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RANDOMIZED CONTROLLED TRIAL

Maternal calcium supplementation during pregnancy and dental caries of children at 12 years of age: follow-up of a randomized controlled trial

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Abstract

Objectives. To evaluate if calcium supplementation during pregnancy could have any influence on primary dentition measured as the reduction of dental caries of the child. *Design.* Individual randomized controlled trial. *Setting.* One hospital in Rosario, Argentina. *Population.* Random sample of 195 12-year-old children from a follow-up study of 614 women who were randomized during pregnancy to calcium supplementation or placebo. *Methods.* An independent researcher blinded to the group where the mothers were assigned performed a dental examination of the children. *Main outcome measures.* Proportion of children with at least one decayed, missing or filled teeth (DMFT/dmft) and mean number of decayed, missing or filled surfaces (DMFS/dmfs) per children. *Results.* Ninety-eight children were assessed in the calcium supplementation group and 97 in the placebo group. 63.3% of the children whose mother took calcium supplementation had at least one DMFT/dmft compared to 86.6% in the placebo group (<0.001). The children whose mother received the intervention had a 27% reduction in the risk of developing at least one DMFT/dmft (RR: 0.73, CI 95%: [0.62; 0.87]). *Conclusions.* This study shows a modeling effect of calcium intake during pregnancy on dental caries of the offspring. At around 12 years of age children whose mothers received calcium supplementation when pregnant showed a significant reduction in dental caries.

Key words: *Fetal modeling, dental caries, calcium intake, pregnancy*

Introduction

Many studies are supporting the hypothesis that adult life can be 'modeled' during intrauterine life implying that nutritional intrauterine deficiencies could be associated with adult diseases such as hypertension, diabetes and obesity (1). Our group have shown that maternal calcium supplementation during pregnancy involves lower rates of hypertension in the progeny in humans and animals (2–3).

In year 1995–1996, we conducted a follow-up of children of around 7 years of age whose mothers participated in a randomized controlled trial during their pregnancy that compared calcium supplementation to placebo for preventing pre-eclampsia (2). Women where blinded to the allocated intervention and still remain blinded at the time of the follow-up. During our interview some mothers mentioned that they had observed that their children had a better dentition and less dental complications compared with other children including their own siblings. Our first reaction was to consider that they could have been influenced by believing they had received calcium supplementation during pregnancy and

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*We deeply regret that Dr Alejandra Luetich, one of the authors of this paper and a prominent and enthusiastic scientist, has deceased after the completion of this study.

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calcium is popularly associated with dentition and the 'strength' of teeth. However, we decided to test such observation by examining dental health in a sample of those children.

Teeth mineralization starts at different stages of fetal life and as a result at term most part of the primary tooth crowns of the fetus has been mineralized (4). It has been described that in children, an insufficient intake of nutrients could limit the ability of the tooth to resist bacterial invasion and caries (5). Some studies have shown that maternal nutrition could influence primary dentition. Maternal deficiency of vitamin A was associated with hypoplasia and irregular mineralization of child enamel (6). An association of maternal deficiency of vitamin D with child enamel hypoplasia has been described and maternal supplementation with vitamin D has shown a reduction of child enamel defects in the primary teeth (7).

Animal studies have shown that insufficient maternal calcium, phosphates, magnesium and iron diet influence normal dental tissues development of the offspring (7). Studies on the effect of drinking water have shown relationship between the calcium content of drinking water and the numbers of decayed, missing or filled surfaces (8).

A study that recruited approximately 50,000 15-year-old schoolchildren in 249 municipalities has shown that the combination of ion fluoride and calcium in drinking water together explained 45% of the variation in the number of decayed, missing or filled surfaces (DMFS) (8).

The objective of this study is to evaluate if maternal calcium supplementation during pregnancy could have any influence on primary dentition measured as the prevalence of dental caries at 12 years of age.

Material and methods

This is a follow-up of a cohort of children and their mothers, who were originally enrolled in a randomized, double blind, placebo controlled trial of calcium supplementation to prevent hypertensive disorders of pregnancy (9).

A detailed description of the methodology of the original trial has been published elsewhere (9). The trial examined the effectiveness of 2 g of elemental calcium supplementation a day, for the prevention of hypertensive disorders of pregnancy. Women were eligible for the study if they were nulliparous, had singleton pregnancies, and had blood pressure values below 140/90 mmHg at the time of random allocation. Supplementation was started at 20 weeks of gestation and continued until delivery. In all, 1,194 nulliparous pregnant women were enrolled in two public hospitals (580 women) and one private hospital (614 women) affiliated to the Centro Rosarino de Estudios Perinatales (CREP), Rosario, Argentina, between August 1987 and November 1990. The women were randomly assigned to receive 2 g of calcium (four tablets of calcium carbonate 500 mg) or similar placebo orally. A previous follow-up study of this trial was restricted, for logistical reasons, to the 614 women and children born at the private hospital (309 women in the calcium group and 305 in the placebo group) (2). The same cohort was used for the present follow-up. The number of children eligible for follow-up was 303 in the calcium group and 299 in the placebo group (11 deaths of newborns and 1 death of the mother).

From March 2000 to December 2000, mothers and their children were contacted and invited to participate in the study. If mothers and children gave written consent they were included in the study. Examiners specifically trained for this follow-up study who were blinded to the assignment group conducted the dental examination in children and completed a questionnaire. The procedures were conducted at the patient's home or at the clinic. In the dental examination, we evaluated if each of the surface of the permanent and primary teeth was decayed, missing or filled. The number of decayed, missing or filled teeth in the permanent (DMFT) and primary (dmft) teeth and the number of decayed, missing or filled surfaces in the permanent (DMFS) and primary (dmfs) teeth were calculated. Due to the low fluoride level in our area, enamel fluorosis is not measured when scoring the enamel defects. The aim of the questionnaire was to investigate maternal and child characteristics related to dental care, such as, use of fluorine pills drops during the mother's pregnancy and provision to the child, regular provision of fluorine mouthwash or topical fluoride and use of fluoride-containing toothpastes for the child and if the mother considers that her child eats the toothpaste while brushing. The examiners measured height, weight and abdominal circumference of the children in light clothes and without shoes. This study was performed in Rosario, Argentina, a city showing a low fluoride concentration in water (0.2 ppm) (10).

The primary outcomes were the proportion of children with at least one DMFT/dmft and the average number of DMFS and dmfs per children. The secondary outcomes were the proportion of children with at least one DMFT, at least one dmft, erupted permanent second molars, mixed dentition and enamel hypoplasia.

Statistical analysis

A random sample of 200 children was taken. We supposed that in children whose mothers were not supplemented, 88% of them would show at least one dental caries (11). Considering a power of 80%, this sample size can show a reduction of at least 18% in the proportion of children with at least one lesion of caries in those whose mothers received calcium supplementation.

Children remained in the group to which their mothers were originally assigned at random, regardless of the women's compliance with the treatment and any postnatal experience (intention to treat analysis).

To describe the characteristics of the mother and the child at pregnancy, birth and after 12 years of the delivery for those with and without dental assessment we used the mean and standard deviation (SD) for continuous variables and percentage relative frequencies for the categorical variables. The maternal and child characteristics related to dental care were compared using a Chi-squared test and a Fisher exact test when required.

We calculated the proportion of children with at least one DMFT/dmft and total number of teeth and we computed the average of the number of DMFS/dmfs per children. The proportion of children with dental eruption in the second molars, mixed dentition and enamel hypoplasia was also measured.

Relative risks (RRs) with their 95% confidence intervals were used to explore the difference in the proportion of children with at least one DMFT/dmft and the proportion of children with dental eruption in the second molars, mixed dentition and enamel hypoplasia between the calcium supplementation and placebo groups.

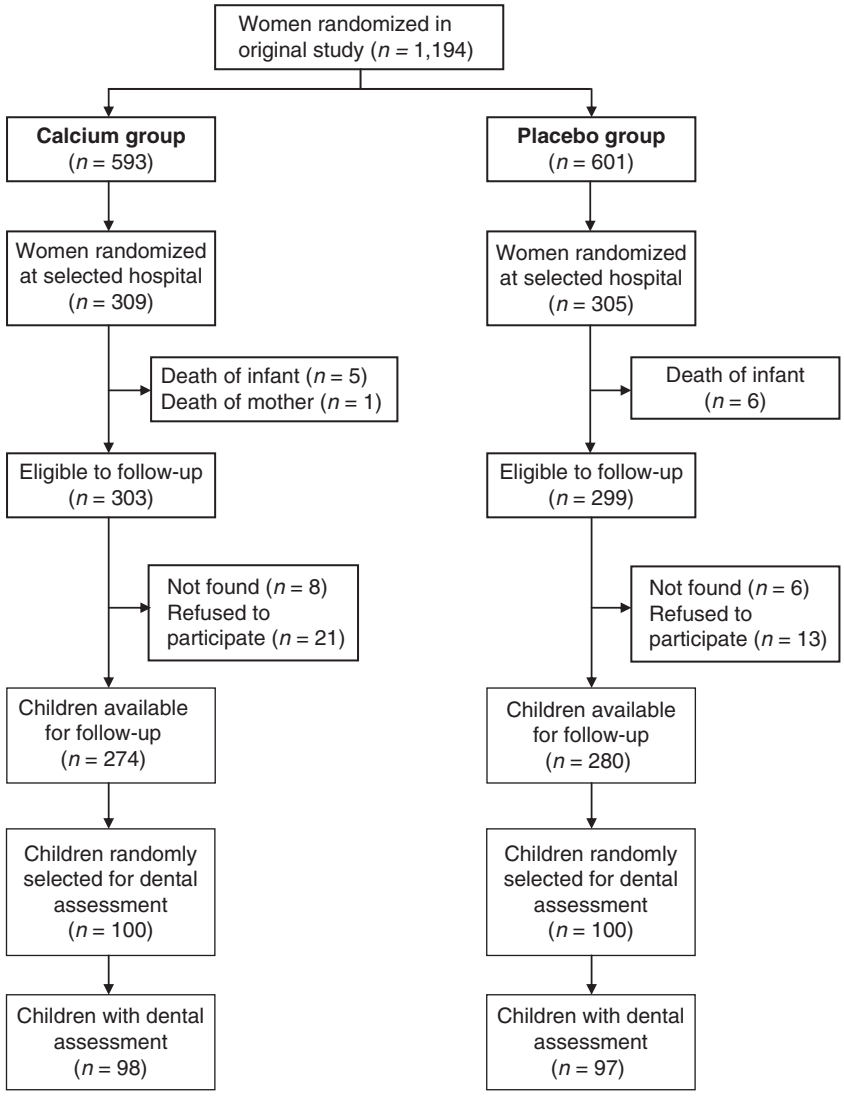

Figure 1. Study population.

Table 1. Characteristics of the child and the mother with and without dental assessment.

	With dental assessment (n = 195)				Without dental assessment (n = 359)			
	Calcium supplementation (n = 98)		Placebo (n = 97)		Calcium supplementation (n = 176)		Placebo (n = 183)	
Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maternal baseline characteristics								
Age (years)	27.5	3.1	27.9	4.3	27.7	4.5	27.3	4.6
Education								
Primary incomplete or less	0/98	0.0	4/97	4.1	7/174	4.0	11/183	6.0
Primary complete or secondary incomplete	20/98	20.4	23/97	23.7	54/174	31.0	59/183	32.2
Secondary complete or tertiary/University incomplete	55/98	56.1	39/97	40.2	58/174	33.3	46/183	25.1
Tertiary/University complete	23/98	23.5	31/97	32.0	55/174	31.6	67/183	36.6
Children characteristics at birth								
Birth weight (g)	3214.1	490.7	3230.8	470.0	3251.1	498.6	3251.5	540.8
Gender (% males)[a]	49/98	50.0	55/97	56.7	91/179	51.7	101/183	55.2
Children characteristics 12 years after delivery								
Age (years)	12.8	0.6	12.8	0.6	12.8	0.7	12.7	0.6
Weight (kg)	47.4	10.5	45.8	10.4	46.9	10.6	45.3	10.8
Height (cm)	153.4	9.1	152.7	7.8	152.8	7.5	151.4	8.6
Abdominal circumference (cm)	69.2	8.8	67.6	8.5	67.2	9.3	66.5	8.7
Maternal characteristics 12 years after delivery								
Weight (kg)	61.2	9.3	62.0	11.5	62.4	11.4	63.6	12.7
Height (cm)	162.3	6.1	162.6	5.8	162.7	6.5	162.3	6.1
Abdominal circumference (cm)	75.9	7.4	76.7	10.4	76.5	9.7	77.2	10.9

[a]n and %.
Note: SD, standard deviation.

For the analysis of the decayed, missing or filled teeth surfaces we used a multilevel analysis. The data followed a hierarchical structure in which the individual participant was the top level unit of analysis, the tooth was the second level of analysis and the surface was the first level of analysis. This approach allowed us to model the random structure. Multilevel models work by splitting the variance in outcome into components for each level of the model, so random effects at tooth and individual level were estimated in the modeling process. Random effects at higher levels are assumed to follow a normal distribution with mean zero and variance that was estimated in the modeling process. The odds ratio (OR) was reported. SPSS and MLWin statistical packages were used for the analyses.

The Ethic Committee of the Centro Rosarino de Estudios Perinatales approved the study protocol.

Results

Figure 1 shows the flow of the population during the 12 years period of the trial. Two hundred children were selected at random. The final number of children assessed was 98 in the calcium supplementation group and 97 in the placebo group.

Table 2. Maternal and child characteristics related to dental care.

	Calcium supplementation (n = 98)		Placebo (n = 97)		
Characteristics	n	%	n	%	p-Value*
Child care					
Used fluorine pills/drops	11/98	11.2	11/97	11.3	0.980
Regularly received fluorine mouthwash or topical fluoride	47/98	48.0	40/96	41.7	0.378
Regularly use fluoride-containing toothpastes	11/97	11.3	9/95	9.5	0.672
Eat the toothpaste during the brushing	16/98	16.3	12/97	12.4	0.431
Maternal care					
Used fluorine pills/drops	4/97	4.1	3/95	3.2	1.000

*Chi-squared test or Fisher exact test if required.

Table 3. Primary and secondary outcomes.

Outcomes	Calcium supplementation (n = 98) n (%)	Placebo (n = 97) n (%)	RR (CI 95%)	p-Value
Primary outcome				
Children with at least one decayed, missing or filled teeth in the permanent and primary teeth (DMFT/dmft)	62 (63.3)	84 (86.6)	0.73 (0.62; 0.87)	<0.001
Number of decayed, missing or filled surfaces in the permanent and primary teeth (DMFS/dmfs)	3.1 (4.05)[a]	4.4 (4.11)[a]	0.67 (0.57; 0.79)[b]	<0.001
Secondary outcomes				
Children with at least one decayed, missing or filled teeth in the permanent teeth (DMFT)	59 (60.2)	79 (81.4)	0.73 (0.61; 0.89)	<0.001
Children with at least one decayed, missing or filled teeth in the primary teeth (dmft)	11 (11.2)	14 (14.4)	0.78 (0.37; 1.63)	0.503
Children with erupted permanent second molars	42 (42.9)	45 (46.4)	0.92 (0.68; 1.26)	0.620
Children with mixed dentition	25 (25.5)	27 (27.8)	0.92 (0.58; 1.46)	0.714
Children with enamel hypoplasia	26 (26.5)	22 (22.7)	1.17 (0.71; 1.91)	0.532

[a]Mean (SD).
[b]Odds ratio calculated from the multilevel analysis.
Note: RR (CI 95%), relative risk (95% confidence interval).

Table 1 presents the characteristics of the mother at the moment of randomization during pregnancy and at 12 years after delivery and the characteristics of the child at birth and at 12 years old. Information is shown according to the treatment group of those included in the current study and those without dental assessment. Similar characteristics are shown between groups.

No relevant differences were found in the dental care of the mother and the child between the placebo group and the group who took calcium supplementation (Table 2).

Table 3 displays the results of the primary and secondary outcomes. The proportion of children with at least one DMFT/dmft was statistically different in the group of children whose mother took calcium supplementation (63.3%) comparing the group of children whose mother was in the placebo group (86.6%). The children whose mother received the intervention had a 27% reduction in the risk of developing at least one DMFT/dmft (RR: 0.73, CI 95%: [0.62; 0.87]). The average number of DMFS/dmfs per children was 4.4 in the placebo group and 3.1 in the intervention group. The multilevel analysis that considered the surface of the teeth as the unit of analysis shows a reduction of 33% (OR: 0.67, CI 95%: [0.57; 0.79]). In relation to the secondary outcomes, an intervention effect was seen in the

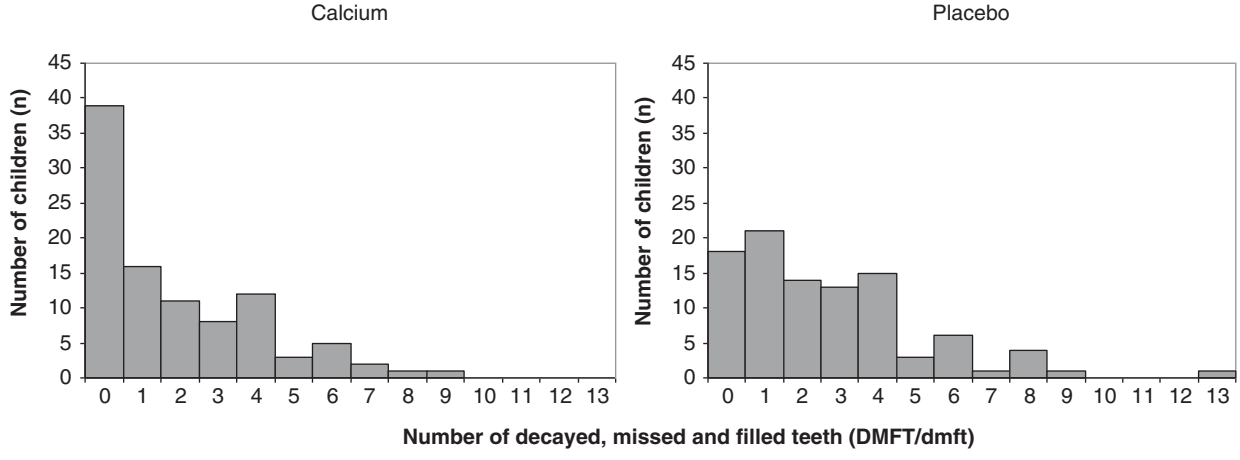

Figure 2. Distribution of children according to the number of decayed, missing and filled teeth (DMFT/dmft) by treatment group.

proportion of children with at least one DMFT (60.2 and 81.4% in the group of calcium supplementation and placebo group, respectively; $p < 0.001$). No significant differences between groups were seen in the proportion of children with at least one dmft, with erupted permanent second molars, with mixed dentition, and with enamel hypoplasia.

Sixty-four percent of the caries were occlusal and 14% were approximal. This distribution was similar in both groups (occlusal 61 and 65% in the placebo and calcium group, respectively; and approximal 14 and 13%, respectively).

Figure 2 shows the distribution of children according to the number of decayed, missing or filled teeth by the two groups under study. The calcium group shows a higher number of children with no dental caries. A higher caries score was observed per children in the placebo group.

Discussion

This study shows a modeling effect of calcium intake during pregnancy on dental caries of the offspring. At around 12 years of age, children whose mothers received calcium supplementation when pregnant showed a significant reduction in dental caries.

Women remained blinded to the group assignment, and also the professional (AL) performing the dental assessment was blinded to this assignment. Data analysis was done also blinded and the group was revealed after finishing the analysis. Another strength of the study is the similarity between groups not only at recruitment but also at the follow-up period. A limitation of the study was that maternal dental status was not measured during pregnancy. However, as it is a randomized controlled trial, the expectations are that maternal dental status should be similarly distributed between the two groups at enrolment.

The association between maternal calcium intake during pregnancy and child's dental caries is plausible. Teeth mineralization starts during fetal life implying that at term a major part of the primary tooth crowns would have mineralized, and an association between maternal nutrition and child enamel mineralization was described (4,7). Maternal calcium intake during pregnancy has been associated with a fetal programming involving hypertension, altered lipid profile, obesity and insulin resistance on those offspring whose mothers received a low calcium diet during pregnancy (2,3,12–14). Fetal programming implies a change that it is prolonged during life span, explaining why children of 12 years old age show this fetal 'imprinting'. Mechanisms of maternal diet on fetal programming are not fully understood. Changes in 1,25-dihydroxyvitamin D3 levels and in the hormone sensitivity at the target cells have been proposed, still requiring further scientific support (14–17).

The observation of an effect of maternal calcium intake on offspring dental health opens an area of interest involving the need of animal and clinical research to confirm these findings and looking for mechanisms involved in such association.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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