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Vascular and Cellular Calcium in Normal and Hypertensive Pregnancy

Zuzana Adamova, **Sifa Ozkan**, and **Raouf A. Khalil**
Division of Vascular Surgery, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

Abstract

Normal pregnancy is associated with significant hemodynamic changes in the cardiovascular system in order to meet the metabolic demands of mother and fetus. These changes include increased cardiac output, decreased vascular resistance, and vascular remodeling in the uterine and systemic circulation. Preeclampsia (PE) is a major complication of pregnancy characterized by proteinuria and hypertension. Several risk factors have been implicated in the pathogenesis of PE including genetic and dietary factors. Ca^{2+} is an essential dietary element and an important regulator of many cellular processes including vascular function. The importance of adequate dietary Ca^{2+} intake during pregnancy is supported by many studies. Pregnancy-associated changes in Ca^{2+} metabolism and plasma Ca^{2+} have been observed. During pregnancy, changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) have been described in red blood cells, platelets and immune cells. Also, during pregnancy, an increase in $[Ca^{2+}]_i$ in endothelial cells (EC) stimulates the production of vasodilator substances such as nitric oxide and prostacyclin. Normal pregnancy is also associated with decreased vascular smooth muscle (VSM) $[Ca^{2+}]_i$ and possibly the Ca^{2+}-sensitization pathways of VSM contraction including protein kinase C, Rho-kinase, and mitogen-activated protein kinase. Ca^{2+}-dependent matrix metalloproteinases could also promote extracellular matrix degradation and vascular remodeling during pregnancy. Disruption in the balance between dietary, plasma and vascular cell Ca^{2+} may be responsible for some of the manifestation of PE including procoagulation, decreased vasodilation, and increased vasoconstriction and vascular resistance. The potential benefits of Ca^{2+} supplements during pregnancy, and the use of modulators of vascular Ca^{2+} to reduce the manifestations of PE in susceptible women remain an important area for experimental and clinical research.

Keywords

calcium; pregnancy; hypertension; preeclampsia; endothelium; smooth muscle

INTRODUCTION

Physiological changes in normal pregnancy (Norm-Preg) adapt the cardiovascular system to the increased metabolic needs of the mother, and allow adequate delivery of oxygenated blood and nutrients to the fetus. Maternal cardiac output, plasma volume and renal blood flow increase during Norm-Preg (Baylis,1987; Sowers et al. 1990). The hypervolemia during the later half of Norm-Preg is related to increased plasma levels of estrogen and progesterone which induce the renin-angiotensin-aldosterone system, cause sodium retention and increase total body

Correspondence and proofs should be sent to: Raouf A. Khalil, MD, PhD Harvard Medical School Brigham and Women's Hospital Division of Vascular Surgery 75 Francis Street Boston, MA 02115 Tel: (617) 525-8530 Fax: (617) 264-5124 raouf_khalil@hms.harvard.edu.

water. Other hormones, such as prolactin, placental lactogen, prostaglandins and growth hormone are increased during Norm-Preg and may contribute to water retention.

Norm-Preg is also associated with decreases in systemic and renal vascular resistance, and blood pressure (BP) (Baylis et al.1987; Sowers et al. 1990). The pregnancy-associated decrease in vascular resistance begins in week 5, with a nadir in weeks 20 to 32, and slowly increases after week 32 until full-term (Duvekot & Peeters, 1994). The pregnancy-associated changes in the systemic and renal hemodynamics are attributed to increased expression of nitric oxide synthase (NOS) and nitric oxide (NO) production by many cell types including renal and vascular cells (Deng et al. 1996, Sladek et al. 1997, Alexander et al. 1999, Nelson et al.2000, Abram et al.2001). Also, during Norm-Preg there is decreased pressor response and vascular reactivity to vasoconstrictor stimuli such as α-adrenergic agonists and angiotensin II (AngII) (Davidge & McLaughlin 1992; Khalil et al. 1998; Khalil & Granger, 2002).

In contrast to the decreased BP during Norm-Preg some women may have hypertension in pregnancy (HTN-Preg). HTN-Preg may present as: chronic HTN that predates pregnancy, preeclampsia (PE)-eclampsia, chronic HTN with superimposed PE, and gestational HTN, a nonproteinuric HTN-Preg (Khalil & Granger, 2002). In 5 to 7% of pregnancies in the US, 15% of pregnancies among African-Americans, women develop PE. PE manifests after the 20^{th} week of pregnancy and is characterized by HTN and proteinuria. HTN is defined as systolic BP >140 or diastolic BP >90 mmHg in a woman known to be normotensive prior to pregnancy. Proteinuria is defined as urinary protein excretion ≥300 mg/24 hr. The clinical phase of PE is associated with enhanced pressor response to vasoconstrictors such as AngII and reduced renal plasma flow. Severe PE may be associated with oliguria, cerebral or visual disturbances, pulmonary edema, cyanosis, impaired liver function, thrombocytopenia, or intrauterine growth restriction. Another feature of PE is abnormal activation of the maternal coagulation cascade with an imbalance in production of the arachidonic acid metabolites thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2), favoring TXA_2, as compared to Norm-Preg where an 8-fold increase in PGI_2 production dominates a small increase in TXA_2. Although PE-eclampsia represent a major cause of maternal and fetal morbidity and mortality, the triggering mechanisms of the hemodynamic and vascular changes have been elusive. PE remits dramatically postpartum after the delivery of the placenta, suggesting a role of the placenta. It has been suggested that reduction of uterine perfusion pressure (RUPP) in late pregnancy causes placental ischemia and initiates the release of biologically active factors such as vascular endothelial growth factor (VEGF) and inflammatory cytokines that cause the changes in maternal circulation and endothelial cell (EC) function observed in PE (Granger et al., 2002).

Due to the difficulty of performing mechanistic studies in pregnant women, several animal models of HTN-Preg have been developed. Surgical RUPP and induction of placental ischemia in late pregnant rats generates a phenotype mimicking many of the characteristics seen in human PE, including HTN, proteinuria and fetal growth restriction. The RUPP rat also demonstrates altered vascular functions as those observed in other animal models and in women with PE (Khalil & Granger, 2002; Gilbert et al., 2008).

PE has been linked to fetal and maternal genes, as well as mother's age, ethnic background, and health condition (Mackay et al. 2001). The incidence of PE may increase with dyslipidemia, hyperglycemia, chronic HTN, and with changes in dietary intake of proteins, vitamins and minerals such as Ca^{2+}. Studies have examined dietary Ca^{2+} intake during the course of normal and complicated pregnancy. Possible correlations between dietary Ca^{2+} intake, plasma levels of Ca^{2+} and the incidence of PE have been examined. Also, the potential benefits of Ca^{2+} supplementation (Ca^{2+}-Suppl) during pregnancy to reduce PE have been explored (Ramos et al. 2006); although the results have not been consistent. Also, while changes in vascular function play a major role in the control of vascular resistance and BP, little is known regarding

the mechanisms of vascular contraction, relaxation and remodeling during pregnancy and PE. Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a major role in regulating the functions of many cell types including blood and vascular cells. Increased $[Ca^{2+}]_i$ in EC stimulates the production of vasodilator substances such as NO and PGI_2. Also, VSM contraction is triggered by increases in $[Ca^{2+}]_i$. However, the regulation of dietary, plasma, and vascular tissue Ca^{2+} during Norm-Preg and the dysregulation of these mechanisms in the setting of the increased vascular resistance and BP in PE are not clearly understood.

The objective of this review is to examine reports in PubMed database and our research work to provide an insight on the inter-relationship between dietary, plasma and vascular cell Ca^{2+} during Norm-Preg and PE. We will first summarize the current understanding of the role of dietary Ca^{2+} and vitamin D, a co-factor in Ca^{2+} metabolism, during pregnancy. We will then describe the mechanisms of Ca^{2+} metabolism during pregnancy, and the changes in plasma Ca^{2+} levels in PE. We will then highlight the changes in $[Ca^{2+}]_i$ in blood cells, platelets and immune cells during pregnancy. We will follow with description of pregnancy-associated changes in the Ca^{2+} regulatory mechanisms of EC and VSM cell function, and the extracellular matrix (ECM) of the vascular wall. The review will end with a perspective and clinical applications of the current knowledge on dietary, plasma and cellular Ca^{2+} during pregnancy, and the possible benefits of Ca^{2+}-Suppl to reduce the incidence or manifestations of PE.

Dietary Ca^{2+} during Pregnancy

The Food and Nutrition Board of the National Academy of Science recommendation for Ca^{2+} intake is 1300mg/day for adolescents 9 to 18yr, and 1000 mg/day for adults 19-51yr (Bronner et al. 2006). The recommended dietary sources of Ca^{2+} are low fat dairy products (milk, cheese, yogurt) and certain green vegetables (broccoli, kale). Ca^{2+}-Suppl are also available as chewable Ca^{2+} or in tablet form. The National Institutes of Health have defined the % Americans in different age groups that do not meet the recommended Ca^{2+} intake: 6-11yr 44% boys and 58% girls, 12-19 yr 64% boys and 87% girls, older than 20 yr 55% men and 78% of women. Among females, dietary Ca^{2+} is influenced not only by age, but also by ethnic background. Among girls 14-18 yr old the daily Ca^{2+} intake as % of the adequate dose is 58.1% by Caucasian, 48.2% by Hispanic and 44.9% by African-Americans (Storey et al. 2004). The daily Ca^{2+} intake can be further compromised by strict dietary regimens particularly among teen-agers. The nutritional status and dietary Ca^{2+} intake are of particular importance during pregnancy as they may affect the outcome of pregnancy and the postpartum condition of mother and fetus. The pregnant woman's body provides 50 to 300 mg/day Ca^{2+} for the developing fetal skeleton. Differences in total Ca^{2+} intake were observed between Caucasian (1556 mg/day) and African-American pregnant women (1421 mg/day) (Harville et al. 2004).

Most studies suggest that Ca^{2+}-Suppl in the range of 375-2000 mg may be beneficial during pregnancy. Ca^{2+} deficiency may increase the risk of PE particularly among teenagers, and Ca^{2+}-Suppl may reduce the incidence of PE. However, the beneficial effects of Ca^{2+}-Suppl appear to vary depending on the mother's age and socioeconomic status, particularly in geographical locations where Ca^{2+} intake is low. The results could also depend on whether the subjects under study include normotensive women or women prone to PE, and whether the subjects have autoimmune disorders or under heparin therapy (Thomas & Weisman, 2006).

Randomized controlled clinical trails (RCCTs) have shown that Ca^{2+}-Suppl is associated with reduced risk of HTN-Preg and PE (Table 1), and suggested that Ca^{2+}-Suppl in a population with low Ca^{2+} intake is a safe, effective and inexpensive measure to reduce the risk of PE (Knight & Keith, 1992;Bucher et al. 1996;López-Jaramillo et al., 1997). Ca^{2+}-suppl reduce the risk of PE and preterm birth particularly in nulliparous women (Crowther et al. 1999). Other studies were more skeptic regarding the effects of Ca^{2+}-Suppl during pregnancy. The Ca^{2+} for PE Prevention (CPEP) trial has shown no significant effect of 2000 mg/day Ca^{2+}-Suppl on BP

or incidence of PE. Also, when the analysis was stratified according to patient's compliance, there was no effect on PE even among women who were most compliant with treatment (Levine et al. 1997). Other studies did not observe a lower risk of PE with greater dietary intake of Ca^{2+}, vitamin C, D, or E, Mg^{2+}, folate or elongated omega-3 fatty acids (Oken et al.2007). Further evaluation of these observations by the FDA have led to the suggestion that it is unlikely that consuming Ca^{2+}-Suppl during pregnancy would reduce the risk of HTN-Preg or PE (Trumbo & Ellwood, 2007). Similarly, a World Health Organization RCCT of Ca^{2+}-suppl among low Ca^{2+} intake pregnant women concluded that it did not prevent PE, although it did reduce PE severity, maternal morbidity and neonatal mortality (Villar et al. 2006).

Interestingly, compiling data from 12 clinical trials, including some of the aforementioned studies, led to the conclusion that Ca^{2+}-suppl may reduce the risk of HTN-Preg by 50%. The composite outcome maternal death or serious morbidity was also reduced. The effect was greatest in women at high risk of PE and those with low baseline Ca^{2+} intake (Hofmeyr, 2007).

The discrepancy in the effects of Ca^{2+}-Suppl among various studies may be related to the study design. Significant heterogeneity was observed within the studies, with less effect of Ca^{2+}-Suppl in the larger RCCTs (Hofmeyr et al., 2007). The discrepancy may also be related to the subjects' age. For example, studies that included large number of pregnant teen-agers, whose demand for Ca^{2+} exceeds that of pregnant adults, have shown that Ca^{2+}-Suppl result in a significant decrease in BP and 12.4% decline in risk of PE (López-Jaramillo et al., 1997).

Experimental studies support beneficial effects of dietary Ca^{2+} during pregnancy. In pregnant ewes, restricted Ca^{2+} intake is associated with decreased plasma Ca^{2+} level and uterine blood flow, increased BP and elevated urinary protein; all symptoms similar to those of PE in women (Prada et al.1994). Also, low Ca^{2+} intake in Norm-Preg rats is associated with increased pressor effects of AngII (Aiko et al.,1992). Also, in both Norm-Preg and nonpregnant rats, a high Ca^{2+} diet (1.7%-2.1%) is associated with reduction in BP, and attenuated VSM reactivity *in vitro*. At the cellular level, the mechanism of attenuated reactivity appears to involve the EC-dependent NO-guanylate cyclase pathway (Ezimokhai & Osman, 1998).

Thus both clinical and experimental studies provided a tangible evidence for beneficial effect of Ca^{2+}-Suppl during pregnancy. The Ca^{2+}-Suppl may exert the strongest influence when dietary Ca^{2+} is low. If Ca^{2+} intake is adequate, no additional Ca^{2+}-Suppl may be needed.

Vitamin D during pregnancy and PE

Vitamin D (cholecalciferol) is a major factor in normal Ca^{2+} absorption and metabolism. Cholecalciferol is formed by the skin during exposure to sunlight and UV irradiation, and also absorbed from ingested food. Absorbed cholecalciferol is converted in the liver to 25(OH) cholecalciferol or 25(OH)D, and further hydroxylated by 1α-hydoxylase in kidney to 1,25 dihydroxycholecalciferol or $1,25(OH)_2D_3$, the most active form of the vitamin D group. $1,25(OH)_2D_3$ is released in the blood stream and distributed to various target organs.

$1,25(OH)_2D_3$ induces genomic responses by diffusing in the cytoplasm and binding with strong affinity to vitamin D receptor, which promotes the transcription and translation of various proteins involved in bone formation (Fischer et al., 2007). $1,25(OH)_2D_3$ is also involved in nongenomic responses that increase intracellular Ca^{2+}. It stimulates Ca^{2+} transport from the intestine and renal tubules. Small concentrations promote bone calcification, while large concentrations augment bone resorption. Vitamin D receptors are also found in the placenta. $1,25(OH)_2D_3$ levels are elevated during pregnancy due to increased activity of kidney 1α-hydoxylase and placental production (Perez-Lopez, 2007).

Adequate concentrations of 25(OH)D (>80 nmol/L) are needed for optimal health of the skeletal system, and also influence the cardiovascular system and BP. More than one-third of the US population suffer from vitamin D deficiency, defined as 25(OH)D <30 to 50 nmol/L. In Middle-Eastern countries vitamin D levels among women are very low. For example, in Morocco the prevalence of vitamin D deficiency (<30 ng/mL) is 91% (Allali et al., 2008).

Serum levels of 25(OH)D differ between men and women, Caucasian and African-Americans, and in winter compared to summer season (Burnand et al.1992). BP is known to increase in winter in the northern hemisphere where vitamin D synthesis in the skin is low. Results from the third National Health and Nutrition Examination Survey revealed an inverse association between circulating 25(OH)D and BP in Caucasian men and women. African-American men and women have significantly lower concentrations of 25(OH)D at all BP classifications than do Caucasian (Judd et al., 2008).

Studies in women supported an inverse relationship between estimated dietary intake of vitamin D and systolic BP (Sowers et al., 1985). In one study involving 148 women, women received 1200 mg Ca^{2+}/day either alone or plus 800 IU vitamin D. Compared with Ca^{2+} alone, vitamin D plus Ca^{2+} resulted in 72% increase in serum 25(OH)D, 17% decrease in serum PTH, and 9.3% decrease in systolic BP (Pfeifer et al., 2001). Vitamin D requirements are increased during pregnancy, and the relation between vitamin D deficiency and the increased BP associated with HTN-Preg needs to be carefully examined. Studies have shown that circulating 1,25-$(OH)_2D_3$ levels in both maternal and umbilical cord compartments are lower in PE compared with Norm-Preg (Halhali et al., 2007).

Experimental studies examined the influence of vitamin D on the renin-angiotensin system. It was found that renin expression and plasma AngII production were increased in vitamin D receptor null mice, and were associated with HTN. Also, in wild-type mice, inhibition of 1,25 $(OH)_2D_3$ synthesis led to increased renin expression, whereas 1,25$(OH)_2D_3$ injection caused renin suppression. Hence, 1,25$(OH)_2D_3$ is a negative regulator of the renin-angiotensin system and thereby plays a critical role in the regulation of BP (Li et al., 2002).

Plasma Ca^{2+}

Ca^{2+} concentration in the extracellular fluid is strictly controlled and maintained at a normal serum level of 2.2-2.6 mmol/L; 40% bound to albumin, 10% in complex with citrate and the rest is ionized Ca^{2+} – the most important fraction. Three main factors affect the level of serum Ca^{2+} – 1,25$(OH)_2D_3$ (vitamin D_3), parathyroid hormone (PTH) and calcitonin (Fig. 1).

PTH increases serum Ca^{2+} levels by inducing bone resorption, intestinal transport, and renal tubular reabsorption. PTH also stimulates the formation of 1,25$(OH)_2D_3$, which in turn stimulates Ca^{2+} transport from the intestine and renal tubules, and in large quantities augments bone resorption. Calcitonin, a peptide hormone secreted by the parafollicular cells in the thyroid gland, tends to decrease plasma Ca^{2+} level mainly by inhibiting osteoclasts and suppressing Ca^{2+} mobilization from bone. Calcitonin also has modest effect on kidney.

Other hormones also influence Ca^{2+} homeostasis. Adrenal steroids decrease osteoblast function and bone formation, and increase osteoclast number and activity. Glucocorticoids decrease intestinal Ca^{2+} absorption and renal Ca^{2+} reabsorption, and augment renal excretion. Excess glucocorticoids cause osteoporosis. Thyroid hormones may cause hypercalcemia and hypercalciuria. Growth hormone facilitates intestinal absorption and renal excretion of Ca^{2+}.

During pregnancy Ca^{2+} is transferred to the fetus via the placenta. The higher Ca^{2+} requirement during pregnancy requires physiologic adaptation of the Ca^{2+} homeostatic mechanisms including intestinal absorption, urinary excretion, and maternal bone Ca^{2+} turnover. These

mechanisms are partly influenced by the placenta estrogen, progesterone, and human chorionic gonadotropin. Intestinal Ca^{2+} absorption rises during the first weeks of pregnancy, and reaches a maximum in the last trimester. In contrast, urinary excretion of Ca^{2+} decreases during pregnancy. Biochemical markers of bone formation (serum osteocalcin and bone alkaline phosphatase) and bone degradation (*N*-telopeptides of type I collagen) increase during pregnancy. Ca^{2+} absorption is positively associated with serum $1,25(OH)_2D_3$ and PTH (Cross et al.,1995). Plasma $1,25(OH)_2D_3$ levels increase two-fold early in pregnancy due to high placental 1α-hydroxylase activity, remain high until delivery and decline to normal values during lactation. The levels of estrogen, prolactin and placental lactogen, which are involved in Ca^{2+} absorption, increase at the same time (Ritchie et al.,1998; Vargas Zapata et al., 2004).

During Norm-Preg, the increased levels of $1,25(OH)_2D_3$ and the facilitation of intestinal Ca^{2+} absorption results in hypercalcemia, which causes PTH levels to decrease (Seely et al., 1992). Also, in response to hypercalcemia, calcitonin is secreted and suppresses Ca^{2+} reabsorption in the renal tubules and results in hypercalciuria. Calcitonin also counteracts the bone resorptive effects of $1,25(OH)_2D_3$, and thus protects the maternal skeleton (Whitehead et al., 1981). In some studies, urinary Ca^{2+} conservation in pregnancy was associated with an increase in serum PTH. However, in women with habitually high Ca^{2+} intake, the association between reduced urinary Ca^{2+} and increased PTH was not evident. When comparing the different stages of pregnancy, PTH levels were low in early pregnancy, declined toward mid-term, but increased in late pregnancy (Davis et al., 1988; Seki et al., 1991). Other studies demonstrated that during pregnancy PTH levels were not different from the levels of nonpregnant controls (Gillette et al.,1982; Saggese et al., 1991). In pregnant rats, PTH levels were higher than in nonpregnant rats (Bourdeau et al.1990). Opposite results have shown that PTH and Ca^{2+} levels were lower in Norm-Preg than nonpregnant rats (Gonen et al., 2005).

Estrogen regulates bone metabolism, prohibiting bone loss and inhibiting stimulatory effect of cytokines on osteoclasts. Therefore, the high gestational estrogen is predicted to protect maternal bone. However, during pregnancy maternal bone mineral density (BMD) decreases, suggesting bone loss (Fukuoka & Haruna, 2003). In rats, Norm-Preg increases BMD, whereas lactation decreases it. Changes in PTH levels contribute to mineralization and demineralization of the skeleton during pregnancy and lactation, respectively (Gonen et al., 2005).

Studies have demonstrated lower levels of $1,25(OH)_2D_3$ and PTH in HTN-Preg and suggested that alterations in Ca^{2+} regulatory hormones could contribute to the development of PE, and the symptom of hypocalciuria (August et al.,1992). Other studies suggest that in PE the low levels of $1,25(OH)_2D_3$ cause a decrease in intestinal Ca^{2+} absorption and serum ionized Ca^{2+}. The lower ionized Ca^{2+} causes an increase in PTH levels, which stimulate Ca^{2+} loss from bones, and tubular reabsorbtion of Ca^{2+} from kidney leading to hypocalciuria *(*Seely et al., 1992). Therefore, urinary Ca^{2+} can be an early marker for PE (Sanchez-Ramos et al., 1991). Hypocalciuria could be a compensatory mechanism for the increased total vascular resistance and decreased renal blood flow in PE (Szmidt-Adjide et al., 2006).

Studies have shown that the plasma Ca^{2+} levels go down a little during Norm-Preg and markedly decrease in women with PE (Seely et al., 1992; Kisters et al., 2000; Sukonpan & Phupong, 2005) (Table 2). However, in one study serum Ca^{2+} was reduced and fractional renal excretion of Ca^{2+} (FECa) increased in Norm-Preg compared to nonpregnant controls. In PE, serum Ca^{2+} was not different from that in Norm-Preg group, but FECa was lower. PTH was slightly lower during Norm-Preg than after delivery, but did not deviate from the nonpregnant group. In PE, PTH did not deviate from the levels in Norm-Preg. Also, calcitonin was the same in the third trimester of pregnancy in both groups. It was concluded that both Norm-Preg and PE are accompanied by considerable alterations in Ca^{2+} metabolism, that PTH and calcitonin

in both groups are mainly unchanged from nonpregnant level, and that the increase and decrease in renal Ca^{2+} excretion in Norm-Preg and PE, respectively, may be attributed to changes in kidney function (Pedersen et al., 1984). Other studies have also demonstrated no difference in serum ionized Ca^{2+} between Norm-Preg and HTN-Preg subjects (Richards et al., 1984).

Experimental studies have shown higher total plasma Ca^{2+} in Norm-Preg than non-pregnant rats. Both the plasma total and ionized Ca^{2+} concentration were lower in L-NAME treated rat model of HTN-Preg compared to Norm-Preg rats (Ebose et al., 2007) (Table 3).

Regulation of $[Ca^{2+}]_i$

Ca^{2+} is a major regulator of the function of various vascular cells. $[Ca^{2+}]_i$ homeostasis is tightly regulated by Ca^{2+} mobilization and Ca^{2+} extrusion mechanisms (Fig. 2). The Ca^{2+} mobilization mechanisms include Ca^{2+} release from the intracellular stores, and Ca^{2+} influx from the extracellualr space through voltage-gated, ligand-gated and store-operated Ca^{2+} channels. Excess Ca^{2+} is either taken up by Ca-ATPase in the intracellular store membrane, or extruded via plasmalemmal Ca-ATPase and Na^+/Ca^{2+}-exchanger. Extremely high cellular Ca^{2+} may be taken up by the mitochondria. The Ca^{2+} regulatory pathways are slightly different in various vascular cells in order to adapt for the specific cell function (Fig. 2).

Ca^{2+} in Red blood cells

Blood volume, as determined by measuring red blood cell volume and plasma volume, is reduced in PE (Silver et al.,1998). Although plasma Ca^{2+} levels may be low in PE, the erythrocytes $[Ca^{2+}]_i$ and membrane Ca^{2+} content are increased, suggesting altered membrane ion transport (Table 2). The highest BP measurements were recorded in PE women with extremely high erythrocytes $[Ca^{2+}]_i$ (Kisters et al.,2000). The increased erythrocytes $[Ca^{2+}]_i$ in PE may be caused by an increase in PTH (Sowers et al., 1989; Kosch et al., 2000).

Ca^{2+} uptake by red blood cell membranes is reduced by ~50% in PE compared with Norm-Preg, suggesting that the membrane Ca-ATPase activity is diminished in PE (Nardulli et al. 1994, Ray et al.1999; Teppa-Garrán et al., 2004; Oviedo et al., 2006). Ca-ATPase activity in the myometrium and placental membranes is also 50% lower in PE than Norm-Preg women, suggesting an endogenous inhibitor of Ca-ATPase in PE (Javed et al., 2000; Carrera et al., 2003). The reduced Ca-ATPase activity of the red cell membranes from PE women was not associated with reduced number of Ca-ATPase molecules or a defective enzyme, but rather with a high level of lipid peroxides (Oviedo et al., 2006). A positive correlation was also found between systolic and diastolic BP and plasma levels of malondialdehyde, a lipid peroxidation product (Kaur et al., 2008). The role of lipid peroxidation and Ca^{2+} in PE is supported by reports that increased lipid peroxides inhibit Na,K-ATPase and Ca-ATPase (Carrera et al., 2003). These changes may occur in other cell types of PE women, and lead to an increase in $[Ca^{2+}]_i$, and some of the symptoms of PE. Lipid peroxidation is partly responsible for the endothelial damage associated with PE (Kaur et al., 2008). Also, a dysfunctional Ca-ATPase could result in increased $[Ca^{2+}]_i$ and vasoconstriction in VSM. Increased lipid peroxides in the placental tissue is also caused by the uteroplacental hypofusion, a triggering event in the pathogenesis of PE (Carrera et al., 2003).

Ca^{2+} in Platelets

In platelets, basal $[Ca^{2+}]_i$ is ~100 nmol/L and is regulated by the intracellular Ca^{2+} stores, membrane channels and Ca-ATPase pump (Kilby et al., 1993). Platelets are activated in early Norm-Preg, but the degree of their activation is controlled by yet unknown buffering factors that maintain hemostasis and prevent further platelet activation. For example, in Norm-Preg the platelets show refractoriness to activation by IL-1 and TNF-α. This refractoriness is lost or diminished in PE, and platelet activation by cytokines occurs weeks to months before the

clinical appearance of PE. Failure to control the platelet activation during pregnancy leads to platelet adhesion, aggregation, and release of TXA_2, which in turn, cause vasoconstriction, further aggregation, and progressive damage to EC (Bar et al.,2004). Also, in PE women during the third trimester, the platelets show more extensive activation, indicated by the increased expression of P-selectin (CD62P), CD63, plasma β-thromboglobulin, and platelet EC adhesion molecule 1 (PECAM-1) (Janes et al.1995; Konijnenberg et al.1997).

Platelet aggregation and secretion are associated with a rise in $[Ca^{2+}]_i$. Several studies suggest that platelet $[Ca^{2+}]_i$ is increased in Norm-Preg women in the third trimester, and highly increased in PE (Haller et al., 1989; Kilby et al.1993) (Table 2). Other studies have shown no significant difference in basal platelet $[Ca^{2+}]_i$ between Norm-Preg and PE women (Barr et al. 1989, Zemel et. al 1990). While some studies showed exaggerated response of platelet $[Ca^{2+}]_i$ to arginine vasopressin early in pregnancy (Zemel et. al 1990), other studies did not show similar results (van der Post et al.1993, Kyle et al.1995).

In Norm-Preg increased platelet $[Ca^{2+}]_i$ stimulates TXA_2 formation and lowers level of cAMP which further increase $[Ca^{2+}]_i$ and enhance platelet aggregation (Sheu et al. 2002). TXA_2 also acts as a vasoconstrictor (Herrera et al., 2006). An increase in platelet $[Ca^{2+}]_i$ in PE, if present, may explain the increased secretion and aggregability seen in this condition. Studies have shown that platelets from PE women require higher concentrations of PGE_1 to inhibit aggregation than those from women with nonproteinuric gestational hypertension indicating increased platelet activity in PE women (Torres et al.1996). PGE_2, a vasodilator produced by ECs, inhibits platelet aggregation (Herrera et al., 2006). In contrast, platelets from women with HTN have high basal secretory levels of TXA_2 (Hawkins et al.1993). Also, the sensitivity and $[Ca^{2+}]_I$ response of platelets to AngII is increased in PE compared to Norm-Preg women (Haller et al.1989). Platelets cGMP is reduced in PE compared to Norm-Preg, indicating decreased amount/action of NO (Teran et al. 2004). In contrast with the inconsistent data in human PE, experimental studies suggest that the platelet resting $[Ca^{2+}]_i$ is higher in L-NAME-treated rat model of HTN-Preg compared with Norm-Preg rats (Ebose et al., 2007) (Table 3).

Ca^{2+} in Immune Cells

There is compelling evidence of peripheral immune cell activation in PE. Leukocyte activation may be secondary to EC activation by circulating syncytiotrophoblast microvillous membranes (STBM) shed from the PE placenta. EC co-cultured with STBM cause significant activation of granulocytes and monocytes as indicated by increased $[Ca^{2+}]_i$, decreased pH_i and release of ROS. Lymphocytes respond mainly with an increase in ROS (von Dadelszen et al. 1999). Serum from PE patients increases ICAM-1 expression on EC surface, which in turn binds and activates leukocytes. Also, expression of integrin counter receptors on leukocytes is increased in PE and Norm-Preg compared with the nonpregnant state, and the expression decreases significantly postpartum (Haller et al.1997). Polymorphonuclear neutrophils (PMN) are the cells most affected in PE, with changes in expression of surface markers and release of granule enzymes. PE is associated with changes in L-selectin on PMN, monocytes and T cells when compared with Norm-Preg. These changes include increased nuclear translocation of NF-κB and levels of IL-6 (Luppi et al.2006). PMN generate superoxide anion ($O_2^{-}\bullet$), and $O_2^{-}\bullet$ generation is reduced in Norm-Preg compared with nonpregnant controls, but is increased in PE and lead to EC dysfunction (Lee et al.2003, Aly et al.2004). Also, during PE, PMN produce TXA_2 and TNF-α in response to oxidative stress (Vaughan et al.2005).

$[Ca^{2+}]_i$ is a key regulator in immune cells. Activation of PMN is associated with rapid elevation in $[Ca^{2+}]_I$ due to Ca^{2+} release from the intracellular stores and Ca^{2+} entry through plasma membrane channels such as store-operated Ca^{2+} channels (Steinckwich et al.2007). A correlation between ROS production and Ca^{2+} entry suggest that endogenous ROS reinforce Ca^{2+} signaling by positive feedback (Giambelluca & Gende, 2008). PMN are essential for the

phagocytosis and killing of pathogenic bacteria, an action that depends on the production of ROS by the phagocyte NADPH oxidase and on the release of proteases. The same effector mechanisms could cause tissue damage in the case of inappropriate PMN activation. Maternal plasma levels of elastase may serve as a marker of PMN activation (Gupta et al., 2006). PMN $[Ca^{2+}]_i$ is higher in PE, compared with healthy pregnant and non-pregnant women (von Dadelszen et al., 1999). Intracellular Ca^{2+} flux is an early step in the signaling cascade that bridges stimulation of selectin and chemokine receptors to activation of adhesive and motile functions (Schaff et al., 2008). The importance of Ca^{2+} is confirmed by the finding that Ca^{2+} channel blockers suppress cytokine-induced PMN activation (Shima et al., 2008). Membrane depolarization accompanies activation of the phagocyte NADPH oxidase. NADPH oxidase regulates PMN membrane potential and Ca^{2+} influx via its electrogenic activity and as a result of generation of ROS (Tintinger et al., 2007).

In PE, changes in $[Ca^{2+}]_i$ occur in other immune cells (von Dadelszen et al., 1999). Peripheral blood monocytes from pregnant women secrete low levels of vasoactive prostanoids and respond to PGI_2, PGE_2 and TXA_2 in a manner similar to that of nonpregnant women. The cells from women with HTN-Preg demonstrate increased reactivity, exaggerated rise in TXA_2 secretion, and prostacyclin (PGI_2) to a lesser extent (Hawkins et al.1993).

In lymphocytes isolated from decidua of PE women, secretion of Th2-type cytokines IL-6 and IL-10 is decreased, while the Th1-type cytokine IFN-γ is increased (Wilczyński et al.2002). Elevation of $[Ca^{2+}]_i$ is one of the triggering signals for T-cell activation by antigen. Stimulation of the T cell receptor, activates IP_3 and ryanodine receptors and Ca^{2+} release from the intracellular stores, and depletion of the stores triggers Ca^{2+} influx through store-operated Ca^{2+} (CRAC) channels. The amplitude and dynamics of the Ca^{2+} signal are controlled by several mechanisms, including K^+ channels, membrane potential, plasma membrane Ca-ATPase, and mitochondria that buffer Ca^{2+} and prevent inactivation of CRAC channels (Lewis et al., 2001).

NF-κB is a key regulator of immune responses. Phosphatidylinositol 3-kinase (PI_3K) and its downstream target Akt, as well as Ca^{2+}/calmodulin-dependent protein kinase and calcineurin are implicated in NF-κB regulation (Chen et al.,2002). Lymphocyte basal $[Ca^{2+}]_i$ is higher in PE than Norm-Preg women (Table 2). Exposure of lymphocytes to low extracellular Ca^{2+} results in an increase in $[Ca^{2+}]_I$, and may serve as a marker of PE (Hojo et al., 1999).

Ca^{2+} in Endothelial Cells (ECs)

$[Ca^{2+}]_i$ is a major regulator of EC function. Both hemodynamic shear stress and Ca^{2+} mobilizing agonists elicit a rise in EC $[Ca^{2+}]_i$ (Fig. 3). Also, Ca^{2+} entry triggered by intracellular Ca^{2+} store depletion regulate many EC functions. ECs release relaxing factors such as NO, PGI_2, and hyperpolarizing factor as well as contracting factors such as TXA_2, endothelin (ET-1), AngII and PAF. A rise of $[Ca^{2+}]_i$ activates Ca^{2+}-dependent enzymes such as NOS and phospholipase A_2, leading to the production and release of NO and PGI_2. $[Ca^{2+}]_i$ also plays a role in cytokine production, induction of adhesion molecules, disassembly of EC junctions and increased EC permeability. Also, in ECs induction of NF-κB by various stimuli requires $[Ca^{2+}]_i$ for proper signal transduction (Nilius & Droogmans, 2001).

EC $[Ca^{2+}]_i$ depends on Ca^{2+} entry through membrane channels, Ca^{2+} pumps, and Ca^{2+} release from intracellular stores. Ca^{2+} entry occurs through nonselective cation channels including: 1) Agonist or receptor-activated cation channels involving phospholipase C, but the downstream second messengers are unclear; 2) Amiloride-sensitive channels may regulate cation fluxes across the blood-brain barrier; 3) Redox nonselective cation channels activated by oxidative stress and equally permeable for Na^+, K^+ and also Ca^{2+}; 4) Cyclic nucleotide-gated channels. Nonselective cation channels are mechanosensitive and increase $[Ca^{2+}]_i$ in response to shear

stress or increased flow. Mechanical forces also affects K^+ and Cl^- channels causing changes in membrane potential and VGCCs. Store-operated or capacitative Ca^{2+} entry is controlled by the filling degree of intracellular Ca^{2+} stores and is a major pathway for Ca^{2+} influx during agonist stimulation. These channels are much more selective for Ca^{2+} and some belong to the *trp* gene family, which encodes the transient receptor potential channels (TRPCs). Two highly Ca^{2+}-selective agonist-activated channels are activated by ATP and bradykinin. Ca^{2+} also enters ECs via reverse-mode Na^+/Ca^{2+} exchanger, where reduction of the Na^+ gradient allows more Ca^{2+} entry and increases $[Ca^{2+}]_i$ (Nilius & Droogmans, 2001).

In ECs, NO synthases (NOS) convert L-arginine to L-citrulline with the release of NO. Endothelial eNOS is a constitutive Ca^{2+}-calmodulin dependent isoform. Increased blood flow causes ECs to synthesize NO. Also, receptor ligands such as Ach activate ECs and stimulate Ca^{2+} release from the intracellular stores. Depletion of intracellular Ca^{2+} stores signals an influx of extracellular Ca^{2+} which activates the eNOS associated with the plasma membrane and promotes NO production. This is supported by reports that inhibiting extracellular Ca^{2+} influx eliminate histamine-stimulated NO production in ECs (Lantoine et al., 1998). NO diffuses to the VSM layer where it activates guanylyl cyclase, producing cGMP, and induces relaxation.

NO plays a major role in gestational vasodialation (Noris et al, 2004). The pregnancy-associated increases in NOS expression/activity are likely due to increased levels of sex hormones. In female guinea pigs, near-term pregnancy and treatment with estradiol (but not progesterone) cause 4-fold increase in Ca^{2+}-dependent NOS activity in the uterine artery. Also, pregnancy-associated increase in NOS activity in the cerebellum is inhibited by the estrogen receptor antagonist tamoxifen. Both pregnancy and estradiol treatment cause increases in eNOS and nNOS mRNA in skeletal muscle, suggesting that the increased NOS activity result from enzyme induction (Weiner et al., 1994).

PE is associated with EC dysfunction. Women with overt PE show increases in circulating levels of markers of EC injury such as cellular fibronectin, clotting factor VII, von Willebrand factor and factor VIII-related antigen (Anumba et al., 1999; Granger et al., 2001; Khalil & Granger, 2002). EC injury is also manifested in one of the most characteristic morphologic lesions of PE, glomerular endotheliosis. In PE, the ischemic placenta and other maternal tissues may release a factor(s) into the systemic circulation that cause EC activation and subsequently dysfunction. EC injury sets in motion a cascade of coagulation, vasoconstriction, and intravascular fluid redistribution that result in the clinical syndrome of PE. Some of the factors released during PE include cytokines such as TNF-α, IL-6, IL-1α and IL-1β, growth factors such as VEGF, lipoproteins or lipid peroxides, ROS, hypoxia inducible factors (HIFs) and neurokinin-B (Vince et al.1995; Davidge et al.1996; Benyo et al.1997; Conrad et al.1998; Lamarca et al., 2007). TNFα destabilizes electron flow in mitochondria, resulting in release of oxidizing free radicals and formation of lipid peroxides, which in turn cause endothelial cell damage (Conrad & Benyo, 1997). Elevated levels of asymmetric dimethyl arginine (ADMA), endogenous inhibitor of eNOS, precede the development of PE (Savvidou et al.2003). Also, STBM shed from the placenta contain factors which could cause EC damage in PE (von Dadelszen et al.1999, Smárason et al.1993). Studies on myometrial and subcutaneous resistance have shown loss of vasodilator effect of Ach, but not substance P in microvessels of PE compared to Norm-Preg women (Wimalasundera et al., 2005).

VEGF may play in the endothelial cell changes associated with PE. Polymorphisms of the VEGF gene (36C/T) are associated with development of PE (Papazoglou et al., 2004; Shim et al., 2007). VEGF may regulate the baseline NO production from ECs. Activation of VEGF, EGF and PDGF receptor tyrosine kinase induces upregulation of eNOS. VEGF also causes a biphasic increase of $[Ca^{2+}]_i$ and stimulates production of NO in human and rabbit ECs (Van

der zee et al. 1997). In resting ECs, eNOS is localized in EC caveolae, small invaginations of the plasma membrane that are abundant in the transmembrane protein caveolin. Caveolin-1 tightly binds to a motif in the oxygenase domain of eNOS and maintains it in an inactive state within caveolae. VEGF receptor stimulation leads to phosphorylation of caveolin-1. Consequently, eNOS dissociates from caveolin-1 and is activated by intracellular Ca^{2+} which is also increased by VEGF (Mukherjee et al.2006). Plasma levels of VEGF and placenta growth factor (PGF) are reduced in severe PE (Livingston et al.2000). Also, the placental expression and secretion of a naturally occurring circulating VEGF antagonist, soluble fms-like tyrosine kinase 1 (sFlt1), is increased in PE (Koga et al.2003, Tsatsaris et al.2003; Karumanchi & Epstein, 2007). When tested in rats, sFlt1 alone induces PE-like phenotype. sFlt1 binds circulating VEGF and prevents its interaction with its EC receptors and thereby lead to EC dysfunction in PE (Kendall & Thomas, 1993, Levine et al.2004). Some studies have shown that VEGF is increased before the clinical onset and further elevated during the vasoconstriction state of PE. The hyperdynamic circulation during the latent phase of PE causes vascular shear stress, which in turn increases the levels of circulating VEGF. Because VEGF normally acts as a vasodilator, its increase may represent an unsuccessful vascular rescue response (Bosio et al.2001).

Some studies have shown that NO levels during PE are equal or higher than in Norm-Preg (Boccardo et al.1996, Anumba et al.1999, Rowe et al.2003). The basal EC $[Ca^{2+}]_i$ is higher in PE compared to Norm-Preg (Haller et al.1997). In contrast, histamine-stimulated Ca^{2+} entry is reduced in fetal umbilical vein ECs from PE compared with Norm-Preg women. Basal and histamine-stimulated cGMP levels were elevated in PE compared with normal cells, implying increased NO production in PE. These data suggest an altered cation membrane permeability and activity of eNOS-sGC pathway in fetal ECs from women with PE (Steinert et al., 2002).

In cultured ECs, exposure to serum from PE women results in increased expression of NOS and NO, release of PDGF, fibronectin which promotes platelet aggregation, increased secretion of ET-1, induction of oxygen radicals, and inhibition of PGI_2 production (Taylor et al.1991a,b, Baker et al.1995, 1996; Davidge et al.1995, Gallery et al.1995). The release of some of these cellular mediators is partly due to increased ECs $[Ca^{2+}]_i$ upon exposure to PE serum. A high $[Ca^{2+}]_i$ in ECs of PE women could be involved in the upregulation of NOS and may produce excess NO which under oxidative stress (also present in PE) produce excess peroxynitrate, a highly reactive intermediate that cause nitration of protein tyrosine residues and cellular oxidative damage. ROS can also activate NF-κB and induce the expression of adhesion molecules in ECs (Saraswathi et al.2004). Some studies suggest that expression of NOS is not different in placentae of normal and PE women (Conrad & Davis, 1995), while other studies suggest that human placental NOS activity is significantly reduced in PE (Brenneckeet al, 1997).

In PE, ECs can activate leukocytes and vice versa (Mantovani & Dejana,1989). Serum from PE patients contain factors that increase EC permeability and ICAM-1 expression on ECs. Thus, increased adhesiveness of leukocytes in PE is likely due to changes on ECs rather than alterations on leukocyte surface. The increased adhesion could contribute to the enhanced coagulation and diminished fibrinolysis seen in PE (Haller et al.1997). In addition to $[Ca^{2+}]_i$, the effect of PE serum on ECs could also involve PKC-α and -ε (Haller et al.1998).

In pregnant rats, both endogenous NO and cGMP production are increased. Also, NO deficiency by administering NOS inhibitors in pregnant rats produces a syndrome of HTN and proteinuria that resemble PE (Yallampalli & Garfield.1993, Khalil et al., 1998; Crews et al. 1999). Supplementation with L-arginine reduces the HTN in the RUPP rat model of HTN-Preg (Alexander et al.2004). Studies have suggested that RUPP and the ensuing placental ischemia/ hypoxia cause an increase in the release of cytokines into the maternal circulation, which in

turn lead to the changes in vascular function and BP [Vince et al., 1995; Conrad et al., 1997; Williams et al., 1998; Khalil & Granger, 2002; Stennett & Khalil, 2006]. In support of the cytokine hypothesis, the plasma levels of TNF-α are elevated in women with PE [Conrad et al., 1997; Williams et al., 1998]. Immune cells may represent another source of the elevated circulating levels of TNF-α in PE [Benyo et al., 2001]. We have shown that infusion of TNF-α or IL-6 in Norm-Preg rats, to elevate its plasma level to levels similar to those in PE, is associated with increased BP and systemic vasoconstriction [Davis et al., 2002; Orshal & Khalil, 2004]. Also, treatment of vascular segments from pregnant rats with TNF-α or IL-6 reduces vascular relaxation and NO production [Giardina et al., 2002; Orshal & Khalil, 2004].

Ca^{2+} in Vascular Smooth Muscle (VSM)

$[Ca^{2+}]_i$ is a major determinant of vascular tone. Ca^{2+} flux to and from the VSM cytosol is regulated by Ca^{2+} release from the intracellular stores, Ca^{2+} entry from the extracellular space, and Ca^{2+} extrusion mechanisms (Fig. 3). Under resting conditions, the opening of K^+ channels permits the exit of K^+ from the cell which hyperpolarizes VSM cell membrane and closes VGCCs. The Na/K-ATPase pump also produces membrane hyperpolarization and further counter-regulates VGCCs. During VSM activation, increased $[Ca^{2+}]_i$ is initiated by IP_3-induced Ca^{2+} release and ryanodine-sensitive Ca^{2+}-induced Ca^{2+} release mechanisms. Vasoconstrictor agonists also stimulate Ca^{2+} entry through ligand-gated and VGCCs. Relaxing factors released from ECs act on VSM and inhibit phospholipase C, open K^+ channels or stimulate $[Ca^{2+}]_i$ extrusion, and thereby decrease $[Ca^{2+}]_i$. EC dysfunction is associated with decreased release of relaxing factors, and decreased VSM Ca^{2+} extrusion. EC dysfunction also causes an increase in VSM contracting factors, which stimulate Ca^{2+} mobilization from the intracellular stores and Ca^{2+} entry from the extracellular space. Contraction of VSM to hypoxia is also mediated by accumulation of $[Ca^{2+}]_i$ (Ramón de Berrazueta et al.1999). Ca^{2+} binds calmodulin to form a complex which activates myosin light chain (MLC) kinase, causes MLC phosphorylation, initiates actin-myosin interaction and produces VSM contraction.

Some studies suggest that vasoconstrictors play a role in PE (Mizutani & Tomoda, 1996). Other studies on women myometrial and subcutaneous resistance vessels have shown that microvessel reactivity to KCl solution, PHE and AngII were not increased in PE compared with Norm-Preg, and suggested that this is an unlikely mechanism of the increased peripheral vascular resistance in PE (Wimalasundera et al, 2005).

Circulating autoantibodies that activate the AngII AT1 receptor are also considered a factor in PE. AT1 autoantibodies (AT-1AA) rise at the time the symptoms of PE develop and subside within 6 weeks after delivery. AT-1AA were purified and characterized as a fraction of IgG antibodies. AT-1AA activity may promote Ca^{2+} signaling and VSM contraction. Also, AT-1AA induced mobilization of intracellular Ca^{2+} initiate a signaling cascade that culminate in activation of NF-κB and activator protein-1 and subsequently tissue factor expression (Dechend et al.2000; Thway et al., 2004). Other studies have postulated the influence of placental proteinases on angiotensin activity and VSM contraction. It has been suggested that the decreased pressor responsiveness to AngII in Norm-Preg is caused by increased inactivation of AngII by angiotensinase in serum and placenta. Angiotensinase activity increases with advancing gestation in Norm-Preg sera, but the enzyme activity is lower in severe PE sera leading to decreased degradation and increased sensitivity to AngII (Mizutani & Tomoda, 1996). Enhanced VSM responses in PE could also be related to increased ROS production. ROS cause increases in $[Ca^{2+}]_i$ by activating VGCCs. ROS can also increase $[Ca^{2+}]_i$ by promoting IP_3-induced Ca^{2+} release from the intracellular stores and by inhibiting plasma membrane Ca^{2+}-ATPase activity (Jin et al., 2004).

Although studies have suggested that the increased vascular reactivity associated with HTN-Preg may be partly due to enhanced activity of VGCCs in VSM (Ebeigbe et al, 1987), the results have not been consistent. Studies compared the effects of sera from Norm-Preg, PE, pregnant women with chronic HTN, non-pregnant women with HTN, and age-matched non-pregnant normotensive women on $[Ca^{2+}]_i$ in cultured rat aortic VSM cells. After 4 hr incubation period with serum, basal $[Ca^{2+}]_i$ was not significantly altered. However, compared with Norm-Preg sera, PE sera markedly reduced hormonally induced Ca^{2+} transients (Green et al.2000).

Our studies in arterial VSM cells of rats have shown that basal and agonist-stimulated $[Ca^{2+}]_i$ are reduced in Norm-Preg compared with virgin rats, but significantly elevated in pregnant rats treated with L-NAME (Murphy et al., 2001) (Table 3). In VSM cells incubated in a Ca^{2+}-containing solution, PHE causes an initial peak followed by maintained increase in $[Ca^{2+}]_i$. The PHE- and caffeine-induced cell contraction and transient increase in $[Ca^{2+}]_i$ in Ca^{2+}-free solution are not significantly different between Norm-Preg and virgin rats nontreated or treated with L-NAME, suggesting that the pregnancy-associated changes in VSM contraction are not due to changes in Ca^{2+} uptake to or Ca^{2+} release from the intracellular Ca^{2+} stores. In contrast, PHE-induced maintained $[Ca^{2+}]_i$ in Ca^{2+}-containing medium, a measure of Ca^{2+} entry from the extracellular space, is reduced in Norm-Preg rats, but enhanced in L-NAME-treated pregnant rats (Murphy et al.2001). Also, KCl-induced VSM cell contraction and $[Ca^{2+}]_i$ are reduced in Norm-Preg compared with virgin rats but enhanced in L-NAME-treated pregnant rats, providing evidence that Ca^{2+} entry through VGCCs is reduced during Norm-Preg but enhanced in L-NAME-treated pregnant rats. The cause of the reduced Ca^{2+} entry into VSM in Norm-Preg rats and its enhancement in L-NAME-treated pregnant rats could be related to possible changes in permeability or number of Ca^{2+} channels. $[Ca^{2+}]_i$ may also play a role in the changes in VSM contraction in RUPP rats. In renal arterial VSM, enhanced mechanisms of Ca^{2+} entry, rather than release from the intracellular Ca^{2+} stores, may be a contributing factor to the increased cell contraction and $[Ca^{2+}]_i$ in response to AngII and KCl in RUPP rats as compared to Norm-Preg rats (Murphy et al., 2003).

VSM Ca^{2+}-Sensitization pathways

In addition to Ca^{2+}/calmodulin-dependent MLC kinase, protein kinase C (PKC), Rho-kinase, and mitogen-activated protein kinase (MAPK) contribute to VSM contraction. Activation of PKC by phorbol esters causes sustained contraction of VSM with no detectable change in $[Ca^{2+}]_i$, suggesting an increase in Ca^{2+} sensitivity of the contractile proteins. Increased VSM PKC expression/activity has been identified in HTN (Horowitz et al.1996; Salamanca & Khalil, 2005). VSM PKC activity may play a role in the vascular changes observed in Norm-Preg and PE. The Ca^{2+} sensitivity of VSM contractile elements is increased in women with PE (Vanwijk et al.2002). PE patients develop IgG autoantibody to VSM AT_1R, and PKC may play a role in the changes in AT_1R-mediated signaling associated with PE. In cultured neonatal rat cardiomyocytes immunoglobulin from PE women enhances AT_1R-mediated chronotropic response, whereas IgG from control subjects has no effect. Treatment of cardiomyocytes with the PKC inhibitor calphostin C prevented the stimulatory effect of IgG from PE women on AT_1R-mediated chronotropic response. Confocal microscopy showed colocalization of IgG from PE women and AT_1R antibody in VSM cells. These studies suggest that PE patients develop stimulatory autoantibodies against AT_1R, a process that appears to be mediated via PKC [Wallukat et al., 1999].

Studies on uterine artery from pregnant sheep and the aorta of late pregnant rats have shown that the decreased vascular contraction during Norm-Preg is associated with decreased PKC activity [Magness et al., 1991; Kanashiro et al., 2000]. Also, the expression and subcellular redistribution of Ca^{2+}-dependent α-PKC and Ca^{2+}-independent δ- and ζ-PKC are reduced in aortic VSM from pregnant compared to nonpregnant rats [Kanashiro et al., 1999; 2000]. BP

is greater in late pregnant rats treated with the NOS inhibitor L-NAME compared with Norm-Preg or virgin rats. Also, PHE-induced contraction is greater in aortas from L-NAME treated pregnant rats compared to Norm-Preg or virgin rats [Khalil et al., 1998; Crews et al., 1999]. Additionally, vascular PKC activity and the expression and subcellular redistribution of α- and δ-PKC were enhanced in L-NAME treated pregnant rats compared with Norm-Preg rats. Thus an increase in the expression/activity of α- and δ-PKC isoforms may play a role in the increased vasoconstriction and vascular resistance associated with HTN-Preg [Kanashiro et al., 1999; 2000]. RUPP and the ensuing placental ischemia/hypoxia in late pregnancy may induce the release of cytokines into the maternal circulation, which in turn lead to the generalized changes in vascular function associated with HTN-Preg [Stennett & Khalil, 2006; Lamarca et al., 2007]. Cytokines likely increase the expression/activity of VSM PKC, leading to increased myofilament force sensitivity to $[Ca^{2+}]_i$ and enhanced VSM contraction.

Rho protein is a family of small GTP binding proteins that are involved in many cellular functions including cell proliferation, migration, cytoskeletal reorganization and contraction. Rho-kinase is activated by GTP binding and is inactivated by hydrolyzing GTP to GDP, and this process is influenced by various stimuli including growth factors and vasoactive substances. Two Rho-kinase isoforms encoded by two different genes have been identified, ROCK1 and ROCK2. Targeting Rho protein or its downstream effector protein Rho-kinase may have therapeutic potential in HTN (Hu et al. 2006). Agonists such as noradrenaline, ET-1 and TXA_2 bind to G-protein-coupled receptors and produce contraction by increasing both $[Ca^{2+}]_i$ and the Ca^{2+} sensitivity of the contractile apparatus. The increased Ca^{2+} sensitivity of VSM results from inhibition of MLC phosphatase activity leading to increased MLC phosphorylation and tension at a constant $[Ca^{2+}]_i$. A major component of the Ca^{2+}-sensitizing effect of vasoconstrictors is ascribed to RhoA-mediated ROCK activation (Loirand et al. 2006; Somlyo et al., 2003). ROCK inhibitors (Y-27632, fasudil) normalize arterial BP in animal models of HTN indicating the importance of ROCK signaling in the vascular hyperreactivity associated with HTN (Uehata et al.1997). The importance of Rho in PE is supported by a study comparing subcutaneous resistance arteries from PE, Norm-Preg and nonpregnant women, and showing that Ca^{2+} sensitivity of the contractile elements is increased in women with PE (VanWijk et al., 2002). Other studies have shown that mRNA expression of Rho-kinase is downregulated in umbilical arteries of PE women (Friel, 2006). Stimulation of AT_1R may induce upregulation of RhoA/ROCK activity in hypertensive rats (Kataoka et al., 2002). An increase in AngII activity during PE may activate ROCK and promote vasoconstriction.

MAPKs are ubiquitously expressed serine/threonine protein kinases that play an important role in cell differentiation, growth, apoptosis, and the regulation of transcription factors and gene expression. MAPKs include extracellular signal-regulated kinase 1/2 (ERK1/2), big MAPK1, c-Jun N-terminal kinase and p38. The ERK family mediates growth factor-stimulated cell differentiation and growth, while JNK and p38 mediate inflammatory cytokine- and stress-induced apoptosis and stress-responsive gene expression. ROS and MAPK promote vascular remodeling in many pathological conditions and may be involved in PE. ROS are increased in PE, and MAPKs are sensitive to oxidative stress (Kyaw et al., 2004). Tyrosine kinase and MAPK activities have been identified in differentiated VSM, suggesting a role in VSM contraction [Khalil et al., 1995]. In differentiated VSM cells, agonist-induced activation and generation of diacyglycerol at the surface membrane promotes the translocation of ε-PKC from the cytosol to surface membrane. The activated ε-PKC promotes the translocation of both MAPK kinase (MEK) and MAPK to the plasmalemma, where they form a complex. PKC induces phosphorylation and activation of MEK, which in turn phosphorylates MAPK at both Thr and Tyr residues (Adam et al., 1992; Khalil et al., 1995). Tyrosine phosphorylation of MAPK targets it to the contractile myofilaments, where it phosphorylates the actin-binding protein caldesmon (D'Angelo et al., 1999; Hedges et al., 2000). The phosphorylation of

caldesmon reverses its inhibition of actin-mediated MgATPase activity, increases the actin-myosin crossbridge cycling and enhances VSM contraction [Khalil et al., 1995]. A decrease in VSM PKC expression during Norm-Preg and potential increase in HTN-Preg likely change the MAPK-caldesmon phosphorylation pathway and thereby VSM contraction.

Ca^{2+} and Extracellular matrix (ECM)

Norm-Preg is associated with significant remodeling of the cytoskeleton and ECM in blood vessels of the uterine and systemic circulation. Matrix metalloproteinases (MMPs) are a family of structurally related, zinc-containing enzymes that promote proteolysis, degrade ECM proteins and play a role in vascular remodeling and cell migration. An essential step in MMP-induced proteolysis is conversion of the zymogen into an active proteinase by various mechanisms including proteolysis, allosteric interactions, oxidative modification and Ca^{2+}. Increased extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) may promote degradation of ECM components by increasing MMP activity. In mouse fibroblasts, increasing $[Ca^{2+}]_e$ induces release and activation of MMP-2 and -9. The MMPs release could be dependent on store-operated Ca^{2+} channels and the NO/cGMP/PKG pathway negatively regulates this Ca^{2+} entry pathway (Huang et al., 2006). Also, PAF induces MMP-9 expression in human vascular ECs through Ca^{2+}- or PI_3K-dependent signaling pathway (Ko et al., 2005).

Studies suggest that plasma levels of MMP-2, MMP-9, TIMP-1, and TIMP-2 are higher in HTN-Preg than Norm-Preg women (Merchant et al.2004; Tayebjee et al.2004). Other studies have shown that the levels of MMP-2 and -9 in decidua cells are not elevated in PE (Huisman et al., 2004). Also, while the levels of MMP-9 may be higher in decidual cells and adjacent interstitial trophoblasts in placenta of PE versus Norm-Preg women, the levels of MMP-2, TIMP-1 and TIMP-2 were similar (Lockwood et al.2008). MMP-9 mRNA expression and protein levels in trophoblasts may be even reduced in PE (Qiao et al.2005). Also, treatment of trophoblast-like cell culture with PE serum causes reduction of MMP-2 (Mahameed et al. 2005). A decreased MMP-2 content/activity during PE would reduce collagen breakdown in the umbilical cord artery. The accumulation of collagen with simultaneous reduction in elastin content in the umbilical cord artery may reduce elasticity of arterial wall and decrease blood flow to the fetus in women with PE (Galewska et al.2003). Also, a low decidual MMP-1 expression in PE may inhibit endovascular invasion by cytotrophoblasts, and partly explain the relative failure of trophoblasts to invade maternal decidual blood vessels (Gallery et al. 1999).

MMPs may also affect vascular reactivity. MMP-2 cleaves big ET-1 to ET-1, which could activate ET_A receptors and produce vasoconstriction, and therefore MMP-2 inhibitors may evoke vasorelaxation (Fernandez-Patron et al.2000). However, MMP-2 can process big ET-1 at the gly-leu bond to $ET_{1–32}$ which activates endothelin B (ET_B) receptor-NO signaling pathway and promote vasodilation (Davison et al.2004). Relaxin upregulates vascular gelatinase activity during pregnancy, and thereby contributes to renal vasodilation, hyperfiltration, and reduced myogenic reactivity of renal arteries through activation of the EC ET_B receptor-NO pathway (Jeyabalan et al.2003). The source of the increased plasma MMPs is unclear, but the placenta is considered a potential source. MMP-2 and -9 are increased in the placenta of diabetic rats at mid-gestation (Pustovrh et al.2007). Also, VEGF has been shown to induce the expression and promote the secretion of MMPs from ECs (Narumiya et al. 2001).

We have shown that MMP-2 and -9 cause relaxation of rat aorta and inferior vena cava (Chew et al., 2004; Raffetto et al.2007). The reversibility of the effects of MMPs on PHE-induced contraction suggests that the actions are not solely due to irreversible degradation of ECM. MMP-2 induced relaxation may not involve increased NO or PGI_2 production, as it is not blocked by the NOS inhibitor L-NAME or the COX inhibitor indomethacin. Other studies on

rats have shown that NO donors enhanced MMP-2 and -9 activities and NOS inhibitor reduced their activities in the maternal side of the placenta, demonstrating that NO may modulate the activation of MMPs (Pustovrh et al., 2007). MMP-2 induced venous relaxation is abolished by blockers of large conductance Ca^{2+}-activated K^+ channels such as iberiotoxin, suggesting involvement of VSM hyperpolarization pathway (Raffetto et al.2007).

MMPs do not inhibit PHE-induced VSM contraction in Ca^{2+}-free solution implying that they do not inhibit the Ca^{2+} release mechanism. In contrast, MMPs inhibit PHE-induced Ca^{2+} influx (Chew et al., 2004). MMP-induced degradation of collagen produces Arg-Gly-Asp (RGD)-containing peptides, which bind to αvβ3 integrin receptors and inhibit Ca^{2+} entry into VSM (Waitkus-Edwards et al., 2002). Also, MMPs stimulate protease-activated receptors (PARs) and activate signaling pathways that could block VSM Ca^{2+} channels (Macfarlane et al. 2001). This is supported by reports that proteases such as thrombin activate PARs and promote endothelium-dependent VSM relaxation by inhibiting Ca^{2+} influx (Hamilton et al.1998).

Clinical Applications and Perspectives

Ca^{2+} plays a major role in the regulation of vascular function particularly during pregnancy. Dietary, plasma and vascular cell Ca^{2+} are influenced by age, ethnic background and geographical distribution. An imbalance in the Ca^{2+} control mechanisms could significantly affect the outcome of pregnancy.

It appears that changes in $[Ca^{2+}]_i$ in erythrocytes, platelets and immune cells as well as ECs and VSM cells play a role in PE. The differences in the results among various studies could be related to differences in the size of the study sub-groups. Also, whether the changes in vascular cell $[Ca^{2+}]_i$ are a cause or consequence of PE remain to be clarified.

Although the results of different studies are not consistent, there is tangible evidence for beneficial effect of Ca^{2+}-Suppl during pregnancy. Ca^{2+} may have the strongest beneficial effects in lowering the incidence of PE in women with inadequate Ca^{2+} intake. If Ca^{2+} intake is adequate, Ca^{2+}-Suppl may not be needed.

Careful monitoring of vascular and cellular Ca^{2+} may also be important in the management of clinical cases of PE. Although the exact cause of the convulsion and excitation during eclampsia is not known, a role of Ca^{2+} is suspected. Magnesium sulfate is considered the first-line therapy to protect against seizures associated with PE-eclampsia, mainly because it prevents vascular spasm in the brain due to its Ca^{2+} antagonist properties. Other medication that could be considered in PE is Ca^{2+} antagonists, which could affect vasoconstriction, cytokine release, and MMPs. Ca^{2+} channel blockers have been tested in PE with positive effect (Walters & Redman, 1984, Papatsonis et al. 2001; Brown et al.2002, Elatrous et al. 2002, Fletcher et al., 1999, Hanff et al.2005). Intravenous diazoxide is one of the earliest drugs to be used in PE, but may cause sudden maternal hypotension, hyperglycemia and uterine atony as well as fetal distress and hypoglycaemia. The most common HTN therapy is nifedipine (given orally or sublingually) and hydralazine. Intravenous labtelalol and glyceryl trinitrate have also been considered. ACE inhibitors are contraindicated in pregnancy because of their harmful effects on the fetus. Further investigation of the regulation of vascular and cellular Ca^{2+} during pregnancy should help further delineate the causes of dysregulation of Ca^{2+} handling mechanisms during PE, and thereby identify cellular and molecular target for prevention and treatment of PE.

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List of abbreviations

$1,25(OH)_2D_3$	1,25 dihydroxycholecalciferol, AngII, angiotensin II
Ca^{2+}	calcium
$[Ca^{2+}]_i$	intracellular free Ca^{2+} concentration
Ca^{2+}-Suppl	Ca^{2+} supplementation
EC	endothelial cell
ECM	extracellular matrix
EGF	epidermal growth factor
ET-1	endothelin-1
HTN	hypertension
HTN-Preg	HTN in pregnancy
MMP	matrix metalloproteinase
MAPK	mitogen-activated protein kinase
MLC	myosin light chain
NO	nitric oxide
Norm-Preg	normal pregnant
O_2^{-}•	superoxide
PE	preeclampsia
PGI_2	prostacyclin
PHE	phenylephrine
PDGF	platelet-derived growth factor
PAF	platelet-activating factor
PGF	placenta growth factor
PKC	protein kinase C
PMN	polymorphonuclear neutrophils
RCCT	randomized controlled clinical trial
ROS	reactive oxygen species
STBM	syncytiotrophoblast microvillous membranes
TXA_2	thromboxane A_2
VEGF	vascular endothelial growth factor
VGCC	voltage-gated Ca^{2+} channel
VSM	vascular smooth muscle

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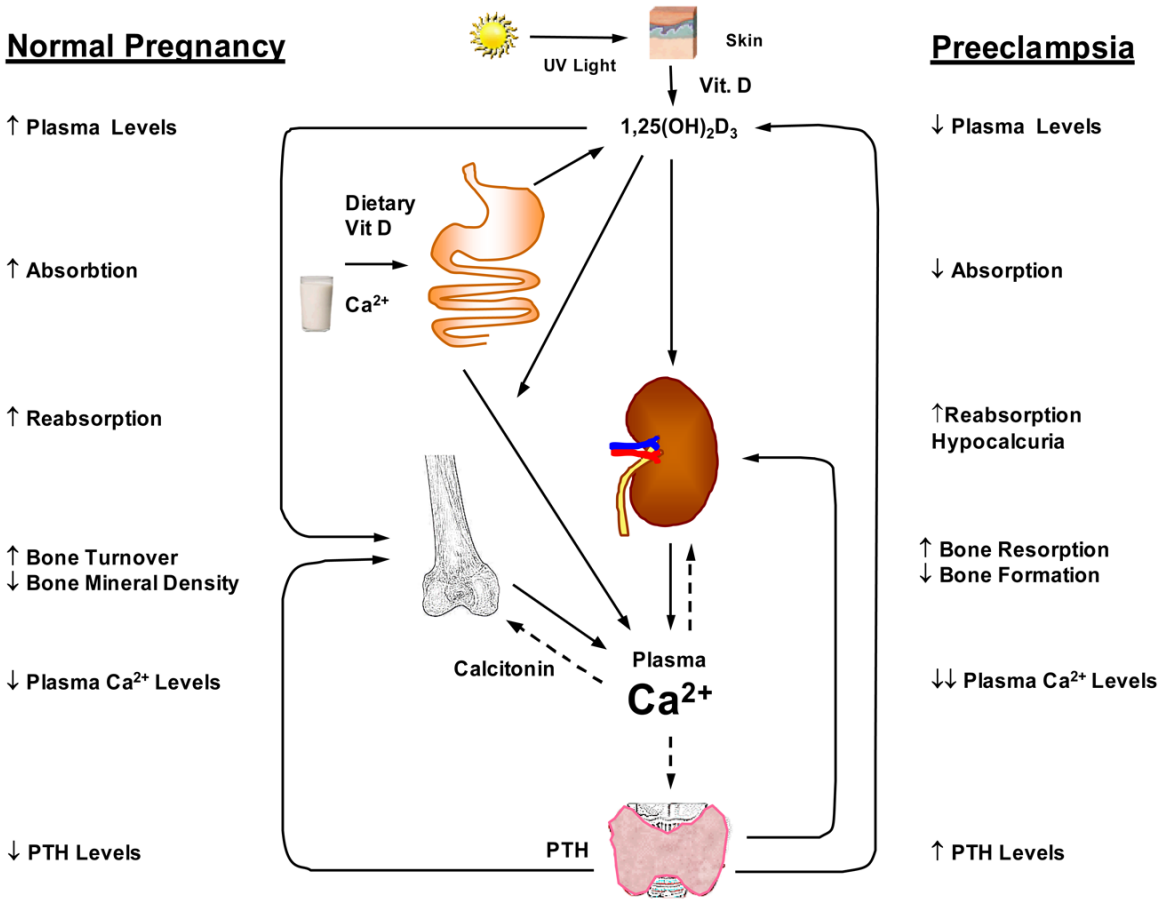

Fig. 1.

Regulation of Plasma Ca^{2+}. Vitamin D cholecalciferol is formed in the skin as a result of UV irradiation or absorbed from the gut, then hydroxylated to 25(OH)D in liver. Under the influence of parathyroid hormone (PTH), 25(OH)D is further hydroxylated in kidney to 1,25 $(OH)_2D_3$, the most active form of vitamin D. PTH stimulates 1,25$(OH)_2D_3$ formation. 1,25 $(OH)_2D_3$ stimulates Ca^{2+} transport from intestine, reabsorption in renal tubules and release from bone. Calcitonin inhibits Ca^{2+} mobilization from bone.

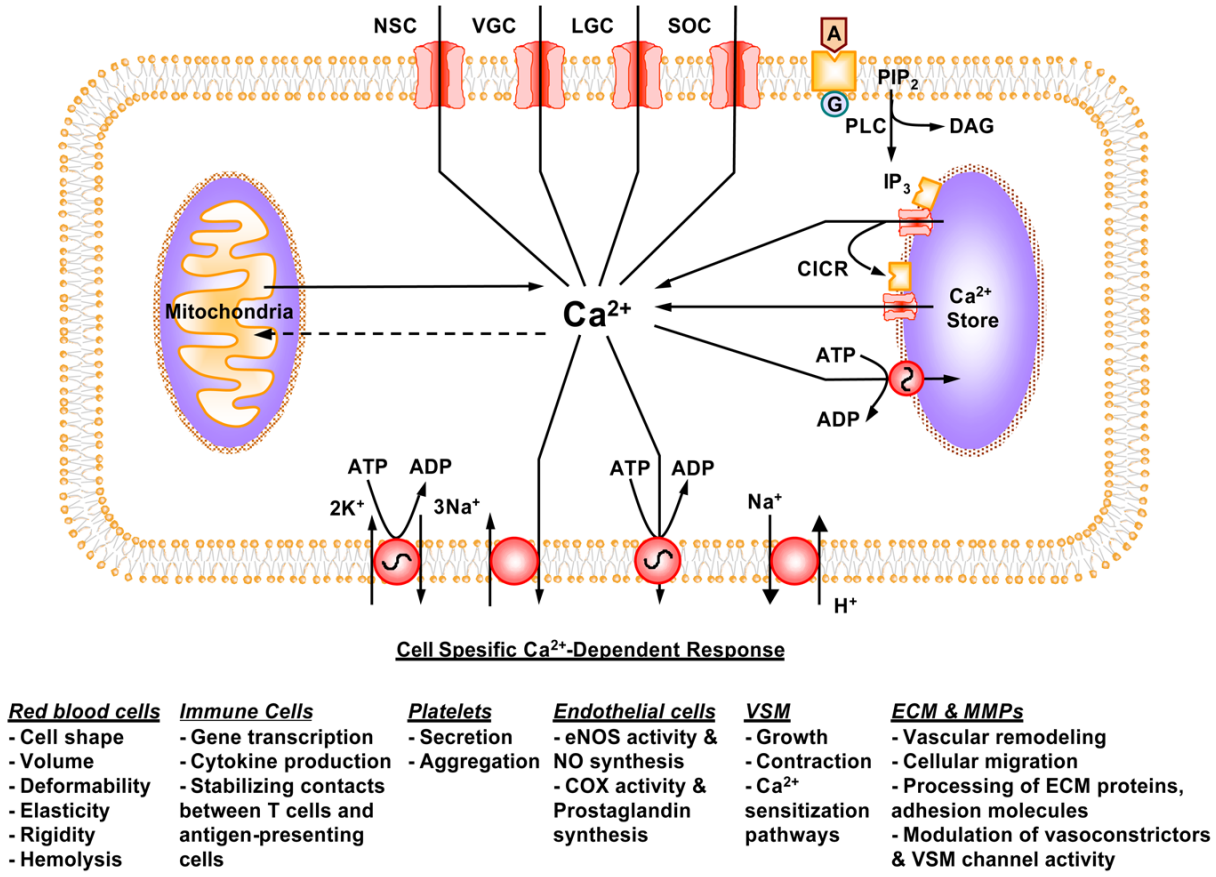

Fig. 2.

Regulation of $[Ca^{2+}]_i$ and Ca^{2+}-dependent cellular response. During cell activation, Ca^{2+} is released from the intracellular stores through IP_3-sensitive Ca^{2+} channels and the ryanodine-sensitive Ca^{2+}-induced Ca^{2+} release mechanism. Extracellular Ca^{2+} enters the cell through voltage-gated, ligand-gated, store-operated, and nonspecific channels. The increased $[Ca^{2+}]_i$ triggers specific responses in red blood cells, platelets, immune cells, endothelial cells, and VSM cells. When the cell stimulus is removed, $[Ca^{2+}]_i$ returns back to normal level via the Ca^{2+}-ATPase pump and Na/Ca exchanger. Also, the Na/K-pump and Na/H exchanger could affect the membrane potential and intracellular pH and further modify the Ca^{2+} response. Extremely high levels of $[Ca^{2+}]_i$ are taken up by the mitochondria.

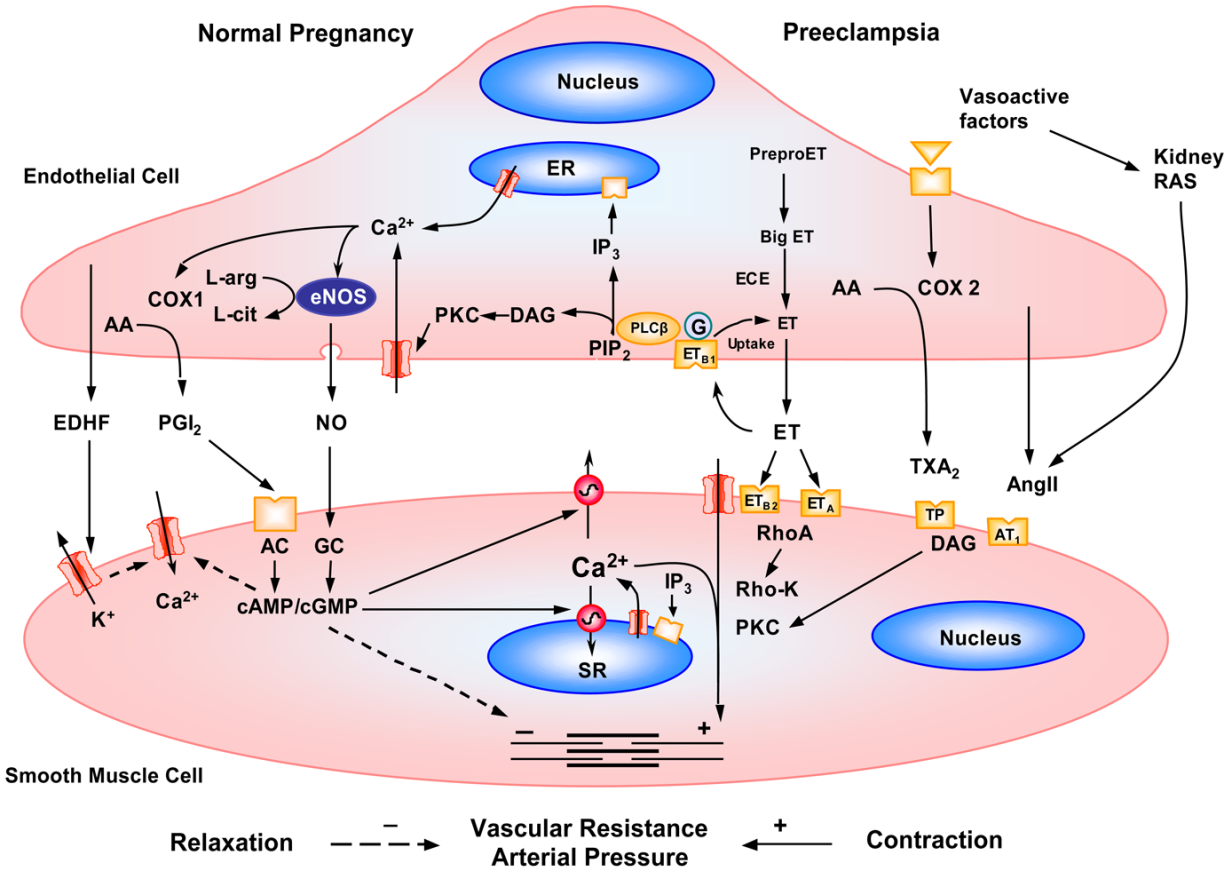

Fig. 3.

Regulation of endothelial and VSM $[Ca^{2+}]_i$ during Norm-Preg and preeclampsia. Norm-Preg is associated with increased expression and Ca^{2+}-dependent activation of endothelial eNOS and COX, leading to increased NO and PGI_2 production. NO and PGI_2 act on VSM, causing an increase in cGMP and cAMP, which activate Ca^{2+} extrusion and VSM relaxation mechanisms. Increased EDHF during pregnancy causes VSM hyperpolarization, and inhibition of Ca^{2+} entry through voltage-gated channels. In preeclampsia, a decreased NO bioavailability mainly due to increased ROS would cause reduction of VSM relaxation. Also, the release of bioactive factors such as cytokines from the placenta and other sources causes an increase in the release of endothelium-derived contracting factors such as ET-1 and TXA_2 and AngII which in turn cause an increase in VSM $[Ca^{2+}]_i$ and the Ca^{2+}-sensitization pathways such as PKC and Rho-kinase, leading to enhanced vasoconstriction, increased vascular resistance and BP.

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Table 1

Effects of dietary Ca^{2+}-Suppl during pregnancy.

Study	Study Design	Number of Women	Ca^{2+}-Suppl (mg/day)	BP	Risk of Preeclampsia
Bucher, 1996	meta-analysis of data from women at risk of PE in 14 RCCTs published in 1966-1994	2549	375-2000	↓ systolic and Diastolic BP	↓ Compared with placebo, Ca^{2+}-suppl reduced the odds for gestational HTN and PE
Knight, 1992	RCCT in normotensive and HTN-Preg women	50	1000	↓ Diastolic BP only in HTN-Preg women	↓ BP-lowering effect of Ca^{2+}-suppl is greater in women with PE
López-Jaramillo, 1997	RCCT in Ecuador of 260 teens <17.5 yr; dietary Ca^{2+} ~600 mg/day	260	2000	↓ systolic BP by 9.1 mmHg and diastolic BP by 6 mmHg	↓ Decreased risk of PE. 3.2% developing PE in the treatment group and 15.5% in placebo group
Crowther, 1999	Multi-center RCCT in Australia to assess the effect of Ca^{2+}-Suppl daily from <24 wk to delivery	456 nulliparous women with a singleton pregnancy	1800	not reported	↓ Ca^{2+}-suppl during pregnancy reduced the risk of PE and preterm birth in this nulliparous population
CPEP	Multi center RCCT in the United States	4589 women of various ethnic and socioeconomic backgrounds with average Ca^{2+} intake 1100 mg/day	2000	_	Ca^{2+}-suppl did not reduce incidence of PE, or maternal and perinatal complications in nulliparous women, or prevent adverse pregnancy outcomes in adolescents or women with low base-line dietary Ca^{2+} intake or urinary Ca^{2+} excretion.
Oken, 2007		1718	Evaluated food intake	_	_
WHO	Randomized trial of Ca^{2+}-suppl among low Ca^{2+} intake pregnant women	8325	1500	_	Ca^{2+}-suppl did not prevent PE but did reduce its severity, maternal morbidity, and neonatal mortality
Hofmeyr, 2007	Meta-analysis of 12 placebo-controlled RCCT	15528	1000	↓ high BP	↓ Decreased risk of PE

↓ reduced risk

_ No change in risk

Table 2

Plasma and vascular cell Ca^{2+} levels in healthy non-pregnant, normal pregnant, and preeclamptic women.

Parameter	Non-pregnant	Normal pregnant	Preeclampsia	References
Plasma				Silver et al., 1998
Volume (ml/m^2)	~2000	2279 +/− 325	1790 +/−332	Seely et al.,1992
Serum [Ca^{2+}] (mmol/L) (mg/dL)	2.2-2.6	1.26+/−0.01	1.20+/−0.01	Kisters et al., 2000
$1,25(OH)_2D_3$ (pg/ml)	2.22-2.54	2.2 +/− 0.1	1.96 +/− 0.5	Sukonpan et al., 2005
PTH (ng/L)	8.9-10.2	9.7 +/− 0.7	9.0 +/− 0.4	Halhali et al.,2007
	50.4 +/− 17.3	50+/−9	43+/−9	Davis et al., 1988
	24.8+/−9.0	15.4+/−1.3	29.9+/−4.3	Seely et al.,1992
24 h Urinary Ca^{2+} Excretion (mg/day)	50-250	189.24±57.06	71.20±22.95	Ray et al.,1999
Red blood cells				
Count (10^{12}/L)	4.7-6.1	4.23±0.40	4.14±0.41	Ceyhan et al.,2006
Cytosolic [Ca^{2+}] (mEq/L)	0.0149±0.0023	0.015±0.001	0.033±0.010	Sowers et al., 1989
Membrane Ca^{2+} (μmol/g)	0.83±0.16	0.77±0.13	1.23±0.36	Kosch et al., 2000
White blood cells				
Count (10^9/L)	4.5-10	10.864±3.350	11.261 ±0.528	Ceyhan et al.,2006
Absolute neutrophil count	2500-7000	7,498.6±2,354.0	9,410±3,066.9	Lurie et al., 1998
Cytosolic [Ca^{2+}] (nmol/L)	47±9	94±3	121±7	Hojo, 1999
Platelets				
Count (10^9/L)	150-450	220±79	227±71	Ceyhan et al., 2006
Main platelet volume (fL)	6.3-9.5	9.45±1.11	9.18±1.52	Ceyhan et al., 2006
Cytosolic [Ca^{2+}] (nmol/L)	112.3±2.9	120.1±5.7	163.6±8.8	Kilby et al., 1990
Endothelial Cells				
Cytosolic [Ca^{2+}]				
sVCAM-1 (marker of activation) (ng/ml)	+	++	+++	Haller et al.,1997
vWf (marker of damage) (IU/dL)	558±20	1,159.8±340	2,269±426	Bouchlariotou, 2008
	90±47	123±24	206.9±40.6	Bouchlariotou, 2008
Cultured rat VSM treated with human sera [$Ca^{2+}]_i$				
Basal (nmol/L)				
AngII (10^{-8}M)	158±14	135±15	160±14	Green et al., 2000
% Increase over baseline	236±16%	370±14%	65±12%	Green et al., 2000

Table 3

Plasma and vascular cell Ca^{2+} levels in non-pregnant, normal pregnant and hypertensive pregnant rat

	Non pregnant	Norm-Preg	HTN-Preg	Reference
Serum Ca^{2+} (mg/dL)	10.14±0.09	10.67±−0.18	10.29±0.08	Ebose et al., 2007
Endothelial cell $[Ca^{2+}]_i$ (nM)	84 ±4	138 ± 5	TBD	Gokina et al, 2006
VSM $[Ca^{2+}]_i$ (nM) - Basal - PHE (10^{-5}M) $[Ca^{2+}]_i$ (nM) Initial Maintained	 86 ±6 417 ± 11 183 ± 8	 63 ± 5 414±13 149 ±8	 109 ± 8 429±19 234 ± 11	 Murphy et al., 2001 Murphy et al., 2001