International Journal of Gynecology and Obstetrics (2005) **91**, 221—227

International Journal of
GYNECOLOGY
& OBSTETRICS

www.elsevier.com/locate/ijgo

CLINICAL ARTICLE

Calcium plus linoleic acid therapy for pregnancy-induced hypertension

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Received 8 June 2005; received in revised form 30 August 2005; accepted 30 August 2005

KEYWORDS
Calcium;
Conjugated linoleic acid;
Pregnancy-induced hypertension;
Pre-eclampsia;
Vascular endothelial function;
Prevention

Abstract

Objective: To determine the effect of dietary supplementation of calcium plus conjugated linoleic acid (calcium—CLA) in reducing the incidence of vascular endothelial dysfunction in pregnant women at high risk of developing pregnancy-induced hypertension (PIH). *Patients and methods:* This randomized, double-blind, placebo-controlled trial conducted at 4 outpatient clinics in 2 developing countries recruited 48 healthy primigravidas younger than 19 years or older than 35 years who had a family history of pre-eclampsia and diastolic notch. Twenty-four participants received daily elemental calcium (600 mg) plus CLA (450 mg) and 24 received placebo from week 18 to 22 of pregnancy until delivery. *Results:* Calcium—CLA supplementation reduced significantly the incidence of PIH (2 cases [8%] in the study group vs. 10 cases [42%] in the placebo group; relative risk, 0.20; 95% confidence interval, 0.05—0.82; $P=.01$). Endothelial dysfunction was also significantly reduced after calcium—CLA supplementation (in 18 women [75%] vs. 4 women [17%]; $P<.001$), compared with the placebo group (in 15 [63%] vs. 9 women [38%]; $P=.08$). *Conclusion:* In pregnant women at high risk for PIH, calcium—CLA supplementation decreases the incidence of PIH and improves endothelial function.

1. Introduction

Pregnancy-induced hypertension (PIH) (i.e., gestational hypertension [GH] and pre-eclampsia [PE])

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0020-7292/$ - see front matter
doi:10.1016/j.ijgo.2005.08.018

remains as a major cause of maternal and perinatal morbidity and mortality, especially in developing countries [1—3]. It has been suggested that multiple conditions, such as nutritional deficiency, insulin resistance, subclinical infections, and immunologic and genetic alterations, contribute to the risk of developing PIH. Most of these conditions have been associated with alterations in endothelial function, which seems to be critical in the development of PIH [4]. In addition, it has been suggested that each of these factors bear a different weight in different populations, and also varies locally with the quality of the health care system, sanitary conditions, and social stratification [3,4]. An insufficient calcium intake has been one of the most studied conditions associated with PIH [5,6]. However, the results of different clinical trials using calcium supplementation to prevent PIH have been contradictory [7—15].

The purpose of the present randomized, double-blind, placebo-controlled clinical trial was to investigate whether oral supplementation of calcium and CLA decreases the incidence of PIH and improves vascular endothelial function in pregnant women at high risk for developing PIH. The participants were recruited from 2 developing countries where calcium intake is low.

2. Patients and methods

2.1. Study population and treatment allocation

Healthy primigravidas at either end of the most common reproductive years (younger than 19 years or older than 35 years) who were between 18 and 22 weeks of pregnancy were recruited at 4 hospital outpatient clinics in Bangladesh and Colombia (Fig. 1). They were considered at high risk for PIH if they had a family history of pre-eclampsia (PE), and had an abnormal finding (e.g., a diastolic notch in uterine or arcuate artery waveforms) on Doppler ultrasonographic examination [16]. At the time of enrollment, none of the participants had a multiple pregnancy, a diastolic blood pressure of 85 mm Hg or higher, cardiovascular or renal disease, or hypertension, and none were taking any medica-

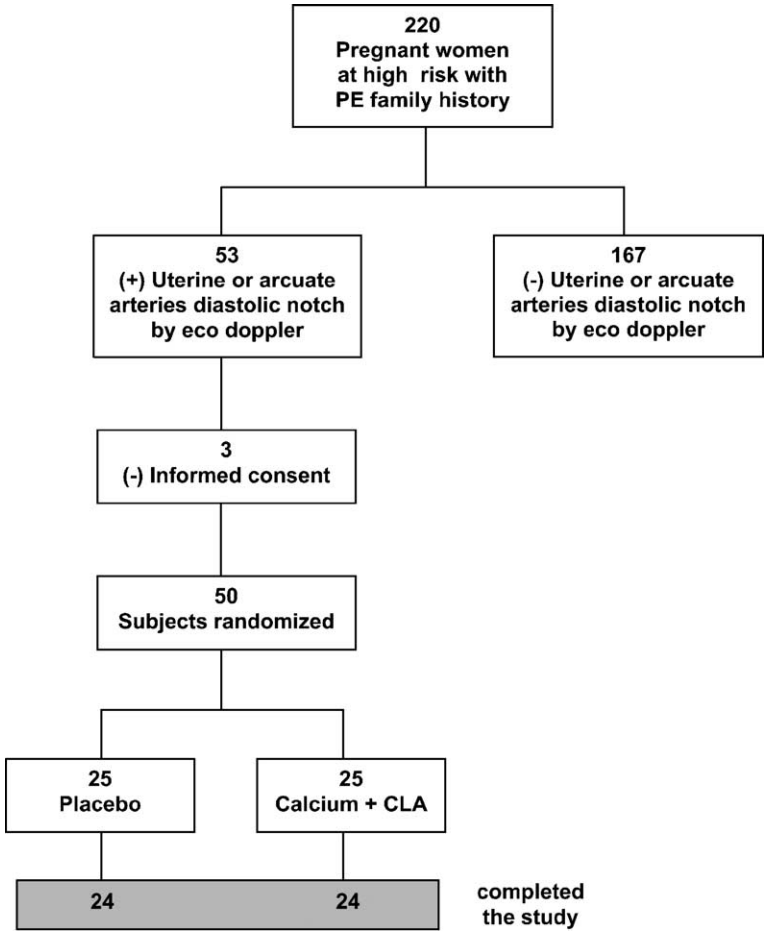

Figure 1 Screening of healthy primigravidas and flow through the trial.

tions. The study was approved by the institutional review board of each of the participating outpatient clinics and all participants gave written informed consent before joining the study.

A qualified nutritionist evaluated the daily calcium intake of the participants, who were then assigned to 1 of 2 groups by opening a sealed, opaque, sequentially numbered envelope containing a card that indicated group allocation. These cards were prepared using a random number table drawn using the True Epistat statistical package, version 5.0 (Epistat Services, Richardson, Texas, USA). The study group received a daily oral dose of 1484 mg of calcium carbonate, i.e., 600 mg of elemental calcium (Pharmanutrients Inc., Illinois, USA), and 450 mg of CLA (Pharmanutrients Inc.). The control group received placebo pills containing 600 mg of lactose and 450 mg of starch, amounting to 5 kcal per capsule (Pharmanutrients Inc.).

The women were instructed to take the pills at the same time every morning until delivery, and to inform their physician about any changes in pill intake or any suspected adverse effects. Compliance was assessed at each antenatal visit using a questionnaire and counting the remaining tablets in the bottle. All members of the research team received training and followed common standard operating procedures at all centers.

2.2. Biochemical analysis

Venous blood was obtained from all participants at inclusion after an overnight fast and before the brachial artery flow-mediated dilatation (FMD) study. Plasma and serum were isolated and stored at −80 °C until needed. Blood samples were collected again after calcium–CLA supplementation, at the end of pregnancy but before the onset of labor. All biochemical assessments were performed at Fundan University, Shanghai, China. Ionized calcium in plasma was measured by the ion-selective electrode method (Nova Biomedical, Massachusetts, USA).

2.3. Flow-mediated vasodilation

Flow-mediated vasodilation was determined at inclusion and after calcium–CLA supplementation, at the end of pregnancy before the onset of labor. The procedure was performed in accordance with the recommendations of the International Brachial Artery Reactivity Task Force [18], with a 7.5-MHz linear-array transducer ultrasonographic system (Vario-View SDD2200; Aloka, Tokyo, Japan). The FMD study technique was cross-validated across participating centers in Colombia and Bangladesh according to previously described procedures [17]. Endothelial dysfunction was defined as FMD lower than 10.4%, according to previous studies performed in the Colombian population [17].

2.4. Follow-up and pregnancy outcomes

All participants received standard antenatal visits every month until the 36th week of pregnancy and twice per month thereafter. Urinary cultures, vaginal smears (when necessary, also vaginal cultures) were performed, and protein urinary levels were assessed at each visit. Participants found to have an infection received appropriate antibiotic treatment.

All participants were followed up until delivery and their medical records were reviewed for the presence of PIH, defined as GH or PE. Pre-eclampsia

Table 1 Sociodemographic characteristics of 48 primigravidas at high risk for pre-eclampsia by treatment[a]

Characteristic	Calcium–CLA group (n = 24)	Placebo group (n = 24)	P value
Marital status			
Single	4 (16.6%)	5 (20.8%)	NS
Married	2 (8.3%)	0	
Common marriage law	18 (75%)	19 (79.2%)	
Recruitment country			
Colombia	9	9	
Bangladesh	15	15	
Education			
Basic	3 (12.5%)	2 (8.3%)	NS
Secondary	10 (41.7%)	18 (75.0%)	
University	11 (45.8%)	4 (16.6%)	
Socioeconomic level			
Low (1–2)	8 (33.3%)	12 (50.0%)	NS
Medium (3–4)	15 (62.5%)	8 (33.3%)	
High (5–6)	1 (4.2%)	4 (16.6%)	
Urban residence	24 (100.0%)	24 (100.0%)	

[a] Data are presented as n (%). Socioeconomic level: Classification from the National Department of Statistics from Columbia and Bangladesh.

Table 2 Obstetric characteristics at study entry of 48 primigravidas at high risk for pre-eclampsia*

Characteristic	Calcium–CLA group ($n=24$)	Placebo group ($n=24$)	P value
Pregnancy duration, weeks	19.7 ± 1.7	20.0 ± 1.9	NS
Body mass index	22.5 ± 2.9	21.4 ± 4.0	NS
Anemia (Hb<10 g/L)	13 (54.1%)	14 (58.3%)	NS
Family history			
Mother with pre-eclampsia	13 (54.1%)	14 (58.3%)	NS
Sister with pre-eclampsia	11 (45.8%)	10 (41.7%)	NS
Family member with essential hypertension	16 (66.6%)	19 (79.1%)	NS
Family member with diabetes mellitus	9 (37.5%)	6 (25.0%)	NS
Infections			
Urinary	5 (20.8)	5 (20.8)	NS
Vaginal	2 (8.7)	3 (13.0)	NS
Urinary and vaginal	1(4.1)	1 (4.1)	NS
Total	8 (33.3)	9 (37.5)	NS
Endothelial dysfunction (FMD (<10.4%))	18 (75.0)	15 (62.5)	NS

* Values are given as mean ± SD or number (percentage).

was defined as normotension before the 20th week of pregnancy, with the subsequent development of hypertension (≥140/90 mm Hg) and significant 24-h proteinuria (>0.3 g/L) in the absence of a urinary tract infection. Gestational hypertension was defined as de novo hypertension arising after mid-pregnancy, without proteinuria. At each visit, blood pressure was measured twice, in accordance with the criteria proposed by the American College of Obstetricians and Gynecologists [19]. Blood pressure measurements were obtained by the Korotkoff method, and Korotkoff phase V was used for determining diastolic pressure.

2.5. Statistical methods

Using a 2-sided test it was calculated that, with a statistical power of 80% at the 5% significance level, 24 women at high risk of developing PIH were needed per group to demonstrate statistical significance with a relative reduction of 75% in the rate of PIH (from 50% to 13%). Assumptions were based on previous studies that demonstrated a high incidence of PE in women with a diastolic notch and a beneficial effect of calcium–CLA supplementation [10,11,16]. The Shapiro–Wilk test was used to assess the normality of continuous data. Continuous variables were analyzed using a 2-tailed t test or the Wilcoxon rank-sum test, and categorical variables using the X^2 test or the Fisher exact test. Statistical significance was set at $P<0.05$. All data were analyzed using the Epiinfo statistical package, version 6.0 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

3. Results

Fifty three primigravidas were invited to participate in the study but 3 refused. Eligibility criteria were (1) being at either end of the most common reproductive years (younger than 19 years or older than 35 years); (2) having a family history of PE and an abnormal result to a Doppler ultrasonographic examination (a diastolic notch in the uterine or arcuate artery waveforms); and (3) being between 18 and 22 weeks of pregnancy. The 50 women who agreed to participate received oral information about the study and signed informed consent forms. One woman from the control group changed residence and was lost to follow-up, and 1 from the study group was excluded because she withdrew from the study. The final analysis was based on 24 women treated with calcium plus CLA and 24 with placebo (Fig. 1).

Socioeconomic characteristics and obstetric risk factors were similar in the 2 groups at study entry (Tables 1 and 2). Mean daily calcium intake was also similar at study entry (601.5 mg [range, 310–1101 mg] vs. 576.0 mg [314–936 mg]; $P=.94$), as was compliance to treatment (80% vs. 81%) and plasma calcium concentrations, both before (1.18 ± 0.03 mmol/L vs. 1.18 ± 0.03 mmol/L) and after supplementation (1.19 ± 0.02 mmol/L vs. 1.19 ± 0.03 mmol/L). Yet, the mean urinary calcium–creatinine ratio increased significantly in the calcium–CLA group after supplementation (11.9 ± 2.7 vs. 13.6 ± 4.4; $P=.005$) but did not vary in the placebo group (12.1 ± 2.5 vs. 12.1 ± 2.3; $P>.05$).

The overall incidence of PIH was significantly decreased at the end of pregnancy in the calcium–CLA group (2 cases [8%] vs. 10 cases [42%] in the placebo group; relative risk, 0.20; 95% confidence interval, 0.05–0.82; $P=.01$). A higher incidence of PE was also observed in the placebo group (3 cases [12.5%]) vs. the calcium–CLA group (0 case).

Mean maternal diastolic blood pressure at delivery was significantly higher in the placebo group

Table 3 Outcome just before delivery for 48 primigravidas at high risk for pre-eclampsia*

Outcome	Calcium–CLA group ($n=24$)	Placebo group ($n=24$)	P value
PIH	2 (8.3)	10 (41.7)	0.01
Blood pressure, mm Hg			
Systolic	116.0 ± 9.6	122.2 ± 21.2	0.19
Diastolic	76.3 ± 7.8	84.3 ± 10.9	0.005
Gestational age (weeks)	38.8 ± 2.2	38.6 ± 1.6	0.61
Cesarean delivery	8 (33.3%)	14 (58.3%)	0.08
Birth weight	2979 ± 448	2705 ± 433	<0.001

Abbreviations: PIH, pregnancy induced hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Values are given as mean ± SD or number (percentage).

($P=.005$), whereas mean birth weight was significantly greater in the calcium–CLA group ($P<0.01$) (Table 3). The cesarean delivery rate was similar in both groups.

At recruitment, vascular endothelial dysfunction (FMD<10.4%) was observed in 18 women assigned to the calcium–CLA group and in 15 women assigned to the placebo group. The percentage of women with endothelial dysfunction (FMD<10.4%) was significantly reduced after calcium–CLA supplementation. It was reduced in 18 women (75%) vs. 4 women (17%) in the calcium–CLA group, compared with 15 women (63%) vs. 9 women (38%) in the placebo group ($P<.001$ vs. $P=.08$). Calcium–CLA supplementation was associated with a 78% decrease in the number of women with endothelial dysfunction.

4. Discussion

Pregnant women from developing countries with low calcium intake are at high risk for developing PIH when they have such well-known risk factors as expecting their first child, being at either end of the most common reproductive years, having a diastolic notch, and/or having a family history of PE. Daily oral administration of elemental calcium and CLA decreased significantly the incidence of PIH, to 8%, in the calcium–CLA group. Moreover, diastolic blood pressure was significantly lower in the calcium–CLA group at the end of pregnancy, which confirmed previous results [8].

During pregnancy, calcium needs greatly increase because of fetal bone formation [20]. Baseline daily calcium intake was similar in the 2 groups. After supplementation, urinary calcium–creatinine ratio was found to be increased in the calcium–CLA group but not in the placebo group. These results suggest that the orally ingested calcium was absorbed.

Multiple studies performed in the Andean population since 1984 have shown a beneficial effect of calcium supplementation in the prevention of PIH and PE [7–9]. However, the Calcium for Preeclampsia Prevention (CPEP) trial [14], which was conducted at 5 universities in the United States and included 4589 healthy nulliparous women, did not show a significant reduction in the incidence of PE or PIH with calcium supplementation. But it must be noted that in the CPEP trial, calcium supplementation was administered to women with a normal calcium intake, whereas in the studies performed in Ecuador and Colombia it was administered to prevent a nutritional deficiency. A recently published meta-analysis of 11 randomized trials comparing calcium supplementation with placebo during pregnancy found a greater reduction in the incidence of PIH among women with low baseline calcium intake [15]. The present study confirms these findings and support the hypothesis that calcium supplementation appears to reduce the risk of PIH, especially in populations living in poor sanitary conditions associated with risk factors for PIH.

Calcium supplementation may play a beneficial role in the prevention of PIH by maintaining plasma ionized calcium levels within the narrow physiological range. Studies have shown that maintaining this range is crucial for the ongoing synthesis of vasoactive substances such as prostacyclin and nitric oxide in the endothelium, and, consequently, for a normal vascular endothelial function [6,21]. In the present study, calcium supplementation was associated with a 78% reduction in the number of women with endothelial dysfunction. This result suggests that the lower incidence of PIH observed in the calcium–CLA group can be associated with a beneficial effect of calcium on vascular endothelial function.

A low incidence of PE (12.5%) was found in the women considered at high risk by the presence of a diastolic notch, which differs from the results from previous studies [16]. Moreover, in a nonselected

Colombian population, a higher incidence of PE was observed [10,11]. The main difference among the present study and those previously conducted in the same population was the screening for and treatment of infections. It is attractive to hypothesize that these encouraging results could be related to the treatment of urinary and vaginal infections. This proposal is based on a previous study performed in Colombia [22] that included 15,354 pregnant women. Early and specific antibiotic treatment was prescribed to 1766 women with asymptomatic bacteriuria and 2150 women with a vaginal infection, and treating these infections decreased the incidence of PE from 5.1% observed in the previous 5 years to 1.8% during the study. This means a reduction of 64.7% in the historical incidence of the disease in the studied population. These results, as well as epidemiological observations from other populations [23,24], suggest that urinary and vaginal infections during pregnancy could be associated with an increased risk of developing PIH.

In conclusion, the present study confirms that calcium—CLA supplementation decreases the incidence of PIH and improves vascular endothelial function in pregnant women with low baseline calcium intake and additional risk factors. Regardless of the small size of the sample, these results indicate a need for improved antenatal care in developing countries, with particular attention to the opportune administration of nutritional supplements to reduce the incidence of PIH.

Acknowledgments

This study was financially supported by the Colombian Institute for Science and Technology development (COLCIENCIAS) (Projects # 1106-04-10275,1106-04-12004) Bogotá, Colombia, and The Partners in Population and Development Institute, Dhaka, Bangladesh.

References

[1] Walker JJ. Pre-eclampsia. Lancet 2000;356:1260–5.
[2] World Health Organization. World Health Organization international collaborative study of hypertensive disorders of pregnancy: geographic variation in the incidence of hypertension in pregnancy. Am J Obstet Gynecol 1988;158:80–3.
[3] López-Jaramillo P, Garcia RG, López M. Preventing pregnancy induced hypertension: are there regional differences for this global problem? J Hypertens 2005;23:1121–9.
[4] López-Jaramillo P, Casas JP, Serrano NC. Preeclampsia: from epidemiological observations to molecular mechanism. Braz J Med Biol Res 2001;34:1227–35.
[5] López-Jaramillo P. Calcium, nitric oxide, and preeclampsia. Sem Perinatol 2000;24:33–6.
[6] López-Jaramillo P, Terán E, Moncada S. Calcium supplementation prevent pregnancy-induced hypertension by increasing the production of vascular nitric oxide. Med Hypotheses 1995;45:68–72.
[7] López-Jaramillo P, Narváez M, Weigel M, Yépez R. Calcium supplementation reduces the risk of pregnancy induced hypertension in an Andean population. Br J Obstet Gynaecol 1989;96:648–55.
[8] López-Jaramillo P, Narváez M, Félix C, López A. Dietary calcium supplementation and prevention of pregnancy hypertension. Lancet 1990;335:293–8.
[9] López-Jaramillo P, Delgado F, Jácome P, Terán E, Ruano C, Rivera J. Calcium supplementation reduces the risk of preeclampsia in Ecuadorian pregnant teenagers. Obstet Gynecol 1997;90:162–7.
[10] Herrera JA. Nutritional factors and lateral rest reduce pregnancy-induced hypertension in positive roll-over test primigravids. Int J Obstet Gynecol 1993;41:31–5.
[11] Herrera JA, Arévalo-Herrera M, Herrera S. Prevention of preeclampsia by linoleic acid and calcium supplementation. Obstet Gynecol 1998;91:585–90.
[12] Belizan JM, Villar J, González L, Campodonico L, Bergel E. Calcium supplementation to prevent hypertensive disorders of pregnancy. N Engl J Med 1991;325:1399–405.
[13] Purwar M, Kulkarni H, Motghare V, Dhole S. Calcium supplementation and prevention of pregnancy induced hypertension. J Obstet Gynaecol Res 1996;22:425–30.
[14] Levine RJ, Aut JC, Curet LB, Sibai BM, Catalano PM, Morris CD, et al. Trial of calcium for prevention of preeclampsia. N Engl J Med 1997;337:69–76.
[15] Atallah AN, Hofmeyr GJ, Duley L. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems (Cochrane Review). Chichester, UK: John Wiley and Sons, Ltd. Cochrane Library; 2004. issue 1.
[16] Zimmermann P, Eirio V, Koskinen J, Kujansuu E, Ranta T. Doppler assessment of the uterine and uteroplacental circulation in the second trimester in pregnancies at high risk for preeclampsia and for intrauterine growth retardation: comparison and correlation between different Doppler parameters. Ultrasound Obstet Gynecol 1997;9:330–8.
[17] Accini JL, Sotomayor A, Trujillo F, Barrera JG, Bautista L, López-Jaramillo P. Colombian study to assess the use of noninvasive determination of endothelium-mediated vasodilatation (CANDEV): normal values and factors associated. Endothelium 2001;8:157–66.
[18] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–65.
[19] Working Group on High Blood Pressure in Pregnancy. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1–22 (suppl).
[20] Pitkin RM. Calcium metabolism in pregnancy and the perinatal period; a review. Am J Obstet Gynecol 1985;151:99–109.
[21] López-Jaramillo P, González MC, Palmer RM. The crucial role of physiological Ca^{2+} concentrations in the production

of endothelial nitric oxide and the control of vascular tone. Br J Pharmacol 1990;101:489–93.
[22] Herrera JA, Chaudhuri G, López-Jaramillo P. Is infection a major risk for preeclampsia? Med Hypotheses 2001;57: 393–7.
[23] Hsu CD, Witter FR. Urogenital Infection in preeclampsia. Int J Gynecol Obstet 1995;49:271–5.
[24] Hill JA, Devoe LD, Bryans JR. Frequency of asymptomatic bacteriuria in preeclampsia. Obstet Gynecol 1986;67: 529–32.