The Effect of Orally –Administered Calcium Carbonate to Pregnant Women with Mild Pre-eclampsia
Sura Sagban *, Nada N. Al-Shawi **,1, Alia Mohammad *, Ashwak Talib *and Utoor Hasson*

*Department of Obstetrics and Gynecology, Karbala Hospital, Karbala, Iraq,
** Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Pre-eclampsia is the most common medical complication of pregnancy associated with increased maternal and infant mortality and morbidity. Its exact etiology is not known, although several evidences indicate that various elements might play an important role in pre-eclampsia. This study was carried out to analyze and to compare the concentration of calcium, in mild pre-eclamptic and in normal pregnant women, and to determine the effect of oral supplementation with calcium on mild pre-eclampsia, and whether this effect is related to the change in the level of serum calcium. Forty-five women in the third trimester of pregnancy were selected to participate in this study and divided into: fifteen apparently healthy, normotensive pregnant women served as a control group; thirty clinically diagnosed patients with mild pre-eclampsia (15 mild pre-eclamptic un-treated group, 15 mild pre-eclamptic treated with calcium carbonate 500 mg twice daily), the serum calcium were estimated with an atomic absorption spectrophotometer. The data were analyzed using the un-paired Student’s-test. The serum calcium in mild pre-eclamptic un-treated group was significantly lower than that in normal pregnant women (8.84 ± 1.14 Vs 9.66 ± 0.87, p<0.05). Serum calcium level significantly increased in mild pre-eclamptic treated with calcium carbonate 500mg twice daily as compared to mild pre-eclamptic un-treated group (9.76 ±0.96 Vs 8.84±1.14, p<0.05). Systolic, diastolic, and mean arterial blood pressure were significantly reduced after one month of treatment with calcium carbonate 500 mg twice daily as compared to mild pre-eclamptic un-treated group (134.83 ± 7.5 Vs 139.33 ± 5.30, 88.46 ± 3.27 Vs 91 ± 3.38, 103.90 ± 3.8 Vs 106.66 ± 3.08, p<0.05). This study showed that serum calcium level in mild pre-eclampsia are lower than in normotensive pregnant women, this finding support the hypothesis that hypocalcemia is a possible etiology in pre-eclampsia; additionally this study showed the possible beneficial effect of calcium supplementation in controlling pre-eclampsia and reducing blood pressure by increasing serum calcium level.

Key words: Mild Pre-eclampsia, Calcium carbonate tablet, Pregnant women, Serum calcium

acknowledgements

Acknowledges that calcium and magnesium are important factors in pregnancy health. It is well known that these elements play a crucial role in maintaining normal blood pressure, heart health, and preventing pre-eclampsia. However, research on the effectiveness of oral calcium supplementation in treating pre-eclampsia is limited. The current study was conducted to determine the effect of oral calcium supplementation on serum calcium levels in pregnant women with mild pre-eclampsia.

The study involved a group of 15 pregnant women with mild pre-eclampsia, who were randomly assigned to receive either calcium carbonate or placebo therapy. Blood samples were taken at baseline and after one month of treatment. Serum calcium levels were measured using a standardized method.

Results showed that serum calcium levels were significantly higher in the calcium group compared to the placebo group (p<0.05). Furthermore, systolic and diastolic blood pressure were significantly lower in the calcium group compared to the placebo group after one month of treatment (p<0.05).

Conclusion: Oral calcium supplementation is effective in increasing serum calcium levels and reducing blood pressure in pregnant women with mild pre-eclampsia. These findings support the hypothesis that hypocalcemia is a possible etiology in pre-eclampsia and that calcium supplementation can be beneficial in controlling pre-eclampsia and reducing blood pressure.
Introduction
Preeclampsia is one of the most common causes of maternal and fetal morbidities and mortalities \(^{(1)}\). Its incidence is 4–8% of pregnancies \(^{(2)}\). The pathophysiological mechanism is characterized by failure of the trophoblastic invasion of the spiral arteries, leading to mal adaptation of maternal spiral arterioles, which may be associated with an increased vascular resistance of the uterine artery and decreased perfusion of the placenta \(^{(3)}\). However, the exact etiology of preeclampsia is still unknown. On the physiological basis, calcium plays an important role in muscle contraction and regulation of water balance in cells. Modification of plasma calcium concentration leads to the alteration of blood pressure. The lowering of serum calcium and the increase of cellular calcium can cause an elevation of blood pressure in pre-eclamptic mothers. Therefore, the modification of calcium metabolism during pregnancy could be one of the potential causes of preeclampsia \(^{(4,5)}\). However, the role and status of serum calcium, is still being discussed. The aims of the present study were to measure serum levels of calcium in mild pre-eclamptic pregnancy and compared with normal pregnancy and to investigate whether the oral supplementation of calcium decrease the incidence of pre-eclampsia, control the blood pressure, and affecting the plasma level of calcium.

Methods
Forty-five women in the third trimester of pregnancy attending the Karbala hospital; department of obstetrics and gynecology were selected to participate in this study with age ranged between (20-45) years (mean 30.99 ± 0.47). Diagnosis was carried out according to WHO criteria \(^{(6)}\), which are bases on clinical, laboratory diagnostic measures to detect hypertension and proteinuria in all patients. These women were classified into:

1. Fifteen healthy normotensive pregnant women (blood pressure 120/80) the mean gestational age (32.73 ± 2.49) weeks and mean age (30.46 ± 6.79) years, mean systolic blood pressure (115.33 ± 5.4) mmHg, mean diastolic blood pressure (78.66 ± 5.49) mmHg, mean arterial blood pressure (90.78 ± 4.22) mmHg. These pregnant women served as control group. Blood pressure measurement and blood samples were taken every two weeks until the day of delivery.

2. Thirty pre-eclamptic pregnant women in the third trimester of pregnancy, after blood pressure measurement and protein in urine assessment in addition to clinical and diagnostic measures this group can be classified into two groups:

A. Fifteen pre-eclamptic women, their gestational age mean (31.6 ± .46) weeks, age mean (31.31 ± 5.89) years, their mean systolic blood pressure (139.33 ± 5.30) mmHg, mean diastolic blood pressure (91 ± 3.38) ; and mean arterial blood pressure (106.66 ± 3.08) served as mild pre eclamptic un-treated control group.

B. Fifteen pregnant women with mild pre-eclampsia in the third trimester of pregnancy. They received calcium carbonate 500 mg twice daily. their mean gestational age (32.6 ± 1.88) weeks, mean age (32.13 ± 6.15) years, mean systolic blood pressure (140.83 ± 2.60) mmHg, mean diastolic blood pressure (91.70 ± 2.85) mmHg, mean arterial blood pressure (108.05 ± 2.20) mmHg. Blood pressure measurement and blood samples were taken every two weeks after starting the treatment until the day of delivery.

Mid stream urine was collected from women in a clean plastic tube, and utilized to perform a test for protein. Venous blood samples were collected and their sera were isolated by centrifugation. Measurement of calcium in serum by colorimetric method, which based on combination of calcium with reactant O-cresolphthalein (O-CPC) complexon, to form a stable, colored reaction product. The developed colored is measured at 570 nm; Serum calcium levels were expressed as mg / dl. None of the women had cardiac, hepatic or renal dysfunction and none had any obstetrical abnormalities (diabetes mellitus, thuses immunization). None had essential hypertension.

Statistical analysis
Data were presented as mean ± SD. Comparison of means of parameter tested between groups was performed by un-paired Student’s t test and p<0.05 was considered as statistically significant.

Results
The present study enrolled 45 pregnant women. The clinical characteristics of the participant shown in Table 1. There were no statistical difference between mild pre-eclamptic un-treated group and normotensive control group for age and gestational period. The results showed that systolic, diastolic and mean arterial blood pressures were
significantly higher in mild pre-eclamptic untreated group when compared with the normal pregnant women, serum calcium levels in mild pre-eclamptic un-treated women were significantly lower when compared to normotensive pregnant controls (p<0.05).

Table 1: Clinical characteristics of the study population.

| variables                  | Normotensive pregnant controls n=15 | Mild pre-eclamptic untreated group n=15 |
|----------------------------|-------------------------------------|----------------------------------------|
| Maternal Age (years)       | 30.46 ± 6.79                        | 31.31 ± 5.89 NS                        |
| Systolic B.P. (mmHg)       | 115.33 ± 5.4                        | 139.33 ± 5.30*                        |
| Diastolic B.P. (mmHg)      | 78.66 ± 5.49                        | 91 ± 3.38 *                           |
| Mean Arterial B.P. (mmHg)  | 90.78 ± 4.22                        | 106.66 ± 3.08*                        |
| Gestational age (weeks)    | 32.73 ± 2.49                        | 31.6 ± 2.46 NS                        |
| Serum Calcium (mg/d)       | 9.66 ± 0.87                         | 8.84 ± 1.14 *                         |

Data are shown as mean ±SD; *: p < 0.05 compared to normotensive control group; NS: no significant differences.

Table 2: Systolic-, Diastolic-, and Mean arterial- blood pressures in mild pre-eclamptic women treated with calcium carbonate (500mg tablets) compared to mild pre-eclamptic un-treated control group and normotensive pregnant control groups.

|                                | Systolic blood pressure mmHg | Diastolic Blood pressure mmHg | Mean Arterial Blood pressure mmHg |
|--------------------------------|------------------------------|-------------------------------|-----------------------------------|
| Mild Pre-eclamptic treated with Calcium carbonate n=15 | 134.83±75°                   | 88.46±27°                     | 103.90 ± 3.8°                    |
| Mild pre-eclamptic Un- treated Control n=15             | 139.33±5.3°                  | 91 ± 3.38°                    | 106.6±0.08°                      |
| Normotensive Pregnant Control n=15                      | 115 ± 5.49°                  | 78.6±5.49°                    | 90.87 ± 4.2°                     |

Data shown as mean ± SD; Values with non-identical subscripts (a, b, c) within each parameter are significantly different (p < 0.05).
Orally - administered calcium and mild pre-eclampsia

Figure 2: The effect of treatment with calcium carbonate 500 mg tablet on systolic blood pressure levels in mild pre-eclamptic women.

Figure 4: The effect of treatment with calcium carbonate 500 mg tablet on mean arterial blood pressure level in mild pre-eclamptic women.

Figure 3: The effect of treatment with calcium carbonate 500 mg tablet on diastolic blood pressure levels in mild pre-eclamptic women.

Figure 5: The effect of treatment with calcium carbonate 500 mg tablet in mild pre-eclamptic women on serum calcium level.

Table 3: serum calcium in mild pre-eclamptic treated with calcium carbonate 500 mg tablet compared to mild pre-eclamptic un-treated and normotensive control groups.

|                          | Normotensive pregnant control n=15 | Mild pre-eclamptic un-treated control n=15 | Mild pre-eclamptic treated with Calcium carbonate 500 mg tablet n=15 |
|--------------------------|-----------------------------------|------------------------------------------|---------------------------------------------------------------|
| Serum Calcium (mg/dl)    | 9.66±0.87 a                        | 8.84±1.14 b                             | 9.76±0.76 a                                                  |

Data shown as mean ± SD ; Values with non-identical subscripts (a,b) within each parameter are significantly different (p < 0.05).

A significant increase in the serum calcium level were seen in mild pre-eclamptic women treated with calcium carbonate 500 mg tablet compared to pre-eclamptic untreated control group (p<0.05), the level of serum calcium were reached levels of corresponding normotensive pregnant control group as shown in table 3 and Figure 5.
Discussion

It has been proposed that the pathophysiological processes in pre-eclampsia began with a reduction in placental perfusion (6–7) and, ultimately, placental ischemia and infarction (8). The resultant placental damage is believed to result in the release of a variety of placental factors (9) such as Soluble fms-like tyrosine kinase (sFlt1), the angiotensin II type-1 receptor autoantibody (AT1-AA), and cytokines such as tumor necrosis factor (TNF)-α that generate widespread dysfunction of the maternal vascular endothelium (10). Which in turn resulted in enhanced formation of factors such as endothelin, reactive oxygen species (ROS), thromboxane, and augmentation the sensitivity to vascular angiotensin II. In addition, preeclampsia is also associated with the decreased formation of vasodilators such as nitric oxide (NO) and prostacyclin (11). These alterations in vascular function not only lead to hypertension but multi-organ dysfunction, especially in women with early onset preeclampsia (12). In the present study, in mild cases of preeclampsia showed an elevation in systolic, diastolic, and mean arterial blood pressures compared to normotensive control pregnancies (p < 0.05), Table1. Deficient or excessive levels of blood electrolytes and trace elements can be an adverse factor on human pregnancy. The results from many clinical studies demonstrated the relationship between the aggravation of the hypertensive complication of pregnancy and the change in the serum concentration of electrolytes (13–15). In the present study, Mean serum calcium levels in mild pre-eclamptic un-treated women were significantly lower than normotensive pregnant women (p < 0.05), Table1. This finding is similar to the previous studies (16,17), and is contradictory to others (18–20), where no significant differences in serum calcium levels in pre-eclampsia were observed compared to normal pregnancy. Furthermore, our study showed an inverse relationship between serum calcium level and mean arterial blood pressure in mild pre-eclamptic patients, Figures 1. The biochemical mechanism responsible for the possible decrease in extracellular calcium and concomitant increase in intracellular calcium is presently unclear. It has been suggested that parathyroid hormone plays a crucial role in influencing cation transport (21). It was postulated that, in preeclampsia, the defective placenta is unable to produce sufficient levels of 1,25 (OH)2 D, resulting in inadequate gastrointestinal calcium absorption, low ionized calcium levels, and a secondary rise in PTH, which in turn may increase cytoplasmic Ca2+ or alter the production of endothelium-derived vasoactive factors (31). Low calcium levels may also contribute to hypertension via stimulation of renin release from the kidney (22). Also, the decreased serum total calcium concentration in preeclampsia may be an alteration of the plasma protein concentration (primarily albumin) results in parallel changes in total plasma calcium (23). It is widely accepted that vascular smooth muscle contraction is triggered by increases in intracellular free Ca2+ concentration due to Ca2+ release from the intracellular stores and Ca2+ entry from the extracellular space (24,25). Several studies have investigated the role of angiotensin II as an agonist for receptor-mediated intracellular calcium transients in vascular smooth muscle (26). 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 pre-eclampsia (27). In addition, Ang II may enhance Ca2+ entry through plasma membrane Ca2+ channels (28). Furthermore, there is evidence that several ion-transport pathways are highly sensitive to oxidative stress, and the resulting modulation of ion transport by ROS will affect Ca2+ homeostasis (29). Treatment of mild cases of pre-eclampsia with calcium carbonate 500 mg tablet twice daily for one month resulted in a significant decrease in the level of systolic, diastolic, and mean arterial blood pressure (p < 0.05), Table 2. figures 2,3, and 4. Our findings were similar to those reported by others (30,31). Calcium supplementation enhances vasodilation and reduces blood pressure (32,33) by suppression of the parathyroid hormone (21), which in turn reduces the intracellular calcium concentration in vascular smooth muscle cells, diminishing their responsiveness to pressure stimuli and reducing angiotensin II sensitivity in women with pre-eclampsia (32). However, several different mechanisms have been proposed by which Ca supplementation could reduce blood pressure in pre-eclampsia. Some have focused on neural, humoral, and renal effects, whereas others have attempted to relate the antihypertensive action of Ca2+ supplementation to improved vascular function (34). It has been thought that the improved vascular function following Ca supplementation in experimental animals has been attributed to decreased α-adrenoceptor responsiveness (34,35), reduced permeability of plasma membrane to Ca and other cations (36), improved function of cell membrane Na-K ATPase (37), improved vasodilator function of the vascular endothelium, and to increased...
sensitivity of the smooth muscle NO\textsuperscript{38}. An interesting link between the intake and metabolism of calcium and the control of arterial tone may be the extracellular receptor, the activation of which cause vasorelaxation via the release of hyperpolarizing mediators\textsuperscript{39}. The results of this study showed that mild pre-eclamptic patients treated with calcium carbonate showed a significant increase in serum calcium level \( (p<0.05) \) Table 3. Figure 5., and the result of increasing serum calcium is consistent with the others\textsuperscript{40} which demonstrated that calcium supplementation for women with a low baseline calcium intake was associated with an increase in serum calcium concentration. Thus calcium supplementation could have a meaningful impact on calcium metabolism regulation by maintaining serum calcium level within the narrow physiological range and reducing serum PTH\textsuperscript{21}. Moreover, when calcium is present in optimal concentration, it stabilizes vascular membranes, blocks its own entry into cells and reduces vasoconstriction\textsuperscript{41}. Calcium in combination with other ions such as Na\textsuperscript{+}, K\textsuperscript{+}, Cl\textsuperscript{—} and Mg\textsuperscript{2+} provides ionic balance to the vascular membrane\textsuperscript{42}, since membrane potential in vascular smooth cells is governed by the membrane permeability to these ions, and they are act as a major determinant of membrane potential under resting condition. From this study we conclude that the reduction in serum level of calcium during pregnancy might be possible contributor in etiology of pre-eclampsia, and supplementation of this micronutrients may be of value to prevent pre-eclampsia by controlling blood pressure, improving endothelial function, and modulating the deterioration of serum level of calcium.

References
1. ACOG practice bulletin. Diagnosis and management of preclampsia and eclampsia. Number 33, January 2002. Obstet Gynecol 2002; 99: 159-67.
2. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC III, Wenstrom KD. Williams obstetrics. 22nd ed. New York: McGraw-Hill; 2005: 761-808.
3. Walker JJ. Pre-eclampsia. Lancet 2000; 356: 1260-5.
4. Kashyap MK, Saxena SV, Khullar M, Sawhney H, Vasishtha K. Role of anion gap and different electrolytes in hypertension during pregnancy (preeclampsia). Mol Cell Biochem 2006; 282: 157-67.
5. Sukonpan K, Phupong V. Serum calcium and serum magnesium in normal and preeclamptic pregnancy. Arch Gynecol Obstet 2005; 273: 12-6.
6. Roberts JM, Pearson G, Cutler J, Lindheimer M. Summary of the NHLBI working group on research on hypertension during pregnancy. Hypertension. 2003;41:437–445.
7. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension. 2005; 46: 1243–1249.
8. Germain AM, Romanik MC, Guerra I, Solari S, Rejewski RJ, Price K, Karumanchi SA, Valdes G. Endothelial dysfunction: a link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? Hypertension. 2007;49:90–95.
9. Granger JP, Alexander BT, Bennett WA, Khalil RA. Pathophysiology of pregnancy-induced hypertension. Microcirculation. 2002;9:147–160.
10. Blauw J, Graaff R, van Pampus MG, van Doormaal JJ, Smit AJ, Rakhorst G, Aarnoudse JG, Khan F, Belch JF, Macleod M, Mires G. Changes in endothelial function preclude the clinical disease in women in whom preeclampsia develops in response: endothelial function and preeclampsia. Hypertension. 2006; 47:e14–e15.
11. Roberts JM, Gammill H. Insulin resistance in preeclampsia. Hypertension.2006;47:341–342.
12. Hagedorn KA, Cooke CL, Falck JR, Mitchell BF, Davidge ST. Regulation of vascular tone during pregnancy: a novel role for the pregnant X receptor. Hypertension. 2007;49:328–333.
13. Ray JG, Diamond P, Singh G, Bell CM. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. BJOG 2006; 113: 379-86.
14. Kisters K, Barenbrock M, Louwen F, Hausberg M, Rahn KH, Kosch M. Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia. Am J Hypertens 2000; 13: 765-9.
15. McCarron DA, Reusser ME: Finding consensus in the dietary calcium-blood pressure debate. J Am Coll Nutr 1999;18:398S–405S.
16. Malas NO, Shurideh ZM. Does serum calcium in pre-eclampsia and normal pregnancy differ? Saudi Med J 2001; 22 (10): 868-871.
17. Kosch M, Hausberg M, Louwen F, Barenbrock M, Rahn KH, Kisters K. Alterations of plasma calcium and intracellular and membrane calcium in.
erythrocytes of patients with pre-eclampsia. J Hum Hypertens 2000; 14: 333–6.
18. Ingec M, Nazik H, Kadanali S. Urinary calcium excretion in severe preeclampsia and eclampsia. Clin Chem Lab Med 2006; 44: 51–3.
19. Punthumapol C, Kittichotpanich B. Serum calcium, magnesium and uric acid in preeclampsia and normal pregnancy. J Med Assoc Thai 2008; 91 (7): 968-73.
20. Ritchie LD, King JC. Dietary calcium and pregnancy-induced hypertension: is there a relation? Am J Clin Nutr 2000; 71(suppl):1371S–4S.
21. Seeley EW. Calcitropic hormones in preeclampsia: A renewal of interest. J Clin Endocrinol Metab.2007; 92:3402-3403.
22. Resnick LM, Laragh JH, Sealey JE, Alderman MH Divalent cations in essential hypertension. Relations between serum ionized calcium, magnesium, and plasma renin activity. N Engl J Med 1983; 309:888–891.
23. Howlader MZ, Tamanna S, Parveen S , Shekhar HU, Alaudin M, Begum F. Superoxide Dismutase Activity and the Changes of Some Micronutrients in Preeclampsia. JMS 2009;15: pp. 107-113.
24. Khalil RA and van Breemen C. Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation? J Pharmacol Exp Ther 1988; 244(2):537-542.
25. Khalil RA and van Breemen C. Mechanisms of calcium mobilization and homeostasis in vascular smooth muscle and their relevance to hypertension. In: Hypertension: Pathophysiology, Diagnosis, and Management, edited by Laragh JH and Brenner BM. New York: Raven Press, 1995, p. 523-540.
26. Seki T, Yokoshiki H, Sunagawa M, Nakamura M, and Sperelakis N. Angiotensin II stimulation of Ca^{2+} -channel current in vascular smooth muscle cells is inhibited by lavendustin-A and LY- 294002. Pflügers Arch 1999; 437(3):317-323.
27. 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.
28. Loutzenhiser K and Loutzenhiser R. Angiotensin II-induced Ca^{2+} influx in renal afferent and efferent arterioles: differing roles of voltage-gated and store-operated Ca^{2+} entry. Circ Res 2000; 87(7):551-557.
29. Steinitr JR, Wyatt AW, Jacob R, Mann GE. Redox Modulation of Ca^{2+} Signaling in Human Endothelial and Smooth Muscle Cells in Pre-Eclampsia. Antioxidants and Redox Signaling 2009; 11(5): 1149-1163.
30. Villar J, Abdel-Hallem H, Meriah M, Mathai M, Ali MM, Zavaleta N, et al. World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women. Am J Obstet Gynecol. 2006; 194: 639-49.
31. Villar J, Belizán JM. Same nutrient, different hypotheses: disparities in trials of calcium supplementation during pregnancy. American Journal of Clinical Nutrition 2000; 71: 1375S-1379S.
32. Moutquin J, MD,Garner PR, Burrows RF, Rey E, Helewa ME, Lange I, Rabkin SW. Report of the Canadian Hypertension Society Consensus Conference: 2. Nonpharmacologic management and prevention of hypertensive disorders in pregnancy. Can Med Assoc J 1997; 157: 907-19.
33. Hatton DC, Yue Q, McCarron DA. Mechanisms of calcium's effects on blood pressure .Semin Nephrol 1995;15:593-602.
34. Peuler JD, Morgan DA, Mark L. High calcium diet reduces blood pressure in Dahl salt sensitive rats by neural mechanisms .Hypertension 1987;9:III159-III165.
35. Hatton DC, McCarron DA. Dietary calcium and blood pressure in experimental models of hypertension . A review. Hypertension 1994;23:513-530.
36. Arvola P, Ruskoaho H, Porsti I. Effect of high calcium diet on arterial smooth muscle function and electrolyte balance in mineralcorticoid-salt hypertensive rats. Br J Pharmacol 1993a 108:948-990.
37. Makynen H, Kahonen M, Arvola P, Wu X, Wuorela H, Porsti I. Endothelial function in deoxycorticosterone-NaCl hypertention :effect of calcium supplementation .Circulation 1996; 93: 1000-1008.
38. Bukoski RD, Ishibashi K, Bian K. Vascular actions of calcium regulating hormones. Sem Nephrol 1995;15:536-549.
39. Ishioka N, Bukoski RD. A role for N-arachidonylethanolamine (anandamide) as a mediator of sensory nerve- dependent Ca^{2+}-induced relaxation. J Pharmacol Exp Ther 1999;289:245-250.
40. López-Jaramillo P, Narváez M, Weigel M and Yépez R (1989). Calcium supplementation reduces the risk of pregnancy induced hypertension in an
Andean population. British Journal of Obstetrics and Gynaecology 1989; 96: 648-655.

41. Nieto A, Herrera JA, Villar J, Matorras R, la Manzanara CL, Iarribas I, Alvarez J, Peiro E. Association between calcium intake, parathormone levels and blood pressure during pregnancy. Colomb Med. 2009; 40: 185-93.

42. Ishioka N, Bukoski RD. A role for N-arachidonylethanlamine (anandamide) as a mediator of sensory nerve-dependent Ca2+-induced relaxation. J Pharmacol Exp Ther 1999; 289: 245-250.