included in the review provided 2 g Ca/d). The 9 studies included (1, 6, 9, 13–18) were double-blind, placebo-controlled trials and the methodology was generally sound in terms of allocation sequence method, rate of exclusions after randomization, concealment of allocation schedule, and double-blinding (**Table 1**). Main outcomes were rates of high blood pressure (with and without proteinuria) and preeclampsia.

Inspection of the funnel plots (the plot of the RRs from individual trials against their sample size) and the results of the individual trials in the new calcium systematic review shows asymmetry of the plot and heterogeneity of results. As was shown previously, asymmetry in funnel plots is a predictor of the lack of agreement between several small trials and the largest trial (11). Although the most common factor associated with asymmetric

TABLE 2
Effect of routine calcium supplementation during pregnancy on relative risk (RR) of high blood pressure

Subgroup	Calcium-supplemented subjects[1]	Control subjects	Typical RR (95% CI)
Low-risk (n = 6 trials)	611/3146	732/3161	0.84 (0.76, 0.92)
High-risk[2] (n = 3 trials)	15/141	54/156	0.35 (0.21, 0.57)
Adequate-calcium diet (≥900 mg/d) (n = 4 trials)	547/2505	614/2517	0.90 (0.81, 0.99)
Low-calcium diet (<900 mg/d) (n = 5 trials)	79/782	172/800	0.49 (0.38, 0.62)

[1] Eg, low-risk, subjects with high blood pressure per total calcium-supplemented subjects.
[2] Those at high risk of hypertensive disorders of pregnancy were selected by the trial authors because they were teenagers, had had preeclampsia previously, had increased sensitivity to angiotension II, or had preexisting hypertension.

funnel plots is "publication bias," this is unlikely to play a large role in this calcium review. The subject has been studied extensively in previous meta-analyses and most researchers working on the topic have been contacted and have offered additional data.

We should therefore explore other factors (eg, population characteristics and treatment compliance) that may be associated with differences in results. The meta-analysis should not focus on a "typical" RR for all trials when heterogeneity is detected even when using statistical strategies such as a random effects model. The discrepancy in this meta-analysis between the point and CIs estimation from the fixed and random effects model (20) further emphasizes the need to search for sources of discrepancy among trials (21).

Stratified analyses were therefore conducted on 2 prespecified subgroups determined by selection criteria used in the individual calcium supplementation trials: baseline dietary calcium intake (mean calcium intake in the population ≥ or <900 mg/d) and the risk of hypertensive disorders of pregnancy (high or low). There were 6 trials with populations classified as having "low calcium intake," all with a mean calcium intake of <650 mg/d ($\bar{x}$: ≈400 mg/d). The risk of high blood pressure was reduced among supplemented women with low baseline dietary calcium [typical RR (TRR): 0.49; 95% CI: 0.38, 0.62]. Among those with adequate dietary calcium, the risk of high blood pressure was 0.89 (95% CI: 0.81, 0.99). The risk of preeclampsia was considerably reduced in trials conducted in populations with low calcium intake (TRR: 0.32; 95% CI: 0.21, 0.49) but was not modified in women with adequate dietary calcium intakes (TRR: 0.92; 95% CI: 0.75, 1.13). Among women at high risk of hypertension, calcium supplementation reduced the risk of high blood pressure (TRR: 0.35; 95% CI: 0.21, 0.57) and preeclampsia (TRR: 0.22; 95% CI: 0.11, 0.43). No such substantial protective effect was seen in women with low risk of hypertension.

We conducted a sensitivity analysis excluding 1 trial (15) with limitations in 3 of the 4 selected methodologic indicators in Table 1. There was a very small reduction in the magnitude of the protective effect of calcium supplementation. In the group with low calcium intake, the TRR of preeclampsia was 0.34 (95% CI: 0.22, 0.52) and that of high blood pressure was 0.52 (95% CI: 0.41, 0.68); in the group with high risk of hypertension, the risk of preeclampsia was 0.24 (95% CI: 0.12, 0.48) and that of high blood pressure was 0.45 (95% CI: 0.26, 0.78). These results do not make any substantive change in the conclusions of the review. There was a reduction in the risk of preterm delivery in women at high risk of hypertension (TRR: 0.42; 95% CI: 0.23, 0.78) but there was no effect in any other subgroup.

Since the most recent update of this systematic review (10 April 1998), the results of 2 new randomized controlled trials have been published (19, 22). The first was a randomized, double-blind, placebo-controlled trial conducted in Colombia in 86 women at high risk of preeclampsia who were randomly assigned to receive 450 mg linoleic acid and 600 mg Ca/d (n = 43) or placebo (22). This trial is unlikely to be eligible for a new calcium supplementation systematic review update because there were 2 concomitant nutritional interventions and because the calcium dose was below the minimum required by the review (1 g Ca/d). In this new trial, the incidence of preeclampsia was lower in the calcium group (9.3%) than in the placebo group (37.2%) (TRR: 0.25; 95% CI: 0.09, 0.69) and the mean gestational age at birth was higher in the calcium group (39.3 ± 1.4 wk) than in the placebo group (38.2 ± 2.3 wk; P = 0.03) (22).

In the second new trial, conducted in Australia (19), 456 nulliparous women were enrolled in a randomized, controlled, double-blind trial that assessed the effect of a supplement of 1.8 g Ca compared with that of an oral placebo. When the funds for the study were exhausted, recruitment to the trial was stopped by the steering group without knowledge of the study outcome—456 women had been randomly assigned (227 were in the calcium and 229 in the placebo group). In the enrolled women, the treatment with calcium reduced the risk of preeclampsia (RR: 0.44; 95% CI: 0.21, 0.90) and the risk of preterm birth (RR: 0.44; 95% CI: 0.21, 0.90). The rate of severe preeclampsia was also lower in the calcium group (1.8%) than in the placebo group (2.6%) (RR: 0.67, 95% CI: 0.19, 2.35), but pregnancy-induced hypertension was not different between the groups (RR: 0.90; 95% CI:

TABLE 3
Effect of routine calcium supplementation during pregnancy on relative risk (RR) of preeclampsia

Subgroup	Calcium-supplemented subjects[1]	Control subjects	Typical RR (95% CI)
Low-risk (n = 6 trials)	188/3146	240/3161	0.79 (0.65, 0.94)
High-risk[2] (n = 4 trials)	8/266	47/291	0.22 (0.11, 0.43)
Adequate-calcium diet (≥900 mg/d) (n = 4 trials)	169/2505	174/2288	0.86 (0.71, 1.05)
Low-calcium diet (<900 mg/d) (n = 6 trials)	27/907	90/935	0.32 (0.21, 0.49)

[1] Eg, low-risk, subjects with preeclampsia per total calcium-supplemented subjects.
[2] Those at high risk of hypertensive disorders of pregnancy were selected by the trial authors because they were teenagers, had had preeclampsia previously, had increased sensitivity to angiotension II, or had preexisting hypertension.

0.59, 1.38). Approximately 30% of women in both groups stopped taking their trial medication during the antenatal period. At trial entry, only 30% of women had calcium intakes <800 mg/d (19), with mean daily calcium intakes similar to those of the NIH trial. The latest trial is eligible for inclusion in the systematic review in the stratum of populations with adequate baseline calcium intake and low risk for hypertension.

Results of the updated meta-analysis after inclusion of the Australian trial in the corresponding strata are presented in **Tables 2** and **3**. There are no changes in the results presented above for the low calcium intake and high-risk strata with the inclusion of the Australian data. Low-risk women supplemented with calcium had a lower RR (0.79; 95% CI: 0.65, 0.94) of developing preeclampsia. For women with adequate calcium intake, the RR of preeclampsia was 0.86 (95% CI: 0.71, 1.05) (Tables 2 and 3).

LONG-TERM EFFECT OF CALCIUM SUPPLEMENTATION

The possibility of an intrauterine programming of later blood pressure and the risk of various chronic diseases later in life has recently attracted considerable interest. This possibility, which implicates diet, impaired maternal nutritional state, and low birth weight (23–25) in the programming, suggests that fetal life is a period for programming physiologic functions. This is a concept naturally extended from the long-term deleterious effects of intrauterine growth retardation already shown on postnatal physical growth and cognitive and neurologic development (26–28). These are issues of tremendous relevance to developing countries, where a large proportion of newborns suffer from intrauterine nutritional restrictions (29).

Using the population of a large, randomized, placebo-controlled trial (6), we explored for the first time in the context of a randomized trial the effect of a nutritional intervention during pregnancy (to correct a deficit) on the blood pressure of the supplemented women's children (30). Children with a mean age of 7 y, whose mothers were randomly assigned during pregnancy to receive 2 g elemental Ca/d (n = 298) or placebo (n = 293), were eligible for the follow-up study. Among these eligible children, 86.2% in the prenatal calcium group and 89.2% in the prenatal placebo group were evaluated at 7 y of age (30).

Overall, systolic blood pressure was lower in the calcium group (mean difference: −1.4 mm Hg; 95% CI: −3.2 mm Hg, −0.5 mm Hg) than in the placebo group. The effect was found predominantly in children whose body mass index (BMI; in kg/m^2) was above the median for this population [mean difference in systolic blood pressure: −5.8 mm Hg (−9.8 mm Hg, −1.7 mm Hg) for children with a BMI >17.5 and −3.2 mm Hg (−6.3 mm Hg, −0.1 mm Hg) for those with a BMI from >15.7 to 17.5]. The risk of high systolic blood pressure was also lower in the calcium group than in the placebo group (RR: 0.59; 95% CI: 0.39, 0.90), particularly among children in the upper quartile of BMI (RR: 0.43; 95% CI: 0.26, 0.71). We conclude from these data that calcium supplementation during pregnancy is associated with lower systolic blood pressure in the offspring, particularly in overweight children.

CONCLUSIONS

It seems clear to us that there is promising evidence of a protective effect of calcium supplementation during pregnancy on preeclampsia when provided to women with deficient calcium intake and that this effect is biologically plausible. There is, however, strong support for the concept that definitive confirmation is still needed in the context of an adequately sized randomized, controlled trial targeted specifically to a population with low calcium intake, ie, the most likely group to benefit from such an intervention. This is because most of the trials in populations with low calcium intakes were small and prone to exaggerate the protective effect, with the largest of them having CIs including the null hypothesis. Also, most of these trials were conducted by the same research groups and thus require external confirmation. Furthermore, because the implementation of calcium supplementation programs will require substantial effort, including early antenatal care and community involvement, it is crucial that implementation of a false-positive intervention be avoided. In the meantime, pregnant women should be encouraged and supported to achieve intakes of 1.2 g Ca/d, as usually recommended. Long-term health benefits for the offspring are also a new and attractive possibility and should be explored by using available data and by conducting future trials.

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