The Role of High Calcium Diet Supplementation on L-NAME induced High Blood Pressure in Pregnant Rats

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ABSTRACT

Background: Hypertension in pregnancy with or without proteinuria is one of the foremost reasons of maternal death and morbidity in the world. This study investigated the role of a high calcium diet (HCa) in N^G-nitro-L-arginine methyl ester (L-NAME) administered pregnant rats.

Methods: Thirty-two female rats of the Sprague-Dawley strain were randomly assigned into 4 groups of control, L-NAME (0.3g/L of water), L-NAME + HCa (2.5%) and HCa diet (2.5%) group. Following confirmation of mating, HCa was administered from day 3 to day 18 of pregnancy while L-NAME was administered from day 12 to 18. The rats were sacrificed on the 19th day and blood pressure measurements were obtained. Blood and placenta were collected for biochemical and oxidative assays.

Results: Blood pressure was reduced in L-NAME + HCa rats compared with L-NAME ($p<0.05$). Placenta and fetal weight were decreased in rats receiving L-NAME, L-NAME + HCa and HCa compared with control ($p<0.05$). HCa diet had no effect on L-NAME impaired oxidative status. No significant difference was observed in the liver enzymes and CRP levels across the groups. However, there was a significant reduction in platelet count of L-NAME + HCa and HCa groups when compared with the control and L-NAME. Calcium and magnesium levels in the serum and placenta homogenate were not different but their excretion was significantly increased in the urine samples of the L-NAME + HCa and HCa groups.

Conclusion: This study showed that HCa reduced the blood pressure of pregnant rats administered L-NAME but had no effect on oxidative stress. This implies that HCa alone might not sufficiently ameliorate the negative effects of hypertension in pregnancy.

Keywords: L-NAME, calcium, placenta, blood pressure, pregnancy.

INTRODUCTION

Calcium is essential for several vital functions in the body. It is the most abundant mineral in man, important in bone formation, muscle contraction, cardiovascular function, neurological function, enzymes, and hormonal activities (1,2). About ninety-nine per cent of the calcium in man is resident in the bones and teeth while the remaining is distributed between the intracellular and the extracellular fluid compartment where it influences neuromuscular transmission, enzymatic activation, and hormonal functions (2). During pregnancy, about 30 g of calcium ion is assimilated by a full-term infant because of increased maternal intestinal calcium absorption (2,3). Calcium is transported actively from the mother to the fetus under placental regulation (4). The WHO, United states and many European nations recommends that pregnant women consume between 1000-1300 mg of calcium per day (5–6) without surpassing the upper limit of 2500 or 3000 mg/day (for pregnant/lactating adult females aged 19–50 years) (7). Inadequate consumption of calcium by pregnant women could present adverse effects on both mother and fetus such as muscle cramping, tremors, osteopenia, tetanus, low birth weight, delayed fetal growth, and poor fetal mineralization (8).

Hypertensive conditions of pregnancy are common difficulties in pregnancy that are accountable for substantial illness and death of the fetus, newborn child, and the mother. It affects about 5.2–8.2% of pregnancies globally and is responsible for about 10 % to 15% of maternal deaths in low- and middle-income countries (9–11). The postulation of the inverse relationship between calcium consumption and hypertensive conditions was first reported among the Mayan Indians in Guatemala with a low prevalence of preeclampsia (12). This was associated with the traditional way in which they prepare their cornmeal by first soaking it in lime before milling thus increasing the calcium content in their diet (12). Low prevalence of preeclampsia has also been reported in another population in Ethiopia with a high calcium intake (13). The mechanism by which low calcium intake results in hypertension during pregnancy has been suggested to be through the stimulation of parathyroid hormone secretion. This results to renin release that causes sodium and water retention because of increased intracellular calcium. These functional changes can lead to the development of preeclampsia (14). Asides decrease in blood pressure; calcium supplementation has been reported to be cardioprotective by increasing the

levels of high-density lipoprotein cholesterol and reducing low-density lipoprotein cholesterol (14–15).

In a study that used ten randomized control trials, their pooled analysis showed that calcium supplementation in pregnancy reduced the risk of preeclampsia by 59%, reduced the tendency of developing gestational hypertension by 45% and reduced the risk of having a preterm birth by 12% in developing countries (16). They recommend calcium supplementation as beneficial for pregnant women developing countries. Another study reported that calcium supplementation benefits in pregnancy, is prominent in populations with a low baseline calcium intake compared to those with adequate calcium intake (17). Although excessive intake of calcium has some undesirable effects such as constipation, reduced iron absorption, flatulence, urinary stone, and myocardial infarction (18–19). However, acceptable calcium intake in pregnancy could prevent fetal growth restriction, preterm birth, and reduce fracture risks (20). This study, therefore, induced preeclampsia by blocking nitric oxide synthesis using L-NAME in mid-late pregnancy. The study aims to determine the role of early high calcium diet supplementation in pregnant rats administered L-NAME.

MATERIALS AND METHODS

Animal Model

Thirty-two female Sprague-Dawley rats weighing between 135g-150g were obtained from the animal house facility of the College of Medicine University of Lagos for this study. The rats were housed in well-ventilated plastic cages with reversed 12-h light/dark cycle. They were acclimatized for ten days, fed with rat pelletized chow, and had free access to water. Male Sprague-Dawley rats were also obtained for mating with the female rats. All animal care and experimental protocols agreed with the updated United States National research council's guide for the care and use of laboratory animals (21).

Animal Groups

The rats were randomly assigned into four groups of eight rats each and were then marked for identification. The experimental groups for the study are as follows:

Group 1: **(Control):** received 0.5% normal calcium diet

Group 2: **(L-NAME):** received L-NAME (0.3mg/kg of H_2O) from day 12 to day 18 of pregnancy. ‘

Group 3: **(L-NAME + Calcium carbonate):** received L-NAME (0.3mg/kg of H_2O) and a diet comprising 2.5% calcium carbonate (from day 3 to day 19 of pregnancy).

Group 4: **(Calcium carbonate):** were fed a 2.5% calcium carbonate diet (from day 3 to day 19 of pregnancy).

Determination of Estrus Cycle and Mating

Estrus cycle of the rats was observed for two weeks and rats with a regular 4–5-day cycle were used for the study. The Marcondes technique was used to determine the phases of the estrus cycle (22). Smears were collected with the aid of a dropper filled with about a few drops of normal saline. The fluid retrieved was then observed under the light microscope for the determination of the cell patterns and the phase of the cycle (X 40). The diestrus phase was identified by the dominance of leucocytes in the smear while the proestrus phase had a dominance of nucleated epithelial cells. The presence of cornified epithelia cells along with some cell debris in the smear indicated the estrus phase, while the metestrus phase had mixed leukocyte and cornified cells. Following 2 weeks of monitoring, rats in the proestrus phase were mated with male rats on the evening of proestrus. Sperm cells present in the smears of the rats the following day, confirmed mating, and was presumed as day 1 of pregnancy

Determination of Calcium Ion Content in the Feed Sample

The prepared feed sample was subjected to atomic emission spectroscopy for the determination of calcium content in the feed. The feed sample was ashed with a furnace at 550^{o}C (Gallenkamp Hotspot furnace, PerkinElmer UK). The concentration of the calcium in the sample was determined from the calibration curve considering the test portion sizes and dilutions. Prior to this, the ash sample was then dissolved in 5mls of 1molar HNO_3 and warmed in a steam bath for 2–3 min to aid the solution. The sample was then made up to 25 ml with 1M HNO_3 and the solution was then measured using a calcium hollow cathode lamp at a wavelength of 422.7 nm (23). The calcium ion content in the feed was 87.251 (ppm) which is about 0.09 g/kg of calcium in the feed. The diets for the control rats were then made up to 0.5% calcium diet and the high calcium diet was made up to 2.5% by addition of calcium carbonate to the prepared feed.

Measurement of Blood Pressure and Fetal Counting

Rats were anaesthetized on day 19 of pregnancy with 5 ml/kg urethane and α-chloralose injected intraperitoneally. The trachea and one carotid artery were cannulated. Blood pressure measurement was obtained from the cannulated carotid artery which was connected to a pressure transducer (model SP 844, AD Instruments) and attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (AD Instruments Pty Ltd., Castle Hill, Australia) (24). Following the above was the dissection of the abdominal cavity for fetus weight and number as well as any resorption sites present at day 19 of pregnancy.

Measurement of Urea, Protein and Creatinine

The rats urine samples were collected with the aid of a metabolic cage on day 17 or day 18 of pregnancy. The urine sample collected during the twelve-hour period was preserved with toluene. The samples collected was used to determine protein level, urea, creatinine, calcium, and magnesium level. Total protein, urea and creatinine were assayed using Randox kits (25).

Determination of Serum and Urinary Calcium and Magnesium Content

Concentrations of calcium and magnesium ion content in the rats' serum, urine, and placenta were assayed using BIOLABO assay kits (FRANCE). 1 mL of reagent 1 (R1) was

pipetted into well-labelled test tubes as blank, standard, and assay test sample tubes. 25 µL of demineralized water was added to the blank while 25 µL of standard R2 was added to the standard test tube. 25 µL of the sample was pipetted and added to the assay test tube and all test tubes were mixed well and incubated for 5 mins at room temperature. Absorbances were read at 570 nm (555–590) against a reagent blank. For magnesium, 1ml of reagent 1 (R1) was pipetted into well-labelled test tubes as blank, standard and assay test samples. 10µl of demineralized water was added to the blank while 10µl of the standard R2(2mg/dl) was added to the standard test tube. 10µl of the sample was pipetted and added to the assay test tube and all tubes were allowed to stand for 5 minutes at a constant temperature. The standard and assay absorbances were read at 530nM against the reagent blank.

Determination of Oxidative Stress Indices

The study accessed Malondialdehyde (MDA) level using the methods described by Mihara and Uchiyama (26). Superoxide dismutase (SOD) enzyme activity in the placenta homogenate was carried out using the method of Sun and Zigma (27), Catalase (CAT) activity was determined based on the exponential disappearance of H_2O_2 as described by Aebi (28). Glutathione levels (GSH) was measured based on the reaction of Ellman's reagent with the thiol group of GSH at pH 8.0 to produce 5 thiol 2 nitrobenzoate (29). All absorbances were recorded using a Shimadzu recording spectrophotometer.

Determination of Liver Enzymes and C-reactive Protein (CRP) Levels

Randox kits were used to determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The protocol for measurement was as described in the manual and the absorbances of the liver enzymes were read as specified for each liver enzyme using a Shimadzu recording spectrophotometer. C-reactive protein level was determined using rat ELISA kits for CRP from ELABSCIENCE China (E-EL-R0506).

Statistical Analysis

The data from this study was analyzed using graph pad prism 6 software. The values were analyzed using one-way ANOVA and student's Newman-Keuls post-hoc test. Results are presented as mean ± standard error of the mean (SEM) with differences considered significant when $p<0.05$.

RESULTS

Influence of High Calcium Diet and L-NAME on Maternal Weights and Fetal Outcome on day 19 of Pregnancy

The effect of a high calcium diet and L-NAME on the fetal outcome on day 19 is presented in Table 1. The rats administered L-NAME + HCa had a significant decrease in weight change and final weights of the dams when compared with the control ($p < 0.05$). The HCa group also had a significant decrease in the weight change and final maternal weight when compared with control and L-NAME administered rats ($p<0.05$). Fetal weight and placenta weights were significantly decreased in the L-NAME, L-NAME + HCa, and HCa groups when compared with the control ($p< 0.05$). The L-NAME group had a significant decrease in litter size when compared with the control ($p<0.05$).

Influence of High Calcium Diet and L-NAME on Blood Pressure Indices

Table 2 reveals the influence of a high calcium diet and L-NAME on the blood pressure of pregnant rats. There was a significant increase in systolic blood pressure and mean arterial

Table 1: Pregnancy Outcome of Rats administered High Calcium Diet and L-N-Nitroarginine Methyl Ester (L-NAME)

	Control	L-NAME	L-NAME + HCa	HCa
Maternal weight Day 19 (g)	208.8 ± 7.45	206.22 ± 6.21	187.22 ± 7.38*	179.88 ± 3.00*#
Weight change in 4weeks (g)	79.77 ± 5.38	75.66 ± 4.79	54.77 ± 9.37*	49.44 ± 4.84*#
Fetal weight Day 19 (g)	4.78 ± 0.10	3.17 ± 0.41*	3.33 ± 0.07*	3.63 ± 0.14*
Placenta weight (Day 19) (g)	0.585 ± 0.02	0.44 ± 0.02*	0.49 ± 0.01*	0.46 ± 0.02*
Litter size	9.75 ± 0.47	6.75 ± 0.43*	8.25 ± 0.47	8.00 ± 0.70

**Significant difference from control and # stands for a significant difference from L-NAME*

Table 2: Blood Pressure Indices Of Pregnant Rats administered L-NAME and High Calcium Diet

	Control	L-NAME	L-NAME + HCa	HCa
Systolic blood pressure (mm/Hg)	100.37 ± 3.48	139.06 ± 3.66*	120.33 ± 4.96*#	105.73 ± 5.113
Diastolic blood pressure (mm/Hg)	73.57 ± 7.23	104.51 ± 7.02*	84.75 ± 3.42	72.26 ± 7.11#
Pulse pressure (mm/Hg)	27.78 ± 3.12	34.56 ± 2.75	35.57 ± 6.57	33.47 ± 3.45
Mean arterial blood pressure	82.50 ± 6.83	116.02 ± 7.12*	96.95 ± 2.53	83.42 ± 6.30#
Heart rate	396.2 ± 29.67	365.2 ± 33.19	416.8 ± 7.08	415.8 ± 22.49
Rate Pressure Product	39605.38 ± 3582	50744.13 ± 5001*	54454.66 ± 2830*	44297.52 ± 3895*#$

**Significant difference from control, # stands for a significant difference from L-NAME and $ stands for significant difference from L-NAME + HCa group (p< 0.05)*

blood pressure in the L-NAME and L-NAME + HCa groups when compared with the control. Systolic blood pressure was significantly decreased in the L-NAME + HCa group when compared with the L-NAME group. The HCa group SBP and MABP was not different from the control however, their values were significantly decreased compared with L-NAME ($p< 0.05$). Diastolic blood pressure was significantly increased in the L-NAME groups compared with the control. Rate pressure product was significantly increased in all the groups compared with the control, however, the increase in the HCa group was significantly decreased when compared with the L-NAME and L-NAME + HCa groups.

Influence of High Calcium Diet and L-NAME on Placenta Oxidative Stress Indices

Table 3 reveals the influence of a high calcium diet and L-NAME on oxidative stress indices in the placenta of rats. The L-NAME and L-NAME + HCa groups had a significantly increased MDA level when compared with the control ($p<0.05$). The MDA levels of the HCa group were not different from the control but were significantly decreased compared with the L-NAME and L-NAME + HCa groups. The activities of catalase and reduced glutathione were significantly reduced in the L-NAME and L-NAME + HCa groups when compared with the control ($p< 0.05$).

Influence of High Calcium Diet and L-NAME on Liver Enzymes, Platelet Count and C-reactive Protein

Table 4 reveals the liver enzymes of pregnant rats administered a high calcium diet and L-NAME. There was no significant difference in the ALP, AST, and ALT enzyme levels in all the groups when compared with the control ($p< 0.05$). Platelet counts were significantly decreased in the L-NAME + HCa and HCa groups when compared with control and L-NAME (Table 4). The CRP level (Figure 1) showed that there was no significant difference in the CRP levels in all the groups when compared with the control ($P< 0.05$).

Influence of High Calcium Diet and L-NAME on Placenta Calcium and Magnesium in Rats

The concentrations of calcium and that of magnesium in the placenta is revealed in Figure 2. The figure shows that there was a significant decrease in placenta calcium level in the HCa group when compared with the L-NAME group ($p< 0.05$). No difference was found in the placenta magnesium concentration of all the animal groups when compared with each other.

Electrolytes and Metabolites Homeostasis

The serum and urine concentrations of calcium, magnesium, creatinine, protein and urea are presented in Table 5. The results showed that there was a significant decrease in serum calcium ion level in the L-NAME + HCa when compared with control and L-NAME. In the urine, calcium excretion was significantly elevated in the L-NAME + HCa and HCa groups

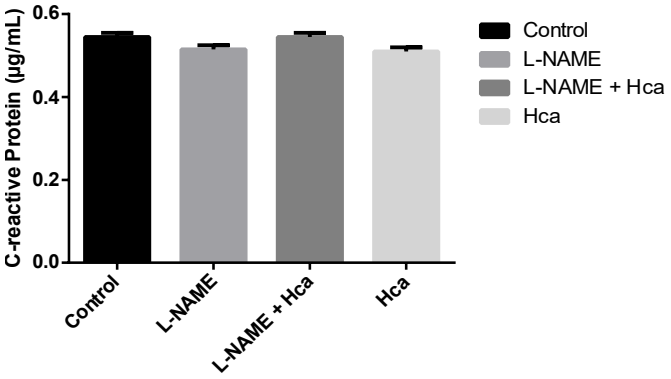

Fig. 1: Effect of High Calcium Diet and L-NAME of C-reactive Protein Levels of Pregnant Rats

Table 3: Placenta Oxidative Stress of Rats administered L-NAME and High Calcium Diet

	Control	L-NAME	L-NAME + HCa	HCa
Malondialdehyde (MDA; µmol/ml/mg pro)	0.013 ± 0.003	0.041 ± 0.007*	0.052 ± 0.006*	0.025 ± 0.005#$
Superoxide dismutase(µmol/ml/mg pro)	3.24 ± 0.44	2.27 ± 0.18*	2.62 ± 0.21	3.28 ± 0.28#
Catalase(µmol/ml/mg pro)	22.53 ± 3.31	17.86 ± 3.47*	18.68 ± 3.07*	33.15 ± 1.45#$
Reduced glutathione(µmol/ml/mg pro)	0.75 ± 0.06	0.43 ± 0.06*	0.41 ± 0.05*	0.64 ± 0.04#$

**Significant difference from control, # stands for a significant difference from L-NAME and $ stands for significant difference from L-NAME + HCa group ($p< 0.05$).*

Table 4: Liver Enzymes and Platelet Count of Pregnant Rats administered L-NAME and High Calcium Diet

	Control	L-NAME	L-NAME + Hca	Hca
ALP (u/L)	35.50 ± 3.14	38.33 ± 3.63	37.83 ± 3.52	31.66 ± 3.30
AST (u/L)	78.00 ± 8.30	76.17 ± 11.84	67.83 ± 12.59	75.00 ± 3.96
ALT (u/L)	25.00 ± 2.19	28.33 ± 4.35	22.00 ± 2.80	21.67 ± 1.36
Platelet (10^9/ L)	287.83 ± 4.77	287.16 ± 10.1	131.66 ± 11.5*#	189.83 ± 8.5*#

**Stands for significant difference from control; # represents significant difference from L-NAME*

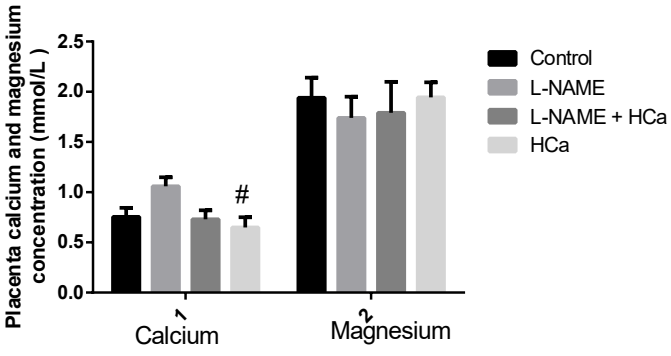

Fig. 2: Effect of High Calcium Diet and L-NAME on the Placenta Calcium and Magnesium Concentration

Stands for significant difference from L-NAME, 1= placenta calcium and 2= placenta magnesium.

Our study showed a decrease in maternal body weight of pregnant rats administered a high calcium diet with L-NAME and high calcium diet only. Increasing evidence supports an association between increased calcium intake and reductions in the weight of the body with specific emphasis on fat mass (33–34). Calcium supplementations increase weight loss by increasing faecal fat elimination, oxidation of body fat and improving insulin sensitivity (35–36). Our study agrees with this finding as both high calcium diet groups had reduced weight gain. In addition, the placenta weight and offspring weight were also reduced in this group relative to the control weights. The reduced placenta size and offspring weight in the L-NAME administered rats might be related to the effects of L-NAME on blood flow resulting in increased blood pressure thus responsible for the similar intrauterine growth restriction observed in preeclampsia.

Table 5: Serum and urine electrolytes of the pregnant rats given L-NAME and HCa diet

	Serum				Urine				
	Control	L-NAME	L-NAME + HCa	HCa		Control	L-NAME	L-NAME + HCa	HCa
Calcium (mmol/L)	2.51±0.03	2.67±0.09	1.77±0.13*#	2.4±0.19	Calcium (mmol/L)	0.57±0.15	0.70±0.13	1.38±0.27*	1.38±0.21*
Magnesium (mmol/L)	1.83±0.11	1.7±0.13	1.53±0.04	1.48±0.15	Magnesium (mmol/L)	1.27±0.06	2.20±0.09	3.7±0.40*#	2.87±0.23*
Creatinine (µmol/L)	46.5±6.02	51.55±6.65	42.61±8.13	51.35±3.58	Creatinine (µmol/L)	1725.33±515.9	2052.8±410.5	2303.8±408.4	1218±366.1
Protein(g/L)	66.8±1.86	61.4±2.64	55.67±4.18	58.28±2.24	Protein (g/L)	0.29±0.05	0.52±0.02*	0.35±0.04	0.32±0.08
Urea (mmol/L)	5.36±0.22	4.68±0.29	5.38±0.48	4.1±0.35					

**Stands for significant difference from control; # stands for significant difference from L-NAME*

compared with control ($p<0.05$). The serum magnesium level had no significant difference but the urine magnesium excretion was significantly elevated in the L-NAME + HCa and HCa groups when compared with control ($p<0.05$). No significant difference was found in the serum and urine creatinine levels when compared across the groups. Serum protein level was not significant but the urine protein level in the L-NAME administered rats was increased when compared with control ($p<0.05$). Finally, there was no significant difference in the serum urea concentration of the rats in this study.

DISCUSSION

Fetal growth places a high demand on maternal calcium turnover (30–31). Low dietary calcium has been postulated to be responsible for the high prevalence of preeclampsia while calcium supplementation in pregnancy is known to lessen the risk of preeclampsia, maternal deaths, and preterm birth (2). Reducing deaths from hypertensive conditions of pregnancy is a global concern (32). We investigated the role of early high calcium diet supplementation on pregnant rats from day three of pregnancy (pre-implantation) to day 18 of pregnancy in rats with inhibited nitric oxide production.

An inverse relationship between an increase in calcium diet and the incidence of preeclampsia has long been established (12,37). Many human studies have confirmed that in populations of those with low calcium intake the incidence of preeclampsia is higher when compared with populations in developed countries with a relatively high calcium intake (10, 38). Findings from this study showed that early high calcium diet supplementation ameliorated the increase in blood pressure by L-NAME. Our results also confirmed the potential of L-NAME to be used as a model for preeclampsia in rats with the presence of increased urine protein. Severe forms of preeclampsia could come with elevated liver enzymes, thrombocytopenia, and renal insufficiency (39). In this study, the liver enzymes of the pregnant rats were not affected. In humans, calcium supplementation has been found to prevent salt-induced blood pressure and inhibit the release of platelets (40). This could be related to the decreased platelet levels reported in this study in an L-NAME model of hypertension. Cardiovascular thrombotic complications include the activities of platelet aggregation which impedes blood flow and results in myocardial infarctions and cerebrovascular accidents (41). The mechanism by which calcium suppresses platelet release and improves platelet

aggregation has been related to its ability to decrease sympathetic activity. This suggests a protective role of calcium supplementation against hypertension-related thrombotic cardiovascular diseases (40). Another study (42) has shown that a high calcium diet (3%) reduced blood pressure in chronic nitric oxide synthase inhibited rats and eliminated the damages in endothelium-dependent and -independent arterial relaxation. They suggest that this vasorelaxation could be because of hyperpolarization and increased sensitivity of the arterial smooth muscles to nitric oxide and decreased production of superoxide and the vasoconstrictor prostanoids (42).

The maternal-fetal border represents a site for the manifestation of oxidative stress. Oxidative stress at this interphase is required for the normal development of the placenta but can also contribute to the pathophysiology of complications such as miscarriages, premature rupture of the membranes, IUGR, and preeclampsia, (43). The major source of reactive oxygen species (ROS) in pregnancy is the placenta which is the central organ that regulates pregnancy (43). Findings from this study showed that L-NAME increased the placenta oxidative stress index and decreased the antioxidant levels. Oxidative stress has been found to inhibit placental endothelial nitric oxide synthase (eNOS) as well as oxidize eNOS cofactor tetrahydrobiopterin (BH_4) (44). Although calcium is not an antioxidant, it communicates with several other systems and pathways; among which are ROS, such as hydrogen peroxide, superoxide anion, and hydroxyl radicals (45–46). In addition, mitochondrial calcium overload has been reported to increase ROS (47–48). Therefore, this could be responsible for the increased placental ROS indices in this study in response to HCa.

Intracellular free calcium ion and magnesium ion concentrations play key roles in vascular smooth muscle contraction. Abnormal homeostatic control of these ions is also recognized in the pathogenesis of preeclampsia, with the underlying mechanism(s) poorly understood (49). Findings from this study showed that placenta calcium and magnesium were not affected as well as serum calcium. However, it was noted that the calcium-supplemented groups compensated for the increased calcium load by excreting a greater amount of calcium and magnesium in the urine. Studies have reported an increased prevalence of preeclampsia in populations with low dietary calcium while populations with high dietary calcium consumption had a lower incidence of preeclampsia (10,38). Calcium's biological functions is majorly derived from its ability to bind and unbind from target proteins, as well as from its charge movement to depolarize membrane potential in the form of calcium currents (45,46). Although the calcium overload is sufficiently excreted by the kidney in the urine, the consistent calcium load brings about some physiological changes in endocrine organs responsible for calcium homeostasis thus reducing smooth muscle contractility and promoting vasodilatation (50).

In conclusion, a high calcium diet reduced blood pressure, could not reverse oxidative stress induced by L-NAME and had no effect on inflammation and liver enzymes of the pregnant rats. The pregnant rats excreted the calcium load appropriately and a decrease in platelet counts was found in the rats that were fed with a high calcium diet. Further research on the mechanisms by which HCa reduces blood pressure and its role on platelet count will contribute to our understanding of how HCa diet prevents hypertensive disorders in populations with low calcium intake.

Conflict of Interests

The authors have no conflict of interest to declare.

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