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Preeclampsia: from epidemiological observations to molecular mechanisms

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Abstract

Preeclampsia is the main cause of maternal mortality and is associated with a five-fold increase in perinatal mortality in developing countries. In spite of this, the etiology of preeclampsia is unknown. The present article analyzes the contradictory results of the use of calcium supplementation in the prevention of preeclampsia, and tries to give an explanation of these results. The proposal of an integrative model to explain the clinical manifestations of preeclampsia is discussed. In this proposal we suggest that preeclampsia is caused by nutritional, environmental and genetic factors that lead to the creation of an imbalance between the free radicals nitric oxide, superoxide and peroxynitrate in the vascular endothelium. The adequate interpretation of this model would allow us to understand that the best way of preventing preeclampsia is the establishment of an adequate prenatal control system involving adequate antioxidant vitamin and mineral supplementation, adequate diagnosis and early treatment of asymptomatic urinary and vaginal infections. The role of infection in the genesis of preeclampsia needs to be studied in depth because it may involve a fundamental change in the prevention and treatment of preeclampsia.

Key words
- Preeclampsia
- Calcium supplementation
- Nitric oxide
- Infection
- Inflammation
- Oxidative stress
- Endothelial dysfunction

Introduction

Preeclampsia is a frequent disease with an incidence of 5 to 7% among the general population; however, geographic, social, economic and racial differences are responsible for an incidence that is up to three times higher in some populations (1). In Colombia, it is the main cause of maternal mortality with up to 42% of maternal deaths being attributed to this disorder (2). Preeclampsia is associated with a five-fold increase in perinatal mortality and its socioeconomic impact on developing countries is huge, even more so if we consider that in Colombia maternal mortality is ten times higher than in the United States (3).

In spite of its importance for public health, the etiology of preeclampsia is unknown. We believe that it is a complex disorder caused by a series of nutritional, environmental and genetic factors that lead to the creation of an imbalance between the free radicals nitric oxide (NO), superoxide (O_2^-) and peroxynitrate in the vascular endothelium (4).

Calcium supplementation and preeclampsia: from epidemiological and nutritional observations to clinical trials and meta-analysis

Earlier observations considered nutritional aspects to be an important risk factor for preeclampsia. It was suggested that protein-calorie undernutrition may have an important role in the etiology of preeclampsia (5). However, more recently a negative correlation between calcium intake and the incidence of preeclampsia was proposed. Belizán and Villar (6) confirmed this initial association among the Mayan Indians in Guatemala. Moreover, they observed that in countries such as Colombia and India, where the mean calcium intake was between 250 and 300 mg per day, the incidence of eclampsia was high (1.6 and 12.0 per 1000 live births, respectively).

These observations were the initial support of our earlier clinical trials carried out to determine the role of calcium supplementation in Andean pregnant women (7-10), a population with a low calcium intake (11).

Our studies, conducted on 408 Ecuadorian pregnant women, suggested a beneficial effect of calcium supplementation in preventing preeclampsia (Table 1) and showed that calcium deficiency plays an important role in the pathogenesis of preeclampsia. These results led other researchers to develop large controlled trials around the world to confirm the beneficial effects of calcium supplementation in preventing preeclampsia (12-18). All of these studies confirmed our results; however, most of them did not have enough statistical power to reach a definitive conclusion. In contrast, the Calcium for Preeclampsia Prevention (CPEP) trial, that included more than 4000 North American women, showed that calcium supplementation did not have any beneficial effect in preventing preeclampsia among healthy nulliparous women (relative risk (RR): 0.94; 95% confidence interval (CI): 0.76-1.16) (19).

Table 1. Effect of calcium supplementation on the outcomes of Ecuadorian pregnant women.

Parameter	Group with supplementation	Group without supplementation
Non-selected pregnancies[a]	N = 49	N = 43
Duration of pregnancy (weeks)	39.3 ± 1.8	38.7 ± 1.7
Birth weight (g)	3097 ± 276	2832 ± 318
Pregnancy-induced hypertension (N)	2 (14%)*	12 (27.9%)
High-risk pregnancies[b]	N = 22	N = 34
Duration of pregnancy (weeks)	39.2 ± 1.2**	37.4 ± 2.3
Birth weight (g)	2936 ± 396	2685 ± 427
Pregnancy-induced hypertension (N)	3 (14%)*	24 (71%)
Teenage pregnancies[c]	N = 125	N = 135
Duration of pregnancy (weeks)	39.6 ± 0.4	38.7 ± 0.3
Birth weight (g)	2907 ± 183	2797 ± 104
Preeclampsia (N)	4 (3.2%)*	21 (15.5%)

Data are reported as means ± SD. *P<0.001 and **P<0.01.
[a,b,c]Data from Refs. 8, 9 and 10, respectively.

Because of these contradictory results, several authors have performed different meta-analyses during the last few years (20-23). These studies have found calcium to be highly effective in preventing preeclampsia with an overall odds ratio (OR) of 0.38; 95% CI: 0.22-0.65 (23). Nevertheless, the real value of meta-analysis in assessing the efficacy of clinical interventions remains controversial (24). In the case of trials with calcium supplementation included in these meta-analyses, the procedures were heterogeneous in several aspects: different doses of calcium supplementation (450 mg to 2 g per day), different starting times, different durations of supplementation, and different definitions of preeclampsia and pregnancy-induced hypertension. These arguments have been used by some authors to limit the impact of the conclusions of meta-analysis, especially when one considers that the only large controlled trial did not show any beneficial effect of calcium supplementation (19).

However, the CPEP (19) has been the target of several criticisms, mainly due to its

limitation in applying its results to other populations. While in the trials involving populations with a low calcium intake calcium supplementation was an intervention to avoid a nutritional problem, in the CPEP study calcium administration was a pharmacological intervention in women with a normal calcium intake (mean baseline intake per day: 1130 mg). Moreover, other methodological criticisms are that the organoleptic characteristics of placebo and calcium were not completely similar, so that the supplement was distributed to the patients in a dark jar; but the more important criticism was that, on average, the women studied took only 64% of the supplement and only 20% of them used more than 90% of the administered dose.

These discrepancies indicate the important aspects of the complex interaction between nutrients and supplements in different populations. For instance, in our studies we consistently found (10,11) a high phosphate intake, even higher than the recommendations of the World Health Organization, provided by vegetables, especially tuberculous and leguminous ones which are the base of the diet of Andean women of low income. This is important because intestinal calcium absorption is decreased by the presence of phosphate and vegetable fiber (25), a situation that could be particularly relevant to the understanding of the contradictory results of calcium supplementation.

The study "Dietary Approaches to Stop Hypertension" (26) carried out on the North American population showed that a diet rich in fruit and vegetables, calcium supplements (800 mg/day derived from dairy products) and reduced fat intake decreased blood pressure (systolic pressure by 5.5 mmHg and diastolic pressure by 3.0 mmHg on average) in relation to a typical North American diet with or without fruits and vegetables, but without the extra calcium. Interestingly, the urinary calcium excretion of the subjects consuming the typical North American diet was similar to that of the subjects with fruit, vegetable and calcium supplementation. However, it was higher than that of subjects consuming extra fruit and vegetables but without calcium supplement. This result could be explained by the concept that calcium was bound to the added fiber introduced in the diet, thus affecting its intestinal absorption, a situation that could be improved by the additional calcium intake.

The decreased calcium absorption in the group that did not take the mineral supplement abolished the beneficial effect of vegetable fiber in reducing blood pressure. Unfortunately, in the CPEP study, Levine et al. (19) did not report the intake of other nutrients, so that it was not possible to find out if the absence of effect of calcium supplementation was due to different interactions with other nutrients in the diet. In view of the heterogeneity of the results included in the meta-analysis and on the basis of the importance of calcium levels, a stratified analysis by baseline dietary calcium intake (mean calcium intake in the population <900 or >900 mg/day) was conducted by Villar and Belizán (23). The risk of preeclampsia was considerably reduced in the six trials conducted on populations with a low calcium diet (typical relative risk (TRR): 0.32; 95% CI: 0.21-0.49) but was not reduced as much in women consuming adequate calcium diets (TRR: 0.86; 95% CI: 0.71-1.05). Based on these results, the authors suggested both that calcium supplementation in women with a low calcium diet is a promising preventive strategy for preeclampsia and that this strategy should be evaluated in a large controlled trial conducted on a population with low calcium intake (23).

Furthermore, a new meta-analysis recently reported that calcium supplementation was associated with a reduction in the risk of preeclampsia (RR: 0.70; 95% CI: 0.58-0.83) mainly for those with a low baseline calcium intake (RR: 0.32; 95% CI: 0.21-0.49). Thus, calcium supplementation ap-

pears to be beneficial for women of populations with a low baseline calcium intake (27).

Benefits of calcium supplementation

The mechanisms that lead to preeclampsia are unknown. However, the hemodynamic changes present during pregnancy, such as increased cardiac output, decreased systemic vascular resistance, increased blood volume and increased renal flow and glomerular filtration (28), led us to think that NO, a vasoactive substance produced by vascular endothelium from the amino acid L-arginine by the action of the enzyme endothelial NO synthase, was responsible for these hemodynamic changes (29). We suggested that dietary calcium supplementation reduces the frequency of preeclampsia by maintaining the serum calcium ion level which plays a crucial role in the production of endothelial NO, the increased generation of which maintains the vasodilation that is characteristic of normal pregnancy (30). This could explain the results obtained in the clinical trials, in which we demonstrated that calcium supplementation for women with a low baseline calcium intake was associated with an increase in serum calcium ion concentrations (7). Moreover, the decreased levels of serum calcium ion observed in our preeclamptic women were associated with lower concentrations of cGMP, the effector of NO actions (31).

Thus, we and others found that during normal pregnancy the production of NO, evaluated by plasma nitrite and nitrate levels, was increased (32), as also was the activity of NO, evaluated by cGMP concentration (31-33). The cause could be the increased activity of NO synthase, probably related to higher shear stress (34) or the physiological increase of estrogens during pregnancy (35).

Nevertheless, the same did not occur with preeclampsia, where we showed that the plasma levels of nitrites and nitrates were similar or increased compared with normal pregnancies (31,32), but the urine and plasma concentrations of cGMP were decreased (31). These contradictory results were confirmed by other authors (36,37). Moreover, divergent results have been obtained by different groups, a fact possibly due to different methods used to quantify nitrites and nitrates and reflecting differences in the populations studied and in the intake of nitrates. Although plasma concentration of nitrites and nitrates may be high in preeclamptic women, the low concentration of cGMP may be due to increased NO inactivation, which might result from excessive production of O_2^- (32,38). This situation had already been demonstrated in other cardiovascular diseases (39). In support of this proposal we found increased levels of antibodies against oxidized LDL, a marker of oxidative stress, in women with preeclampsia (32). Similar results have been reported by others using different markers of oxidative stress (38,40).

The role of oxidative stress in preeclampsia is also supported by the observation that supplementation with vitamin antioxidants during pregnancy decreases the incidence of preeclampsia (41). At present there is good evidence supporting the proposal of increased oxidative stress in preeclampsia. For instance, nitrotyrosine residues which are a marker of peroxynitrate formation, a product of the reaction between NO and O_2^-, are found in placental vessels of preeclamptic and diabetic pregnancies, indicating increased oxidative stress (42). Peroxynitrate causes dysfunction of placental vessels and alters the normal increase in blood flow, thus leading to damage to the fetus (42).

Factors that affect the production of nitric oxide and superoxide

Healthy vascular endothelium provides a tonic dilator tone and prevents the adhesion of white cells and platelets to the vessel wall (29). These actions are mediated by the pro-

duction of NO and prostacyclin. Blockade of NO synthesis reproduces many of the cardiovascular changes of preeclampsia in animals (43). For example, acute blockade of endothelial NO generation causes constriction of resistance vessels, hypertension, altered platelet reactivity, and adhesion of white cells to the endothelium. Chronic blockade, which promotes neointima formation, results in renal damage evidenced by proteinuria. Inhibiting NO generation also worsens endotoxin-induced glomerular thrombosis in pregnant rats (44). Thus, endothelial dysfunction is likely to trigger white cell activation and a sequence of events that lead to further endothelial damage.

Recently, it was proposed that acute infections and the resulting inflammation might impair the function of vascular endothelium (45). Hingorani et al. (46) studied healthy volunteers vaccinated with typhoid vaccine who presented an inflammatory response and cytokinemia. Eight hours after vaccination, forearm blood flow response to local intra-arterial infusion of bradykinin was almost abolished and endothelium flow-dependent dilatation of the brachial artery was also reduced. In our population we have observed that C-reactive protein is an independent risk factor for essential hypertension (47).

The production and release of reactive oxygen intermediates in endothelial cells, such as O_2^- and hydrogen peroxide, are induced by TNF-α, IL-1 and IL-6 via the activation of NAD(P)H oxidase (48).

Evidence for systemic inflammation in preeclampsia

Proinflammatory cytokines appear to be involved in cellular events that establish and maintain pregnancy (49); however, their role has not yet been well defined. The fetal allograft, through activation of the maternal immune system, may induce the secretion of cytokines by maternal immunocompetent cells. In healthy pregnant women, TNF-α is thought to modulate the growth and invasion of trophoblasts in maternal spiral arteries (50). In keeping with a role for cytokines in normal pregnancy, we have recently found that circulating levels of TNF-α, C-reactive protein, and IL-6 were elevated in healthy pregnant Andean women in Quito during the third trimester (51).

Abnormal cytokine responses in the mother and fetus may be involved in the pathogenesis of preeclampsia. Circulating levels of TNF-α (50) were elevated in women who developed preeclampsia in the third trimester when compared with plasma concentrations in normotensive women matched for gestational age. Recently, several investigators (52-54) have reported that serum concentrations of IL-2, IL-6 and TNF-α and its soluble receptor sTNFp55 were significantly higher in the first and second trimester among pregnant women who subsequently developed preeclampsia compared to those in the control group. These results suggest that the perturbation of immune regulation may precede the clinical manifestation of preeclampsia.

We had observed that pregnant women in the Andes, who have a high risk for preeclampsia and chronic subclinical infection, have increased levels of C-reactive protein during the third trimester (51). These results are consistent with the proposal that preeclampsia is associated with a greater inflammatory response than observed in normal pregnancy (53). Furthermore, the levels of IL-6 and TNF-α that we detected in the Ecuadorian women studied were significantly higher than those reported for European and North American women (52,54) and increased more substantially during both normal pregnancy and preeclampsia. Whether these differences are related to genetic (inflammatory response and L-arginine:NO pathway) and/or environmental factors (e.g., infection) remains to be determined.

We have also shown that preeclamptic

women had higher leukocyte counts and increased total number of neutrophils compared to a matched normal pregnancy group (51). These results provide further evidence of enhanced inflammation in preeclampsia, as recently reported by other groups in different populations (53,54). These studies were undertaken in women with established preeclampsia; therefore, it cannot be determined whether the increase in C-reactive protein and proinflammatory cytokines was a cause or a consequence of the disease (51,54).

Is infection a major risk factor for preeclampsia?

A growing body of evidence links infection and inflammatory processes with preeclampsia. Hill et al. (55) found that the incidence of asymptomatic bacteriuria was higher in pregnant women with preeclampsia (19%) than in normal gravidas (3 to 6%). Hsu and Witter (56) reported that the incidence of urinary tract infection in preeclamptic pregnant women was higher than in normotensive pregnant women. Urinary tract infection was shown to be a strong risk factor for eclampsia, with OR of 4.23 (95% CI: 1.05-5.09) in a multivariate logistic regression analysis of characteristics associated with eclampsia (57). These findings have been confirmed recently by Mittendorf et al. (58), who observed that urinary tract infection during pregnancy was associated with nearly a two-fold increased risk for preeclampsia. Moreover, primiparas who had a urinary tract infection were five times more likely (OR 5.3; 95% CI: 2.9-9.7) to develop preeclampsia than primiparas who did not have urinary tract infection during pregnancy.

The role of infection in the pathogenesis of preeclampsia is particularly relevant in developing countries, where the high incidence of chronic subclinical infection may contribute to the high incidence of preeclampsia. Recently we have developed a program of biopsychosocial risk assessment that adds to standard prenatal care (59). This method allows to identify pregnant women at high risk of preeclampsia, helps to define nutritional interventions like supplementation of calcium and linoleic acid to prevent preeclampsia, and to screen and treat asymptomatic urinary and cervical infections, thus reducing maternal and perinatal morbidity and mortality. The innovative aspects were the early identification and corrections of risk factors for preeclampsia and preterm delivery in all pregnant women receiving prenatal care at 129 health centers and hospitals of the public health service in the western part of Colombia.

A total of 15,354 pregnant women between 14-20 weeks of gestational age were included, 1,443 (9.4%) of whom were at high risk of developing preeclampsia and received nutritional supplementation during pregnancy with 450 mg/day of linoleic acid and 1.5 g/day of elemental calcium started at 25-32 gestational weeks. Asymptomatic bacteriuria was identified in 1,766 (11.5%) pregnant women and vaginal infections and bacterial vaginosis were detected in 2,150 women (14.0%). Both bacteriuria and vaginal infections were identified in 783 (5.1%) pregnant women. Physicians prescribed oral antibiotics for 7-10 days according to the type of organism grown in culture, with a compliance of 88%. Moreover, the tests were repeated following completion of the treatment to assess its efficacy and treatment was repeated when necessary. These interventions led to a 64% decrease in the incidence of preeclampsia, from 5.1% observed during the five years preceding the program to 1.8% during the program (59).

We believe that this dramatic reduction in the incidence of preeclampsia was due to the early identification of risk factors, the administration of nutritional supplements and principally the treatment of asymptomatic infections. In fact, only 1,443 women received nutritional supplementation but 3,133

pregnant women received oral antibiotics for 7-10 days to treat infection. It is highly tempting to speculate that the early treatment of asymptomatic infections may have been the predominant factor that contributed to the improvement of pregnancy outcomes. Unfortunately, the results described here are from an open population-based program. Consequently, it is not possible to make a definitive statement that infection is the major risk factor for preeclampsia. However, based on these results, we have proposed that chronic subclinical infections may increase maternal cytokines to levels high enough to affect vascular endothelial function in individuals with a predisposition to subsequent development of preeclampsia (59).

Conclusions

After 20 years of medical research on the etiology and the mechanisms that lead to preeclampsia, we believe that this disease results from the interaction between economic, psychosocial, nutritional, environmental and genetic factors that lead to a common alteration, i.e., an imbalance in the production of free radicals, NO, O_2^- and peroxynitrate (60). In Figure 1 we present an integrative model where all the known risk factors and the new ones are listed together to explain the clinical manifestations of preeclampsia.

An adequate interpretation of this model would allow us to understand that the best way to prevent preeclampsia in a universal and effective manner is the establishment of an adequate prenatal control system, whose procedures should contain an adequate vitamin (A, C and E) and mineral (calcium and iron) supplementation. In addition, adequate prenatal control would allow physicians to diagnose and promptly treat asymptomatic urinary and vaginal infections. Finally, the role that infection and inflammation may play in the imbalance of free radicals that leads to preeclampsia needs to be studied in depth because it may involve a fundamental change in the prevention and treatment of preeclampsia.

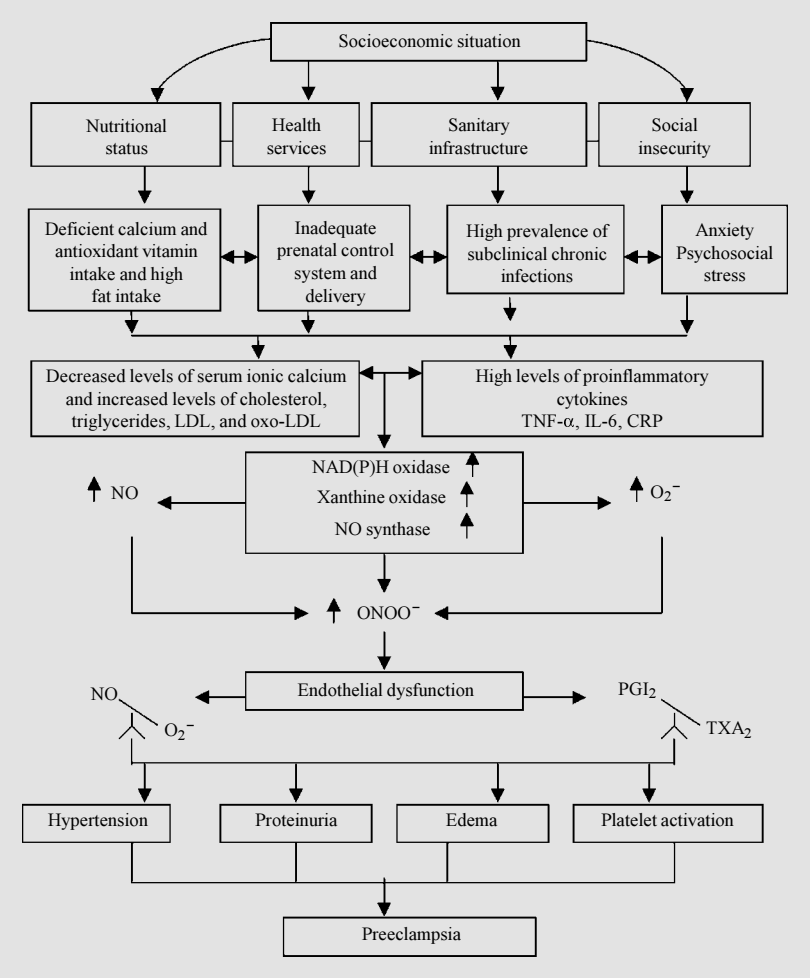

Figure 1. Interaction between different social, economic, nutritional and environmental factors that contribute to creating an imbalance between nitric oxide/superoxide (NO/O_2^-) that leads to the subsequent development of preeclampsia. CRP, C-reactive protein; PGI_2, prostaglandin I_2; TXA_2, thromboxane A_2.

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