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Pregnancy

Calcium and Conjugated Linoleic Acid Reduces Pregnancy-Induced Hypertension and Decreases Intracellular Calcium in Lymphocytes

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Background: The aim of the present study was to determine whether the beneficial effect of oral supplementation with calcium and conjugated linoleic acid (CLA) in the reduction of the incidence of pregnancy-induced hypertension (PIH) is related with changes in plasma levels of prostanoids, renin, angiotensin II, calciotropic hormones, and plasma and intracellular ionized free calcium.

Methods: These mediators were determined using the blood samples obtained from a randomized, double-blind, placebo-controlled trial that included 48 healthy primigravidas with a family history of preeclampsia and with diastolic notch, recruited from four outpatient clinics from two developing countries. Participants were randomized to daily oral doses of elemental calcium and CLA or lactose-starch placebo from week 18 to week 22 of gestation until delivery.

Results: The incidence of PIH was significantly reduced in women receiving the supplement (2 women [8.3%]) compared with placebo (10 women [41.7%]) (relative risk = 0.20, 95% confidence interval 0.05–0.82, $P = .01$). There were no significant differences in the plasma concentrations of ionized calcium, prostaglandin E_2, renin, angiotensin II, parathormone,and calcitonine. The concentration of intracellular ionized free calcium presented a significant reduction after interventions (92.0 nmol/L [range 62.5 to 220 nmol/L] *v* 62.5 nmol/L [range 28 to 200 nmol/L; $P = .01$) in the supplemented group but not in the placebo group. The women who developed PIH ($n = 12$) presented a significant increase in the concentrations of intracellular calcium after interventions (120 nmol/L [range 89.2 to 240 nmol/L] *v* 137.5 nmol/L [range 89.2 to 138 nmol/L; $P = .02$).

Conclusions: Calcium and CLA supplementation during pregnancy reduces the incidence of PIH, and decreases the intracellular concentration of ionized free calcium in peripheral blood lymphocytes. Am J Hypertens 2006;19: 381–387 © 2006 American Journal of Hypertension, Ltd.

Key Words: Calcium supplementation, conjugated linoleic acid, intracellular ionized calcium, pregnancy-induced hypertension, prostaglandins.

Pregnancy-induced hypertension (PIH) is one of the most common contributors to maternal morbidity and mortality, premature birth, intrauterine growth retardation (IUGR), and low birth weight.[1] The etiophysiopathology of PIH remains unknown.[2] An imbalance in the vascular production of prostaglandins has been proposed as a possible mechanism in the alterations of the vascular reactivity that characterizes PIH.[3] Prostacyclin and prostaglandin E_2 (PGE_2), produced mainly in the vascular endothelium, are potent vasodilators and inhibitors of platelet aggregation, whereas thromboxane A_2, produced primarily by platelets, is a potent vasoconstrictor and promoter of platelet aggregation.[4] It has been observed that a decrease in the PGE_2 levels is associated with an increase in the vascular response to angiotensin II in pregnant patients who are susceptible to develop preeclampsia (PE), probably through the increase of intracellular ionic calcium.[5–7]

The oral administration of the combination of conjugated linoleic acid (CLA) as precursor of prostaglandins

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and calcium as an activator of membrane phospholipases could lead to an increase in the production of vasoactive prostaglandins and contribute to reduce the incidence of PIH. In previous randomized clinical controlled trials we have demonstrated that calcium supplementation decreases the incidence of PIH[8–10] and that in combination with linoleic acid produces an increase in PGE_2 in pregnant patients at high risk of developing PE.[11] However, in experimental studies the supplementation with CLA reduced PGE_2 formation.[12] The aim of the present randomized, double-blind, placebo-controlled clinical trial was to investigate whether the oral supplementation of calcium and CLA decreases the incidence of PIH and affects the plasma levels of prostanoids, renin, angiotensin II, calciotropic hormones, and plasma and intracellular ionized free calcium.

Methods

Study Population

Healthy primigravid women younger than 19 or older than 35 years from week 18 to 22 of gestational age were recruited from four outpatient clinics in Bangladesh and Colombia and were screened to enter the study. Pregnant women without a reliable family history of PE (antecedent from clinical record of mother or sister with PE) were excluded. None of the patients had multiple pregnancy, diastolic blood pressure (BP) of 85 mm Hg or more at the first antenatal visit, cardiovascular or renal disease, nor were they hypertensive and none of them was taking any medication at the time they were enrolled. The gestational age was determined according to the date of the last menstrual period. The study was approved by the institutional review board of each of the participating outpatient clinics and all subjects gave written informed consent before entering the study.

Upon enrollment, physicians completed a questionnaire including sociodemographic and obstetric characteristics. A properly qualified nutritionist evaluated the calcium daily intake of the enrolled pregnant women.[13,14] The participants were allocated in two random groups: the supplemented group ($n = 24$) who received a daily oral dose of 1484 mg of calcium carbonate (600 mg of elemental calcium) and 450 mg of CLA, and the control group ($n = 24$) who received placebo pills (600 mg of lactose and 450 mg of starch with a calorie content of 5 kcal per capsule). The CLA refers to a mixture of positional and geometric conjugated dieonic isomers of linoleic acid, specifically the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. The CLA was produced by Pharmanutrients Inc., Lake Bluff, IL. The dose of calcium was calculated from the differences between physiologic needs and the calculated oral intake in the study populations, whereas the linoleic acid was calculated on the basis of substrate for the production of prostaglandins.[11] There were no differences in weight, size, flavor, or color between treatment and placebo pills. Eligible women were assigned to groups by opening the sequentially numbered, sealed, opaque envelope containing a card that indicated the study allocation. These random cards were prepared and sealed by an independent administrative staff member using a random number table prepared with the True Epistat statistical package version 5.0 (Epistat Services, Richardson, TX).

The women were instructed to take the pills at the same time every morning until delivery. Compliance was assessed at each antenatal visit answering a questionnaire and counting the remaining tablets in the bottle. All the patients received the standard antenatal monthly visit until the 36th week of gestation and twice a month thereafter.

A follow-up to term was made and the medical records were reviewed for each patient to determine the presence of PIH. Pregnancy-induced hypertension was defined by the presence of gestational hypertension (GH) or PE. Preeclampsia was defined as normotension before the 20th week of gestation with the subsequent development of hypertension (>140/90 mm Hg) and significant concomitant 24-h proteinuria (>0.3 g/L) in the absence of urinary tract infection. Gestational hypertension was defined as de novo hypertension arising after midpregnancy without proteinuria. At each visit, BP was measured twice, according to the criteria proposed by the American College of Obstetricians and Gynecologists. Blood pressure measures were obtained by Korotkoff's method and the mean values of two consecutive measurements were used for analysis. The standard mercury sphygmomanometers were calibrated every 3 weeks.

Blood samples and nutritional inventory were obtained after calcium/placebo administration before delivery. All the members of the research team received adequate training and were working under common standard operating procedures in all the centers.

Biochemical Analysis

Blood samples were collected from the antecubital vein at inclusion time and after supplementation at the end of pregnancy before delivery or treatment for PIH. Plasma was separated immediately by centrifugation, fractionated into several aliquots, and stored frozen at −80°C until use.

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-hypaque (Pharmacia & Upjohn, Bridgewater, NY) density gradient centrifugation and used to measure the intracellular ionized free calcium concentrations in each local center using a method described elsewhere.[15] Briefly, after PBMC separation, cells were washed in Hank's balanced salt solution containing 1% fetal calf serum and were suspended at a concentration of 10^7 cell/mL. Cells were then incubated for 30 min at 37°C in a solution containing a final concentration of Fluo-3/AM (4 μg/mL) and FuraRed (10 μg/mL). Next, PBMC were diluted in 4 mL of Hank's balanced salt solution containing 1% fetal calf serum incubated for 10 min at 37°C and then were washed, diluted again, and kept at

Table 1. Sociodemographic characteristics of the primigravidas at high risk of developing PIH

	Calcium–CLA (*n* = 24)	Placebo (*n* = 24)	*P*
Age (y)	23.7 ± 7.0	22.7 ± 7.8	.2
Recruitment country			
Colombia/Bangladesh	9/15	9/15	
Ethnicity			.53
Black	1 (4.1%)	3 (12.5%)	
White	8 (33.3%)	6 (25.0%)	
Other	15 (62.5%)	15 (62.5%)	
Education			.31
Basic	3 (12.5%)	2 (8.3%)	
Secondary	10 (41.7%)	18 (75.0%)	
University	11 (45.8%)	4 (16.6%)	
Residence			
Urban	24 (100.0%)	24 (100.0%)	
Socioeconomic level			.21
Low (1,2 levels)	8 (33.3%)	12 (50.0%)	
Medium (3,4 levels)	15 (62.5%)	12 (50%)	
High (5,6 levels)	1 (4.2%)	0	
Gestational age at entry (wk)	19.7 ± 1.7	20.0 ± 1.9	.44
Body mass index	22.5 ± 2.9	21.4 ± 4.0	.05
Plasma levels			
Calcium (mmol/L)	2.17 ± 0.27	2.12 ± 0.35	.53
Creatinine (μmol/L)	58.7 ± 15.2	53.3 ± 7.6	.53
Anemia (Hb <100 g/L)	13 (54.1%)	14 (58.3%)	.77
Family history			
Mother with preeclampsia	13 (54.1%)	14 (58.3%)	.77
Sister with preeclampsia	11 (45.8%)	10 (41.7%)	.77
Hypertension	16 (66.6%)	19 (79.1%)	.32
Diabetes mellitus	9 (37.5%)	6 (25.0%)	.35

PIH = pregnancy-induced hypertension.

Data are presented as *n* (%) and means ± SD. Socioeconomic level: classification from the National Department of Statistics of Colombia and Bangladesh (1–6 range).

room temperature for 15 min in the dark. The PBMC were maintained at 37°C until analysis by flow cytometry using a read FACSort cytometer (Series B 0266; Becton Dickinson, San Jose, CA). The analyses were focused on the lymphocyte subpopulation that was selected using granularity (SSC) and size (FSC) parameters.

Standardized methods were used to determine serum levels of PGE_2, renin,[16] angiotensin II,[17] parathormone,[18] and calcitonin[19] by radioimmunoassay (RIA) technique in the Shanghai Medical University, Shanghai (China), using reagents from Dia Sorin Inc, MN, Beijing North Institute of Biological Technology, Beijing, China and Nanjing Biological Engineering Research Institute, Nanjing, China.

Statistical Analysis

A sample of 24 women per group was calculated to demonstrate statistical significance of a relative reduction of 75% in the rate of PIH (from 50% to 13%) with an α of 0.1 and β of 0.2 assumptions based on previous studies with pregnant women at high risk of developing PIH[11] (relative risk [RR] = 0.25, 95% confidence interval [95% CI] 0.09–0.69). Differences between continuous variables were evaluated with the two-tailed test for variables that were normally distributed and the Kruskall-Wallis test for variables that were not normally distributed. The χ^2 or Fischer's exact test (if the smallest value is under 5 in any

Table 2. Daily intake at inclusion of the primigravidas at high risk of developing PIH

	Calcium–CLA (*n* = 24)	Placebo (*n* = 24)	*P*
Energy (kcal/d)	1666.1 (1196–2583)	1722.7 (995–2919)	.72
Proteins (g/d)	71.4 (46.4–145.0)	70.06 (46.5–145.1)	.57
Calcium (mg/d)	601.5 (310–1101)	576.0 (314–936)	.75
Iron (mg/d)	14.7 (7.11–25.5)	16.3 (9.2–39)	.28

Abbreviation as in Table 1.

Data are presented as median (range) and means ± SD.

one of the cells) was used to analyze differences in categorical variables. The RR of PIH and its 95% CI were also calculated using the Epiinfo statistical package (version 6.0 Centers for Disease Control and Prevention, Atlanta, GA). Statistical significance was estimated as $P < .05$.

Results

Between March 2001 and March 2003, 220 primigravid women of reproductive ages and with a family history of PE were selected and screened for abnormal Doppler ultrasound in uterine or arcuate arteries (diastolic notch) from week 18 to 22 of gestation. The eco-Doppler ultrasound was positive in 53 (24%) women (21 in uterine arteries and 32 in arcuate arteries). Three eligible women refused to participate in the study. The 50 women who agreed to participate received oral information about the study and signed informed consent forms. One woman from the control group was lost during the follow-up (change of residence) and one woman from the supplemented group withdrew. The final analysis was based on 24 women treated with calcium and CLA and 24 with placebo. At study entry, groups were comparable with respect to sociodemographic characteristics (age, ethnicity, socioeconomic status, educational level, and residence) and obstetric risk factors (gestational age at inclusion, body mass index [BMI], anemia, and family history of PE) (Table 1). There were no significant differences in the average daily diet (Table 2). No differences were found in basal values of systolic (106.6 ± 7.4 mm Hg *v* 104.4 ± 8.3 mm Hg) and diastolic BP (71.6 ± 6.2 mm Hg *v* 68.1 ± 8.7 mm Hg) ($P = .1$) between both groups.

The mean BP at delivery was significantly higher in the placebo group (systolic BP, 122.2 ± 21.2 mm Hg *v* 116 ± 9.6 mm Hg, $P < .01$; diastolic BP, 84.3 ± 10.9 mm Hg *v* 76.3 ± 7.8 mm Hg, $P < .01$).

Ten women (41.7%) (7 GH and 3 PE) from the placebo group developed PIH compared with 2 (8.3%) from the supplemented group. No woman from the supplemented group met the criteria for PE diagnosis. The overall incidence of PIH was significantly reduced in women receiving the calcium–CLA supplement (RR = 0.20, 95% CI 0.05–0.82, $P = .01$). Gestational hypertension was treated with bed rest and oral nifedipine. Fifty percent of the patients with GH had cesarean delivery. The three patients with PE had cesarean section, 2 were treated with magnesium sulfate and oral nifedipine and 1 with alpha-methyldopa and diazepam.

The compliance of the treatment was similar in both groups (80% *v* 81%). The spontaneous or induced reported side effects attributed to their treatment were 1/24 (4%) in both groups (flatulence, gastritis). The levels of creatinine in plasma were not affected during pregnancy in both groups (supplemented, 0.77 ± 0.24 mg/dL *v* 0.7 ± 0.14 mg/dL, $P = .58$; control, 0.71 ± 0.10 mg/dL *v* 0.77 ± 0.16 mg/dL, $P = .56$). The mean ratio between calcium and creatinine increased in the supplemented group (11.9 ±

Table 3. Serum prostanoids, calciotropic hormones, renin, angiotensin, and plasma-ionized calcium in primigravidas at high risk of developing PIH

	Calcium–CLA (*n* = 24)			Placebo (*n* = 24)		
	Basal	Post supplementation	*P*	Basal	Post supplementation	*P*
Prostaglandin E_2 (pg/mL)	16.4 (8.4–62.0)	11.7 (7.4–35.3)	.23	11.1 (5.6–37.0)	12.2 (4.9–48.1)	.65
Renin (ng/mL/h)	3.1 (0.43–32.3)	2.5 (1.3–23.1)	.39	1.86 (0.71–31.7)	1.59 (0.30–37.7)	.46
Angiotensin II (pmol/L)	212.54 (42.2–636.1)	140.6 (65.0–554.9)	.72	111.7 (56.4–713.7)	190.5 (31.1–726.9)	.36
Parathormone (ng/L)	17.6 (6.4–54.7)	16.2 (3.7–33.7)	.35	19.8 (5.4–61.6)	19.9 (3.8–105.0)	.84
Calcitonin (ng/L)	64.6 (26.5–102.7)	61.2 (28.6–101.0)	.86	59.9 (43.2–86.8)	59.5 (44.9–98.8)	.96
Plasma-ionized Calcium (mmol/L)	1.18 ± 0.03	1.19 ± 0.02	.82	1.18 ± 0.03	1.19 ± 0.02	.86

Abbreviation as in Table 1.
Data are presented as median (range) and mean ± SD.

2.7 *v* 13.6 ± 4.4, *P*= .005) but no differences were observed in the placebo group (12.1 ± 2.5 *v* 12.1 ± 2.3).

There were no significant differences in the median of PGE_2, renin, angiotensin II, parathormone, and calcitonine before and after interventions in both groups (Table 3). The plasma levels of ionized calcium were similar in both groups before and after interventions (Table 3). In the supplemented group the concentration of intracellular ionized free calcium decreased (92.0 nmol/L [range, 62.5 to 220 nmol/L] *v* 62.5 nmol/L [range, 28 to 200 nmol/L], $P = .01$) after intervention, but there were no changes in the placebo group (Fig. 1). The women who developed PIH ($n = 12$) presented a significant increase in intracellular ionized free calcium concentrations in peripheral blood lymphocytes after interventions (120 nmol/L [range, 89.2 to 240 nmol/L] *v* 137.5 nmol/L [range, 89.2 to 138 nmol/L], $P = .020$). In the analysis by treatment group, the women who remained normotensive in the calcium–CLA group presented a statistically significant decrease in intracellular ionized free calcium concentrations in peripheral blood lymphocytes after supplementation, whereas women who developed PIH in the placebo group showed an increase in intracellular calcium concentrations in these cells (Fig. 2). In the supplemented group the correlation index (R^2) between PGE_2 and renin increased from 0.01 (95% CI −0.39–0.41, $P = .25$) to 0.55 (95% CI 0.27–0.72, $P < .001$) ($P = .02$) and between PGE_2 and angiotensin II increased from 0.09 (95% CI −0.32–0.47, $P = .09$) to 0.46 (95% CI 0.05–0.72, $P = .001$). No other significant correlations were observed.

The birth weight was significantly greater in the supplemented group (2979 ± 448 g *v* 2705 ± 433 g, $P < .001$) (Table 4). There were no significant differences in the gestational age at delivery between both groups (38.8 ± 2.2 *v* 38.6 ± 1.6 weeks, $P = .61$).

Discussion

The pregnant women included in this clinical trial were healthy women at high risk of developing PE, identified for well-known epidemiologic factors such as primigravids younger than 19 or older than 35 years, low income, antecedents of PE in mother or sister, and presence of diastolic notch by Doppler ultrasound in uterine or arcuate arteries.[20] In these high risk women the supplementation with calcium and CLA reduced the incidence of PIH.

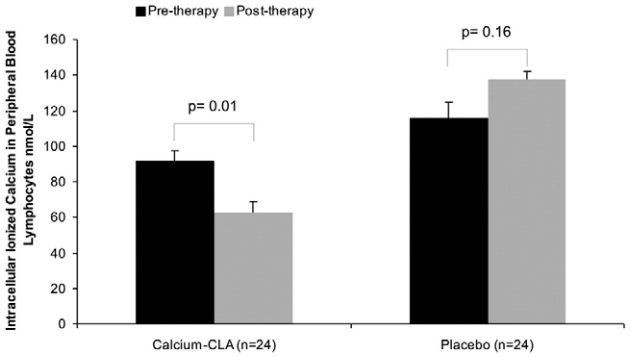

FIG. 1. Intracellular ionized free calcium concentrations in peripheral blood lymphocytes of included pregnant women before and after administration of calcium–CLA or placebo. Intracellular ionized free calcium was significantly decreased after supplementation with calcium and CLA ($P = .01$).

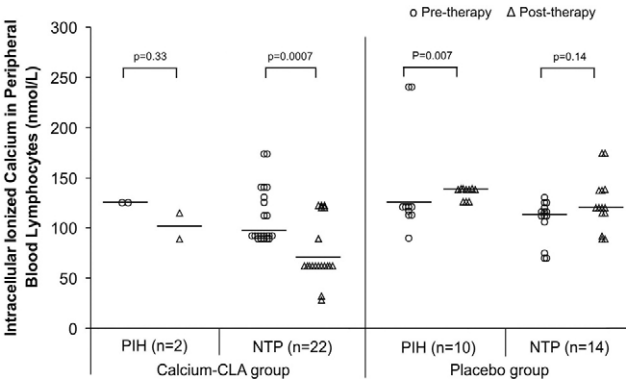

FIG. 2. Intracellular ionized free calcium concentrations in peripheral blood lymphocytes of included pregnant women who developed pregnancy-induced hypertension or remained normotensive before and after administration of calcium–CLA or placebo. Women who remained normotensive in the calcium–CLA group presented a statistically significant decrease in intracellular ionized free calcium concentrations after supplementation ($P = .0007$), whereas women who developed pregnancy-induced hypertension in the placebo group showed an increase in intracellular calcium concentrations ($P = .007$). Mean intracellular free calcium concentration and individuals values for each group are shown.

During pregnancy, there is a great demand for calcium intake to respond to the higher demands caused by the process of fetal bone formation. Furthermore, there is a dilution of the cation due to the expanded extracellular fluid volume, and to the normal hypercalciuria of pregnancy consequent to increased glomerular filtration. Thus, serum-ionized calcium concentrations depend on an adequate calcium intake.[8] In the present study, calcium nutrient intake was low at the beginning of the study and during the follow-up period in both groups (601 *v* 602 mg/d and 576 *v* 673 mg/d, respectively).

Previously, it was reported that preeclamptic women present a reduction in extracellular calcium concentration with low levels of the serum-ionized calcium and abnormal calciotropic hormone levels.[21] Moreover, it was proposed that the beneficial effects of calcium supplementation in the prevention of PIH could be related with the maintenance of the plasma-ionized calcium levels within the narrow physiologic ranges.[22] The concentration of extracellular-ionized calcium is crucial for the synthesis in the endothelium of vasoactive substances, such as prostacyclin and nitric oxide.[23,24] The creatinine plasma levels were not affected during pregnancy in both groups; however, the plasma ratio of calcium-to-creatinine in the supplemented group increased significantly, whereas in the control group it did not change. These results suggest that

Table 4. Perinatal outcomes in the primigravidas in high risk of developing PIH

	Calcium–CLA (*n* = 24)	Placebo (*n* = 24)	*P*
Cesarean sections	8 (33.3%)	14 (58.3%)	.08
Birth weight (g)	2979 ± 448	2705 ± 433	<.001
Small newborns (<10th percentile)	1 (4.1%)	4 (16.6%)	.31
Low (<2500 g)	1 (4.1%)	5 (20.8%)	.17
Respiratory distress	2 (8.3%)	0	.44
Preterm delivery	1 (4.1%)	2 (8.3%)	.88
Premature rupture of membranes	2 (8.3%)	4 (16.6%)	.60
Perinatal mortality	1 (4.1%)	1 (4.1%)	.47
Cause	Extreme premature	Genetic malformation	

Data are presented as *n* (%) and means ± SD.

the supplemented calcium was absorbed. However, we cannot confirm this fact because we did not measure the urinary calcium excretion. The levels of plasma-ionized calcium were within the physiologic ranges in both groups. Thus, it is unlikely that the preventing effect of calcium supplementation could be related to the plasma concentration of ionized calcium. The discrepancy of these results with previous clinical trials[8] in the behavior of the plasma-ionized calcium in the calcium–CLA group may be related with the quantity of the calcium supplemented. In the previous study the supplemented group was given 2 g/d of elemental calcium, but in the present study the supplementation was only 600 mg/d. Moreover, these results could explain the lack of significant differences in the calciotropic hormones between the supplemented and the placebo groups. The secretion and biosynthesis of parathormone and calcitonin are regulated by the concentration of plasma-ionized calcium. Thus, parathormone is secreted when the plasma-ionized calcium decreases below the parathyroid gland set point of about 1.10 mmol/L.[25]

Therefore, the reduction of intracellular free calcium in peripheral blood lymphocytes in the pregnant women who received low doses of calcium and CLA could not be attributed to the effect of calciotropic hormones. In patients with high BP, an inverse correlation between intracellular platelet calcium and plasma-ionized calcium has been observed, supporting the hypothesis that hypertensive individuals may possess a factor that allows intracellular calcium to be elevated beyond the expected values based on extracellular calcium levels.[26] Moreover, higher intracellular free calcium concentrations have been observed in lymphocytes obtained from women with PE but not from normotensive pregnant women or pregnant patients with chronic hypertension.[27]

The concentration of intracellular free calcium is increased by the opening of cell membrane channels, mobilization of intracellular deposits, or by limitation of cellular extrusion.[28] Several studies have investigated the role of angiotensin II as an agonist for receptor-mediated intracellular calcium transients in vascular smooth muscle.[28] These studies have consistently shown an increase of intracellular free calcium concentration in platelets and lymphocytes in response to stimulation with angiotensin II and vasopressin in patients with PE.[6] In this context, our observations that the women in the supplemented group presented with lower intracellular free calcium concentration in peripheral lymphocytes and that women who developed PIH presented with a significant increase in intracellular calcium concentrations in these cells, suggesting an alteration in the membrane calcium transport, which is corrected by the calcium–CLA supplementation. Also, it is not possible to discard the fact that the supplementation with CLA could interact with calcium to reduce the intracellular ionized free calcium concentrations.

Previously, we have demonstrated[11] that linoleic acid plus calcium supplementation in pregnant women decreases the risk of PIH and increases the formation of PGE_2. However, in the present study using calcium–CLA supplementation, we observed no significant decrease in the concentration of PGE_2 in the supplemented group. This contradictory result could be explained because CLA is a mixture of positional and geometric double bonded isomers of eicosanoids, in most cases biologically inactive. Hence, these isomers of linoleic acid, unlike linoleic acid, are likely not efficient substrates or precursors for metabolism by cyclooxygenase or lipoxygenase to generate bioactive eicosanoids. This view is supported by experimental studies demonstrating that the supplementation with CLA reduced PGE_2 formation.[12]

In conclusion, our results demonstrate that the administration of low doses of oral calcium and CLA during pregnancy significantly reduces intracellular free calcium concentration in peripheral blood lymphocytes. This finding could explain the beneficial effect of the nutritional intervention in reducing the incidence of PIH. Moreover, our results could be related with the well-known effects of the calcium channel blockers, especially nifedipine, as safe and effective therapeutic agents in controlling BP in PIH.[29,30] Additional studies are needed to elucidate the specific mechanisms by which calcium and CLA supplementation reduces intracellular ionized free calcium concentrations in lymphocytes and other cell types in pregnant patients.

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References

1. López-Jaramillo P, Garcia RG, López M: Preventing pregnancy induced hypertension: are there regional differences for this global problem? J Hypertens 2005;23:1121–1129.
2. López-Jaramillo P: Calcium, nitric oxide, and preeclampsia. Semin Perinatol 2000;24:33–36.
3. Moncada S, López-Jaramillo P: Actividad Biológica del Endotelio Vascular. Investigación y Ciencia. Scientific American 1991;176: 60–66.
4. Dusting GJ, Moncada S, Vane JR: Prostacycline: its biosynthesis, actions, and clinical potential. Adv Prostaglandin Thromboxane Leukot Res 1982;10:59–67.
5. Zemel MB, Zemel PC, Berry S, Norman G, Kowalczyk C, Sokol RJ, Standley PR, Walsh MF, Sowers JR: Altered platelet calcium metabolism as an early predictor of increased peripheral vascular resistance and preeclampsia in urban black women. N Engl J Med 1990;323:434–438.
6. Haller H, Oeney T, Hauck U, Distler A, Philipp T: Increased intracellular free calcium and sensitivity to angiotensin II in platelets of preeclamptic women. Am J Hypertens 1989;2:238–243.
7. Kilby MD, Broughton Pipkin F, Cockbill S, Heptinstall S, Symonds EM: A cross-sectional study of basal platelet intracellular free calcium concentration in normotensive and hypertensive primigravid pregnancies. Clin Sci (Lond) 1990;78:75–80.
8. 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;96:648–655.
9. López-Jaramillo P, Narvaez M, Felix C, Lopez A: Dietary calcium supplementation and prevention of pregnancy-induced hypertension. Lancet 1990;335:293–300.
10. López-Jaramillo P, Delgado F, Jacome P, Teran E, Ruano C, Rivera J: Calcium supplementation and the risk of preeclampsia in ecuadorian pregnant teenagers. Obstet Gynecol 1997;90:162–167.
11. Herrera JA, Arevalo-Herrera M, Herrera S: Prevention of preeclampsia by linoleic acid and calcium supplementation. Obstet Gynecol 1998;91:585–590.
12. Li Y, Watkins BA: Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 1998;33:417–425.
13. Cameron M: 24-hour recall, in Cameron M, Staveren W, Loken E (eds): Manual on Methodology for Food Consumption Studies. Oxford, Oxford University Press, 1988, pp 83–88.
14. Haraldsdottir J: Food frequency, in Cameron M, Staveren W, Loken E (eds): Manual on Methodology for Food Consumption Studies. Oxford, Oxford University Press, 1988, pp 92–95.
15. Novak EJ, Rabinovich PS: Improved sensitivity in flow cytometric intracellular ionized calcium measurement using fluo 3/fura red fluorescence ratios. Cytometry 1994;17:135–141.
16. Fyhrquist F, Soveri P, Puutula L, Stenman UH: Radioimmunoassay of plasma renin activity. Clin Chem 1976;22:250–256.
17. Fyhrquist F, Kala R, Standertskiold-Nordensta, Eisalo A: Angiotensin II plasma levels in hypertensive patients. Acta Med Scand 1972;192:507–511.
18. Armitage EK: Parathyrin (parathyroid hormone): metabolism and methods for assay. Clin Chem 1986;32:418–424.
19. Abe K, Adachi I, Miyakawa S, Tanaka M, Yamaguchi K, Tanaka N, Kameya T, Shimosato Y: Production of calcitonin, adrenocorticotropic hormone, and beta-melanocyte-stimulating hormone in tumors derived from amine precursor uptake and decarboxylation cells. Cancer Res 1977;37:4190–4194.
20. 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: 330–338.
21. Villar J, Belizan J: Same nutrient, different hypotheses: disparities in trials of calcium supplementation during pregnancy. Am J Clin Nutr 2000;71:1375–1379.
22. Seeley EW, Wood RJ, Brown EM, Graves SW: Lower serum ionized calcium and abnormal calciotropic hormone levels in preeclampsia. J Clin Endocrinol Metab 1992;74:1436–1440.
23. López-Jaramillo P, Guarner F, Moncada S: Effects of calcium and parathyroid hormone on prostacyclin synthesis by vascular tissue. Life Sci 1987;40:983–986.
24. López-Jaramillo P, González MC, Palmer RMJ: The crucial role of physiological Ca2+ concentrations in the production of endothelial nitric oxide and the control of vascular tone. Br J Pharmacol 1990;101:489–493.
25. Osborne CG, McTyre RB, Dudek J, Roche KE, Scheuplein R, Silverstein B, Weinberg MS, Salkeld AA: Evidence for the relationship of calcium to blood pressure. Nutr Rev 1996;54:365–382.
26. Baker PN, Kilby MD, Broughton FP, Pipkin F: The effect of angiotensin II on platelet intracellular free calcium concentration in human pregnancy. J Hypertens 1992;10:55–60.
27. Hojo M, Suthanthiran M, Helseth G, August P: Lymphocyte intracellular free calcium concentration is increased in preeclampsia. Am J Obstet Gynecol 1999;180:1209–1214.
28. Dostal DE, Murahashi T, Peach MJ: Regulation of cytosolic calcium by angiotensin in vascular smooth muscle. Hypertension 1990;15: 815–822.
29. Aali BS, Nejad SS: Nifedipine or hydralazine as a first-line agent to control hypertension in severe preeclampsia. Acta Obstet Gynecol Scand 2002;81:25–30.
30. Hall DR, Odendaal HJ, Steyn DW, Smith M: Nifedipine or prazosin as a second agent to control early severe hypertension in pregnancy: a randomised controlled trial. BJOG 2000;107:759–765.