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REVIEW ARTICLE

The role of arginine, homoarginine and nitric oxide in pregnancy

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Abstract Normal pregnancy leads to profound maternal hemodynamic changes, including increased blood volume and vasodilatation. Several vasodilator mediators are implicated, including prostaglandins, carbon monoxide and nitric oxide (NO). Pre-eclampsia (PE) affects 3–10 % of pregnancies and is associated with increased maternal and perinatal morbidity and mortality. Around 8 % of pregnancies are complicated by intra-uterine growth restriction (IUGR), also associated with increased perinatal mortality and morbidity. PE and IUGR often co-exist. NO is essential for the formation of healthy endothelium, and in pregnancy promotes endovascular invasion by the cytotrophoblast. As interstitial trophoblasts invade the maternal spiral arteries in the uterine wall, they produce NO which acts on artery walls to create a low-resistance, high-caliber uteroplacental unit. If this process fails, the result is a high-resistance uteroplacental circulation. The hypoperfused and ischemic placenta releases antiangiogenic factors which mediate generalized endothelial dysfunction, oxidative stress and inflammatory mediators. It is these mediators that are implicated in both the fetal and maternal syndromes of PE and IUGR. Studies of NO and its modulator amino acids, including the precursors arginine and homoarginine and the NO synthesis inhibitor asymmetric dimethylarginine (ADMA), have investigated their role in both normal and pathological pregnancies. Many studies of PE (and, to a lesser extent, IUGR) have investigated maternal circulating ADMA, arginine and homoarginine levels. This article reviews and discusses the role of these amino acids in pregnancy. The results have shed some light on their role in these pathologies, but some of the findings have been conflicting and more research is needed. Nevertheless, therapeutic interventions that manipulate these guanidine–amino acids and their interactions hold real promise for the management of pregnancies complicated by PE and/or IUGR, and the results of ongoing studies are eagerly awaited.

Keywords ADMA · Arginine · Homoarginine · Nitric oxide · Pregnancy · Pre-eclampsia

Abbreviations

ADMA	Asymmetric dimethylarginine
cGMP	Cyclic guanosine monophosphate
DDAH	Dimethylarginine dimethylaminohydrolase
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
FMD	Flow-mediated dilatation
GC–MS	Gas chromatography–mass spectrometry
GC–MS/MS	Gas chromatography–tandem mass spectrometry
GTN	Glyceryl trinitrate
HPLC	High-performance liquid chromatography
ISDN	Isosorbide dinitrate
IUGR	Intra-uterine growth restriction
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
NO	Nitric oxide
NOS	Nitric oxide synthase
PE	Pre-eclampsia
PlGF	Placental growth factor
ROS	Reactive oxygen species

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sEng	Soluble endoglin
sFlt-1	Soluble Fms-like tyrosine kinase-1
SGA	Small for gestational age
sGC	Soluble guanylyl cyclase
TRPC	Transient receptor potential cation (channels)
VEGF	Vascular endothelial growth factor

Introduction

Normal pregnancy leads to profound maternal hemodynamic changes, including an increase in blood volume and vasodilatation. Several vasodilator mediators are implicated, including prostaglandins, carbon monoxide and nitric oxide (NO) (Noris et al. 2005). Recent literature has focused on the role of NO and its regulatory compounds, in particular the clinical relevance of aberrant NO pathways which are implicated in pre-eclampsia (PE). The association of NO with vasodilation was first appreciated in 1980 and it was first linked with the pathogenesis of PE in 1990 (Sarrel et al. 1990). Since then, research into the relationship between the substrates of nitric oxide synthase (NOS), i.e., arginine and homoarginine, and the endogenous inhibitors of NOS, notably asymmetric dimethylarginine (ADMA), continue to provide insights into the pathophysiology of the impaired endothelial function underpinning PE. PE contributes to up to 16 % of direct maternal deaths in developed countries (Khan et al. 2006). Moreover, it is associated with serious medical complications, such as eclampsia, stroke, renal failure and pulmonary edema. As PE affects 3–10 % of pregnancies and accounts for 40 % of non-spontaneous (iatrogenic) premature births in the UK (Meher and Duley 2007; Savvidou et al. 2003), the clinical significance of even a modest improvement in the outcome of individual pregnancies would be profound.

This article provides an overview of the literature on the arginine/NO pathway in pregnancy. Evidence supporting a role for these amino acids in the physiology of normal pregnancy and in the pathophysiology of PE and intrauterine growth restriction (IUGR) will be discussed. The potential for arginine regulators to be used as biomarkers for PE and for identifying pregnancies at increased risk will be described and their therapeutic potential as NO donors will be explored.

Physiology of arginine, homoarginine and nitric oxide in pregnancy

NO was first identified as the endothelium-derived relaxing factor (EDRF) and shown to be associated with vasodilation by one of the Nobel prize winners Furchgott and colleagues in 1980. It is an autocrine and paracrine signaling molecule with an impressive range of regulatory functions. It has been shown to play a specific role in the physiological vascular adaptation of normal pregnancy (Sladek et al. 1997). NO is essential for the formation of healthy endothelium, and in pregnancy promotes endovascular invasion by the cytotrophoblast (Zhou et al. 1997). As interstitial trophoblasts invade the maternal spiral arteries in the wall of the uterus, they produce NO which acts on artery walls to create a low-resistance, high-caliber uteroplacental unit (Noris et al. 2005). It has been shown in vitro that NO mediates angiopoietin-induced growth and migration of extravillous trophoblast cell lines (Dunk et al. 2000).

L-arginine is the substrate for NOS and ADMA is its endogenous inhibitor (Bo¨ger 2006; Kielstein and Zoccali 2005). Therefore, infusion of ADMA is associated with endothelial dysfunction (Calver et al. 1993). L-Arginine can be replaced by L-homoarginine as a substrate for the biosynthesis of NO (Lyengar et al. 1987; Hecker et al. 1991). The ratio of L-arginine to ADMA could be used as a marker of altered NOS activity (Tsikas et al. 2000a, b) and hence can be used in evaluating the effects of ADMA (Kielstein and Zoccali 2005; Bode-Boger et al. 1996).

Conversely, when normal trophoblastic invasion fails, a high-resistance uteroplacental unit is formed, as endovascular invasion does not extend beyond the superficial part of the spiral arteries, which means that the arteries still have the capacity to constrict flow. Studies looking at the placentas of pre-eclamptic women show that both the numerical density and the depth of interstitial trophoblast invasion of uterine tissues are significantly reduced (Kadyrov et al. 2003). This can be assessed by Doppler ultrasonography of the uterine arteries at 23–25 weeks of gestation (Savvidou et al. 2003). The hypoperfused and ischemic placenta releases antiangiogenic factors, which mediate generalized endothelial dysfunction, oxidative stress and inflammatory mediators, e.g., cytokines (Böger et al. 2010; Savvidou et al. 2003). It is these mediators that are implicated in both the fetal and maternal syndromes of PE and may account for the wide spectrum of its clinical complications (Steegers et al. 2010), although direct evidence for this in humans is limited (Johal et al. 2014). Studies of the role of NO and its arginine modulator amino acids in pregnancy have investigated their role in both normal pregnancy and in pathological states such as PE and IUGR.

NO acts on a multitude of pathways. It diffuses into vascular smooth muscle and increases cyclic guanosine monophosphate (cGMP) resulting in vasodilation, while simultaneously reducing the effect of vasoconstrictors (Myatt et al. 1992). It also inhibits platelet aggregation and adherence to endothelial surfaces. Furthermore, it modifies the expression of inflammatory cytokines and inhibits interactions between immune and endothelial cells. Finally, NO

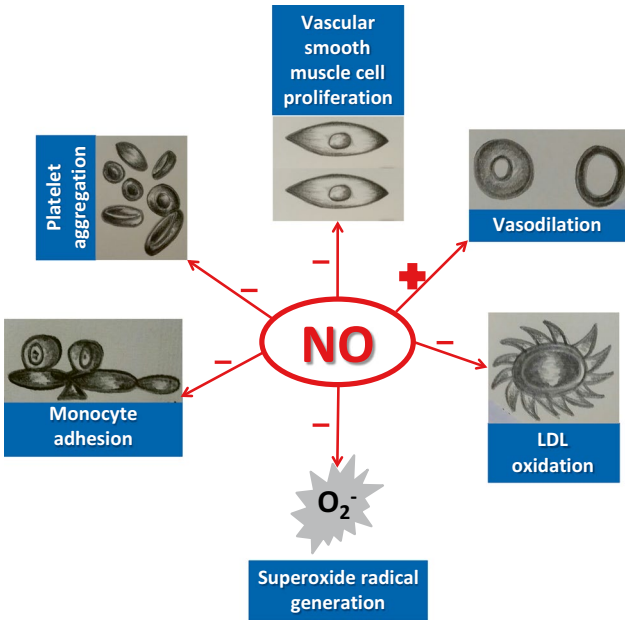

Fig. 1 Vascular actions of nitric oxide (adapted from Alexander et al. 2004 and Böger et al. 2010). *LDL* Low density lipoproteins

reduces the release of oxygen-derived free radicals. Figure 1 depicts these interactions (Böger et al. 2010).

More recently the mechanisms leading to the vasodilation associated with NO have been shown to be more complex than previously appreciated, being integrated with many biochemical pathways (Boeldt et al. 2014). In any endothelial cell, the level of NO produced by endothelial NOS (eNOS) is determined by the maximum capacity of the cell (eNOS expression levels), eNOS phosphorylation state, and the intracellular Ca^{2+} concentration in response to circulating hormones or physical forces. In pregnancy, modification of NO output occurs at the level of sustained phase 'capacitative entry' [Ca^{2+}] response; this adaptive response is lacking in pre-eclamptic pregnancies. Moreover, gap junction function is an essential permissive regulator of the capacitative response, and impairment of NO output results from any inhibitor of gap junction function, or capacitative entry using transient receptor potential cation (TRPC) channels. Identifying these Ca^{2+} signaling mechanisms underlying normal pregnancy, the adaptation of the NO output not only provides novel targets for future treatment of diseases of pregnancy, but may also apply to other common forms of hypertension (Boeldt et al. 2014). NOS is expressed on the syncytiotrophoblast and endothelial cells in the placenta during pregnancy (Kakui et al. 2003; Myatt et al. 1997a, b).

It is unclear whether the altered physiology (particularly increased vasodilation) in pregnancy is secondary to an increase in endogenous NO or increased sensitivity of vascular smooth muscle to NO (Meher and Duley 2007). Baylis et al. (1998) showed that placentas of pre-eclamptic women express normal levels of eNOS and form normal amounts of NO. The mechanisms controlling vasodilation through NO are complex and integrated with many biochemical pathways (Boeldt et al. 2014). Elevated estrogen levels promote endothelium-dependent vasodilatation during normal pregnancy, which is mediated partly via NO through vascular smooth muscle relaxation (Nevzati et al. 2015; Hayashi et al. 1995). To understand the altered physiology of normal pregnancy via NO, an appreciation of NO metabolism is important.

NO is synthesized from the amino acid substrates L-arginine and L-homoarginine by a family of calcium-calmodulin-dependent enzymes called NOS. eNOS is the most important member of this family and is the key enzyme when considering the role of NO in normal pregnancy. The activity of this enzyme is inhibited by ADMA which competes with L-arginine at the substrate-binding site (Tsikas et al. 2000a, b). NO might also play an anti-inflammatory role by inhibiting the expression of adhesion molecules, including ICAM-1, vascular cell adhesion molecule-1, E-selectin, and P-selectin (Davenpeck et al. 1994; Gauthier et al. 1995). PE, which has an inflammatory component, is characterized by elevated levels of these adhesion molecules (Abe et al. 2008; Szarka et al. 2010; Matsubara et al. 2003). The level of ADMA in the maternal circulation falls during the first half of pregnancy, reaching its nadir by 24 weeks' gestation, and then rises again to pre-pregnancy levels toward term (Holden et al. 1998). This pattern of the initial fall and subsequent rise mirrors that of maternal vascular tone and blood pressure in pregnancy (Khalil et al. 2009).

The maximal binding rate of L-arginine to NOS is twice that of L-homoarginine (Abu-Soud et al. 1999), and pregnancy studies, particularly those in pathological pregnancies, indicate that L-arginine is the more significant substrate in the regulation of the NO pathway. However, Valtonen et al. (2008) examined levels of homoarginine, arginine and ADMA in pregnant women and non-pregnant controls and found interesting results indicating the importance of homoarginine in the adaptive changes of pregnancy. Serum homoarginine concentration was shown to be significantly higher during the second and third trimesters than in the non-pregnant controls (4.8 ± 1.7 and 5.3 ± 1.5 versus 2.7 ± 1.0 µM, respectively, $P < 0.001$). In line with this finding, brachial artery flow-mediated dilatation (FMD), a marker for endothelial function, increased in pregnancy. FMD was positively correlated with serum homoarginine levels in pregnant, but not in non-pregnant, women. These results indicate that homoarginine plays a direct role in NO upregulation. It has been postulated that this acts through a direct increase in NO production, though previous studies have reported that homoarginine can also

modulate NO production in endothelial cells by modifying cellular L-arginine transport mechanisms (Bird et al. 2003).

In a study by Valtonen et al. (2008), maternal arginine concentrations fell significantly in the first trimester ($P < 0.05$), then increased toward term, to almost the same level as in the controls. The arginine/ADMA ratio, a determinant of NO production by NOS, remained unchanged throughout pregnancy. Both the arginine level and the arginine/ADMA ratio are significantly associated with PE, but how levels influence normal pregnancy is less clearly understood.

In 1992, Vallance and colleagues first reported that ADMA is an endogenous inhibitor of NOS (Vallance et al. 1992). The following year, Fickling and coworkers (1993) found that plasma concentrations of ADMA differed between non-pregnant and pregnant women, and that women with PE had significantly higher levels than those with normotensive pregnancy. Since then, multiple studies have reported that ADMA levels fall during the course of normal pregnancy in parallel with the fall in systemic arterial blood pressure (Holden et al. 1998). As well as playing a role in normal and pathological pregnancy, ADMA plays an important role in cardiovascular disease. It exerts negative cardiovascular effects by displacing L-arginine from NOS, thereby reducing NO production in the vascular endothelium (Böger et al. 2010). ADMA levels fall until 24 weeks of gestation before rising to pre-pregnancy levels in the third trimester (Holden et al. 1998). Interestingly, extracellular ADMA concentrations were reported to be tenfold higher than the intracellular ADMA levels (Cardounel 2007). Moreover, there is a relationship between ADMA concentration and NOS activity at varying arginine levels, suggesting that ADMA, as well as the ratio of ADMA to arginine, influences the activity of NOS at pathological, as well as physiological, levels (Cardounel 2007; Tsikas et al. 2000b).

Pre-eclampsia and the arginine–NO pathway

PE is multisystem disorder and remains a leading cause of maternal and perinatal mortality and morbidity in both developed and developing countries (Cantwell et al. 2011; Duley 2009; WHO 2005). Despite extensive research, it remains an enigmatic condition. PE is characterized by impairment of trophoblast invasion of the maternal spiral arteries, leading to placental hypoxia and the release of inflammatory factors, endothelial cell activation and the clinical manifestation of PE (Redman 1991; Roberts and Redman 1993; Granger et al. 2001). However, the underlying mechanisms that impair trophoblastic invasion are not fully elucidated.

PE is usually defined as hypertension and proteinuria after 20 weeks of gestation in a previously normotensive pregnant woman. In the Western world, it affects between 2 and 7 % of all pregnancies, but can be up to three times more common in certain ethnic groups (Noris et al. 2005). Sandrim et al. (2010) found significantly higher circulating concentrations of ADMA in Afrocaribbean pre-eclamptic women, suggesting a possible mechanism for these racial disparities. Early-onset PE, defined as PE requiring delivery prior to 34 weeks' gestation, is associated with the greatest risk of maternal and perinatal complications (MacKay et al. 2001; von Dadelszen et al. 2003; von Dadelszen et al. 2009). Women who develop PE are also at increased long-term risk of cardiovascular disease and stroke (Irgens et al. 2001; Smith et al. 2001; Ray et al. 2005; Bellamy et al. 2007). Until now, delivery is the only cure for PE. However, the complications and inherent costs of prematurity represent a major challenge to patients and health-care systems. The additional National Health System (NHS) costs of caring for a neonate born before 33 or 28 weeks of gestation have been estimated at £61,509 and £94,190, respectively (Mangham et al. 2009). According to Mangham et al., up to £260 million a year would be saved if we could delay the delivery of all premature babies by 1 week (Mangham et al. 2009). The incidence of PE is likely to increase, secondary to the rise in obesity and advanced maternal age at the first pregnancy.

Noris and colleagues (2005) neatly outlined the complex contribution of the L-arginine/NO pathway to the pathogenesis of PE (Fig. 2). It is postulated that reduced NO activity in the pre-eclamptic placenta is secondary to rapid NO degradation to peroxynitrite (ONOO–), an anion devoid of angiogenic properties. The fact that the production of reactive oxygen species (ROS) including peroxynitrite is significantly increased in PE supports this theory. eNOS facilitates the production of both NO and the superoxide anion (O_2^-), which oxidizes NO into the inactive peroxynitrite (Förstermann 2006). The relative amounts of NO and O_2^- formed are tightly regulated by intracellular levels of L-arginine; in vitro depletion of L-arginine has been shown to lead to production of NO and O_2^-, and subsequent formation of $ONOO^-$. Arginine levels have been found to be lower than normal in pre-eclamptic villous tissues (Noris et al. 2004). However, studies comparing levels of arginine in normal and PE pregnancies have yielded heterogeneous results. Some studies have reported an increased level in PE, others a reduced level, and others still no significant difference (Khalil et al. 2013). A list of the studies investigating the maternal circulating ADMA and arginine levels, and their ratio, in pregnancies complicated by PE compared with controls is shown in Table 1.

Molnar et al. (1994) showed that a pre-eclamptic syndrome was induced in rats by the chronic administration of an L-arginine antagonist. Higher levels of blood and tissue markers of oxidative stress were seen, supporting the

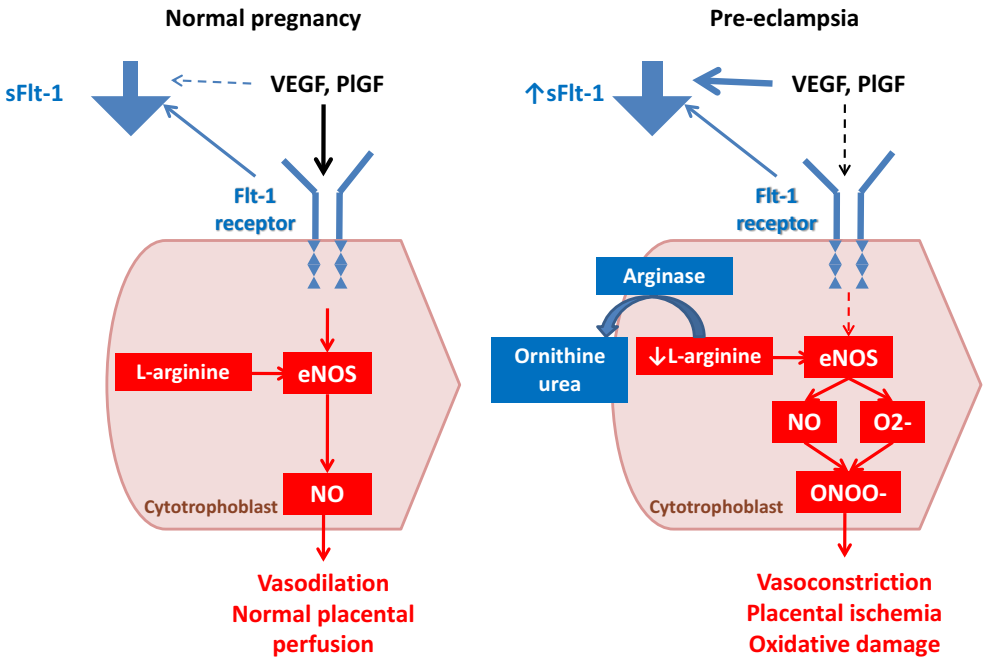

Fig. 2 The interaction between L-arginine and nitric oxide in the pathogenesis of pre-eclampsia (adapted from Noris et al. 2005)

hypothesis that arginine deficiency contributes to oxidative stress which is known to induce PE (Fig. 2). Noris et al. (2005) further points to the significantly raised levels of the L-arginine-hydrolyzing enzyme arginase II in pre-eclamptic trophoblastic cells in comparison to normal placenta (Noris et al. 2004). He refers to potential upregulation of the enzyme by testosterone as seen in rats and mice.

Many studies have demonstrated increased ADMA levels in women with PE (Khalil et al. 2013; Böger et al. 2010). ADMA levels measured at 23–25 weeks' gestation were raised in women who subsequently developed PE (Savvidou et al. 2003). This finding was confirmed by Speer and coworkers (Speer et al. 2008). Compared to normotensive controls, women who later developed PE had significantly higher circulating concentrations of ADMA. However, a large study in Colombia (Maas et al. 2004) did not find any significant difference in ADMA levels between controls and women with PE. It has been postulated that the relative cardiovascular and infective risk of that population may explain this leveling of risk associated with ADMA concentration (Böger et al. 2010).

ADMA concentration is controlled by the activity of dimethylarginine dimethylaminohydrolase (DDAH) enzymes. Genetic polymorphisms of DDAH-1, which modifies ADMA activity, have been described, while DDAH-2 activity has been shown to be affected by oxidative stress (Böger et al. 2010; Anderssohn 2012). Another observation in pregnancies complicated by PE is decreased placental DDAH activity, leading to an increase in ADMA levels, and so reduced NO production. Studies comparing the NO level in PE and normal pregnancy have reported conflicting results (Matsubara et al. 2010; Sandrim et al. 2011). This observation might be explained by the reduced bioavailability of NO, rather than its levels in the maternal blood (Sandrim et al. 2011). An imbalance between NO and ROS has been proposed to contribute to the pathogenesis of PE (Matsubara et al. 2010). Maternal circulating ADMA levels are significantly higher in early-onset severe PE than in normotensive pregnancies or even in cases with late-onset PE; again, this suggests that the lower the level of NO production, the more severe is the degree of PE (Alpoim et al. 2013). However, the levels of ADMA were not significantly different between late-onset PE and normotensive pregnancies (Alpoim et al. 2013). These findings support the theory that the pathophysiology of early and late PE is distinct.

Studies examining the relationship between ADMA, arginine, homoarginine and PE have been summarized previously (Khalil et al. 2013). Twelve studies from 1993 to 2010 including a total of 740 patients reported the circulating ADMA and arginine concentrations and their ratio in women before and after the onset of clinical PE and compared them with normotensive controls. Most studies reported that in PE, ADMA levels were significantly increased.

In a nested case–control study in the first trimester, we made a distinction between early-onset PE (delivery before 34 weeks) and late-onset PE (Khalil et al. 2013). Interestingly, early PE was found to have significantly

Table 1 Studies reporting the concentrations of maternal circulating asymmetric dimethylarginine (ADMA) (μM) and arginine (μM) and their ratio in pregnancies complicated by pre-eclampsia compared with controls

References	Analytical method	Gestation (weeks)	Control		Pre-eclampsia	
			N	Median (IQR)	N	Median (IQR)
ADMA						
Fickling et al. (1993)		3rd trimester	10	0.46b	8	1.23a, b
Pettersson et al. (1998)	HPLC	32–40	12	0.36 (SEM 0.01)	12	0.55 (SEM 0.02)a, b
Holden et al. (1998)	HPLC	25–40	44	0.56 (SD 0.23)	18	1.17 (SD 0.42)a, b
Ellis et al. (2001)	HPLC	24–32	19	0.56 (range 0.36–1.10)	12	0.68 (range 0.51–0.82)a
Ellis et al. (2001)	HPLC	36–40	16	0.53 (range 0.40–0.73)	32	0.68 (range 0.43–1.70)a
Maas et al. (2004)	HPLC	33–40	93	0.42 (IQR 0.29–0.55)	67	0.43 (IQR 0.31–0.56)
Kim et al. (2006)	HPLC	28–40	13	0.28 (IQR 0.17–0.33)	16	0.21 (IQR 0.16–0.24)
Siroen et al. (2006)	HPLC	First stage of labor	15	0.37 (SD 0.06)	16	0.40 (SD 0.06)b
Powers (2008)	HPLC	26–42	31	0.49 (SD 0.08)	15	0.55 (SD 0.07)a, b
Mao et al. (2010)	HPLC	3rd trimester	30	0.82 (SD 0.11)	62	1.27 (SD 0.31)a, b
Braekke et al. (2009)	LC–MS/MS	24–42	40	0.39 (IQR 0.33–0.43)	43	0.44 (IQR 0.38–0.50)a
Sandrim et al. (2010a, b)	ELISA	35.9 (SD 3.7)	47	2.11 (SD 0.01)	47	2.20 (SD 0.02)a, b
Turan et al. (2010)	HPLC	3rd trimester/labor	54	1.24 (SD 0.20)	55	3.25 (SD 1.42)a, b
Savvidis et al. (2011)	ELISA	24–32	36	1.01 (range 0.30–1.64)	38	1.38 (range 1.04–1.74)a
Demir et al. (2012)	HPLC	36–40	40	0.66 (range 0.60–0.92)	40	0.97 (range 0.61–1.99)a
Anderssohn (2012)	LC–MS/MS	3rd trimester	28	0.42 (SD 0.07)	18	0.51 (SD 0.15)a, b
Rizos et al. (2012)	ELISA	3rd trimester	41	0.58 (SD 0.16)	10	0.68 (SD 0.11)b
Tamas et al. (2013)	LC–MS/MS	3rd trimester	15	0.37 (range 0.29–0.45)	36 early-onset	0.44 (range 0.27–0.74)b
					17 late-onset	0.44 (range 0.29–0.67)b
Laskowska et al. (2013a)	ELISA	3rd trimester (≥34)	65	0.49 (SD 0.11)	62 early-onset	0.59 (SD 0.16)
		3rd trimester (≥34)		0.49 (SD 0.11)	53 late-onset	0.56 (SD 0.17)
Alpoim et al. (2013)	ELISA	3rd trimester (<34)	50	0.48 (IQR 0.42–0.58)	29 (early severe)	0.66 (IQR 0.52–0.78)a
		3rd trimester (≥34)	50	0.48 (IQR 0.42–0.58)	24 (late severe)	0.47 (IQR 0.42–0.60)a
Arginine						
Pettersson et al. (1998)	HPLC	32–40	12	74.5 (SEM 3.8)	12	80.7 (SEM 5.8)b
Maas et al. (2004)	HPLC	33–40	93	29.4 (IQR 18.1–56.6)	67	43 (IQR 18.9–58.9)
Kim et al. (2006)	HPLC	28–40	13	85.4 (IQR 55.1–117.8)	16	54.2 (IQR 39.0–77.5)a
Siroen et al. (2006)	HPLC	First stage of labor	15	41.1 (SD 12.1)	16	45.9 (SD 12.2)b
Powers (2008)	HPLC	26–42	31	36.3 (SD 17.9)	15	35.4 (SD 13.9)b
Mao et al. (2010)	HPLC	3rd trimester	30	78.55 (SD 3.09)	62	78.58 (SD 4.76)b
Braekke et al. (2009)	LC–MS/MS	24–42	40	39.1 (IQR 34.6–45.2)	43	45.4 (IQR 37.7–54.4)a
Turan et al. (2010)	HPLC	Third trimester/labor	54	50.3 (SD 27.5)	55	90.7 (SD 56.6)a, b
Tamas et al. (2013)	LC–MS/MS	Third trimester	15	58.0 (range 14.8–280.7)	36 early-onset	49.9 (range 17.2–113.5)
					17 late-onset	53.4 (range 16.5–95.1)
Pimentel et al. (2013)	HPLC	Third trimester	7	97.86 (SD 25.50)	6	88.33 (SD 18.42)b
Ehsanipoor et al. (2013)	ELISA	34–42	17	2.14 (SD 0.33)	12	1.88 (SD 0.19)a, b
Arginine/ADMA ratio						
Pettersson et al. (1998)	HPLC	32–40	12	211.0 (SEM 14.3)	12	145.6 (SEM 10.5)a, b
Maas et al. (2004)	HPLC	33–40	93	88.9 (IQR 41.8–137.8)	67	88.9 (IQR 41.8–137.8)
Kim et al. (2006)	HPLC	28–40	13	375.1 (IQR 322.0–531.1)	16	215.6 (IQR 132.0–424.2)a
Powers (2008)	HPLC	26–42	31	74.2 (SD 31.7)	15	64.6 (SD 24.5)b
Braekke et al. (2009)	LC–MS/MS	24–42	40	106.5 (IQR 90–121)	43	105.8 (IQR 91–126)

ELISA enzyme-linked immunosorbent assay, *HPLC* high-performance liquid chromatography, *LC–MS/MS* liquid chromatography–mass spectrometry

a $P < 0.05$

b Mean values

Table 2 Studies reporting the concentrations of maternal circulating asymmetric dimethylarginine (ADMA) concentration (μM) and L-arginine (μM) and their ratio in pregnancies before the clinical onset of pre-eclampsia compared with controls

References	Analytical method	Gestation (week)	Control		Pre-eclampsia	
			N	Median (IQR)	*N*	Median (IQR)
ADMA						
Savvidou et al. (2003)	HPLC	23–25	43	0.81 (IQR 0.49–1.08)	10	2.7 (IQR 2.21–3.21)a
Powers (2008)	HPLC	8–21	31	0.34 (SD 0.08)	15	0.45 (SD 0.09)a, b
Khalil et al. (2013)	GC–MS/MS	11–14	300	0.37 (0.34–0.41)	25 early-onset	0.38 (0.33–0.45)
					50 late-onset	0.37 (0.34–0.40)
Rizos et al. (2012)	ELISA	First trimester	41	0.51 (SD 0.14)	10	0.58 (SD 0.10)b
Rizos et al. (2012)	ELISA	Second trimester	41	0.52 (SD 0.13)	10	0.63 (SD 0.14)a, b
Arginine						
Savvidou et al. (2003)	HPLC	23–25	43	21.9 (IQR 19.4–25.8)	10	31.1 (IQR 24.6–35)a
Powers (2008)	HPLC	8–21	31	49.5 (SD 17.2)	15	37.7 (SD 10.9)a, b
Khalil et al. (2013)	GC–MS	11–14	300	51.56 (47.63–62.05)	25 early-onset	46.06 (41.00–55.50)a
					50 late-onset	51.32 (48.00-59.41)
Arginine/ADMA ratio						
Savvidou et al. (2003)	HPLC	23–25	43	31.1 (IQR 21.4–39.7)	10	11.27 (IQR 8.74–15.17)a
Powers (2008)	HPLC	8–21	31	155.8 (SD 62.4)	15	84.7 (SD 24.3)a, b
Khalil et al. (2013)	GC–MS	11–14	300	0.007 (0.006–0.008)	25 early-onset	0.008 (0.007–0.009)a
					50 late-onset	0.007 (0.006–0.008)

ELISA enzyme-linked immunosorbent assay, *HPLC* high-performance liquid chromatography, *GC–MS* gas chromatography–mass spectrometry, *GC–MS/MS* gas chromatography–tandem mass spectrometry

a $P < 0.05$

b Mean values

reduced levels of arginine and homoarginine, but late PE did not. A similar pattern was observed by Alpoim and colleagues (Alpoim et al. 2013); ADMA levels were increased in early-onset PE (0.66 μM) versus late PE (0.47 μM) ($P = 0.001$) and normotensive pregnant controls (0.48 μM) ($P = 0.001$). Laskowski and coworkers also found higher levels of ADMA in early PE compared to late PE and controls, but this difference was not statistically significant (Laskowska et al. 2013a, b). These findings support a hypothesis that aims to explain the heterogeneity of PE pathology: NO metabolism is implicated in early PE, whereas late PE can be seen as associated with the pathophysiology of the metabolic syndrome. An updated literature review included studies reporting on the maternal circulating ADMA and arginine levels and their ratio in pregnancies complicated by PE, both at the time of the disease and before its clinical onset (Tables 1, 2). Early-onset PE is associated with impaired placentation, which is reflected in increased maternal circulating levels of the antiangiogenic factor soluble endoglin (sEng) and decreased levels of the angiogenic factor placental growth factor (PlGF). These changes can be detected as early as the first trimester, long before the clinical manifestation of the disease (Akolekar et al. 2008; Foidart et al. 2010). Soluble Fms-like tyrosine kinase-1 (sFlt-1) is a circulating splice variant of the vascular endothelial growth factor (VEGF) receptor and binds to VEGF and PlGF, reducing their bioavailability (Sandrim et al. 2008). Endoglin is a transforming growth factor β1 and β3 co-receptor that is expressed on the surface of endothelial cells and its soluble form is sEng (Levine et al. 2004, 2006). PE is associated with the release of sEng into the maternal circulation, which interferes with the normal transforming growth factor β signaling (Levine et al. 2004, 2006). Furthermore, there is an inverse relationship between circulating levels of the major NO metabolite nitrate and antiangiogenic factors (Sandrim et al. 2008). Studies have reported that sEng modifies the expression and activity of eNOS, ultimately leading to the endothelial dysfunction characteristic of the pathogenesis of PE (Toporsian et al. 2005; Venkatesha et al. 2006; Santibanez et al. 2007; Ten Dijke et al. 2008). Identification of women who will develop PE is likely to facilitate targeted antenatal surveillance and possibly intervention. Up to 5 % of women with PE develop complications (Churchill and Duley 2002) and up to 30 % of women who develop early-onset PE can develop severe complications requiring admission to the intensive care unit (Sibai and Stella 2009).

The results of the studies which investigated the maternal circulatory ADMA and arginine levels are not entirely consistent. These discrepancies may be secondary to

differences in populations examined, study designs, and more importantly the different analytical techniques used to analyze the samples. These techniques include enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), gas chromatography–tandem mass spectrometry (GC–MS/MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). The advantages and limitations of each of these techniques are beyond the scope of this review (for a discussion see Schwedhelm 2005; Tsikas 2008; Martens-Lobenhoffer and Bode-Böger 2012).

A recent study (Sandvik et al. 2013) that investigated cardiovascular risk after PE pregnancies found that women with previous PE and low birth weight babies still had alterations in arginine, homoarginine and ADMA levels 10 years after pregnancy. This was an interesting finding that suggested that there is long-term endothelial morbidity following PE. Moreover, the continued alteration of arginine biomarkers begs the question of causality—whether the balance of arginine-related substrates is the primary factor leading to endothelial dysfunction, or the primary pathology is endothelial dysfunction which then alters regulation of NO and the arginine-related biomarkers. Interestingly, the circulatory levels of sFlt-1, a known antiangiogenic factor, were also elevated in these women (Sandvik et al. 2013).

The relationship between homoarginine and PE is far less commonly reported than its relationship with arginine, NO or ADMA. Maternal serum homoarginine levels in the first trimester are similar to those in non-pregnant women; from then on, there is an estrogen-mediated rise with gestation (Valtonen et al. 2008; Zhu and Evans 2001). We have demonstrated that the maternal circulatory homoarginine levels are significantly lower in women who go on to develop early-onset PE, and that these changes can be seen as early as 11 weeks' gestation (Khalil et al. 2013). It is possible, therefore, that homoarginine could be used as a marker in first trimester screening for early-onset PE. However, first trimester homoarginine levels were similar in late-onset PE and normotensive pregnancies, so hold little promise for screening for the late-onset form of the disease (Khalil et al. 2013). Levels of homoarginine are positively correlated with baseline brachial artery diameter and FMD, supporting its role in modulating endothelial function (Valtonen et al. 2008).

Intrauterine fetal growth restriction and the arginine/NO pathway

It is estimated that 8 % of pregnancies are complicated by IUGR (Mandruzzato et al. 2008), which is associated with increased perinatal mortality and morbidity. Approximately, 50 % of stillbirths and 10 % of perinatal deaths occur in pregnancies complicated by IUGR (Mandruzzato et al. 2008; Froen et al. 2004; Richardus et al. 2003). IUGR is also associated with long-term morbidity, including neurodevelopmental disability, poor intellectual and psychological performance, increased risk of developing cardiovascular disorders, lower growth hormone secretion and delayed onset of puberty (Strauss 2000; Hediger et al. 1998; Lee et al. 2003; Lundgren et al. 2001; Bardin et al. 2004; Sung et al. 1991; Lienhardt et al. 2002; Stein et al. 1996).

Small for gestational age (SGA) is traditionally defined as birth weight below the 10th centile for gestational age and sex according to population references (Battaglia and Lubchenco 1967; Pallotto and Kilbride 2006). The majority of infants who are born SGA have a normal outcome. Abnormal fetal Doppler, in particular the umbilical artery and middle cerebral artery Doppler, are useful in the antenatal identification of pregnancies complicated by IUGR, as opposed to just being constitutionally small (Morales-Roselló et al. 2014).

Impaired trophoblastic invasion and placental dysfunction are common features in PE, especially early-onset PE, and in IUGR. The resulting impaired placental transfer of nutrients and oxygen leads to suboptimal fetal growth. eNOS expression was shown to be increased in the placental tissue of pregnancies complicated by IUGR or PE, which could be an adaptive response to the increased resistance and poor trophoblastic invasion (Myatt et al. 1997a, b). Interestingly, maternal circulatory ADMA levels in the first and second trimesters are positively correlated with birth weight (Rizos et al. 2012). Despite the considerable literature on the decreased NO synthesis and/or bioavailability and impaired NO/cGMP signaling pathway in pregnancies complicated by PE, studies investigating this association in IUGR are far less common and more controversial (Laskowska et al. 2013a, b; Powers 2008; Rizos et al. 2012). One study found that maternal serum ADMA levels were elevated in pregnancies complicated by IUGR when compared with uncomplicated controls (Laskowska et al. 2013b). However, in another study, ADMA levels were significantly lower in SGA pregnancies than in controls, in the first (11–14 weeks), second (20–24 weeks) and third trimesters (28–35 weeks) (Rizos et al. 2012). ADMA levels were also lower in young adults born preterm at extremely low birth weight compared with healthy adults born at term (Bassareo et al. 2012).

An earlier study has reported that ADMA levels were not significantly different in pregnancies with SGA, in either the second or third trimester (Powers 2008). Similar to the findings in studies focusing on PE, these discrepant findings reported in IUGR could be due to differences in the

study populations (whether SGA or IUGR), study design or the techniques used to analyze the maternal samples.

The crux of the current management of pregnancies complicated by IUGR is monitoring and timely delivery. This usually requires balancing the risks of stillbirth (if the pregnancy is allowed to continue) and prematurity (if the baby is delivered early), especially in early-onset IUGR. The role of the NO pathway in antenatal treatment of IUGR has recently been explored. Animal studies have shown a sildenafil-induced normalization or improvement of the growth and/or fetal Doppler in IUGR mice, rat, guinea pig and sheep models (Satterfield et al. 2010; Stanley et al. 2012; Refuerzo et al. 2006; Wareing et al. 2005; Sanchez-Aparicio et al. 2008). In an observational study including ten women with early-onset severe IUGR and who received sildenafil, fetal abdominal circumference growth velocity increased following the treatment (Von Dadelszen et al. 2011). When compared with 17 women with early-onset severe IUGR who did not receive sildenafil, the intact survival was significantly better. Furthermore, a single dose of sildenafil citrate improved both the umbilical and middle cerebral artery Doppler in a randomized controlled trial of 41 pregnant women with IUGR at 24–37 weeks of gestation (Dastjerdi et al. 2012).

Based on these encouraging data, the STRIDER (Sildenafil Therapy In Dismal prognosis Early-onset intrauterine growth Restriction) Individual Participant Data (IPD) Study Group has begun a series of trials and meta-analysis to explore whether sildenafil improves the outcomes in these babies that currently have a very poor prognosis. Several other studies of this question are under way around the world.

Arginine/NO pathway and other perinatal pathology

Studies have demonstrated a link between ADMA and gestational diabetes (Mittermayer et al. 2002; Gumus et al. 2012). Circulatory ADMA levels are elevated in women who develop gestational diabetes, at the time of the diagnosis (Akturk et al. 2010; Telejko et al. 2009) and even after the incident pregnancy (Mittermayer et al. 2002; Gumus et al. 2012). Moreover, ADMA was found to be associated with early carotid atherosclerosis in women with a history of gestational diabetes, suggesting that it could be a screening marker for adverse cardiovascular outcome in these women (Xia et al. 2015).

An association between arginine and depression was noted in 2014 (Raw et al. 2014). In a case–control study of 21 depressed pregnant women and 42 matched controls, arginine levels were 10 % lower in the women with depression. The authors suggested that arginine supplementation might benefit women with depressive symptoms in pregnancy.

Possible therapeutic role of the arginine/NO pathway

The current management of PE includes antihypertensive therapy, prophylaxis against eclampsia using magnesium sulfate and earlier delivery. In early-onset PE a course of steroids is given to promote fetal lung maturation. Nevertheless, the risks of prematurity are substantial, especially before 30 weeks' gestation.

The use of L-arginine and NO donors is relatively common in cardiovascular disorders in non-pregnant populations. All of the evidence described above supporting the role of NO in PE and IUGR pregnancies prompted several studies investigating the use of NO donors, including the organic nitrates glyceryl trinitrate (GTN), also known as nitroglycerin, isosorbide dinitrate (ISDN) and S-nitrosothiols to try to improve pregnancy outcomes. Most of these therapeutic options focus on the restoration of the NO-soluble guanylyl cyclase (sGC) pathway by using inhibitors of cGMP breakdown, such as the phosphodiesterase inhibitor sildenafil. This in turn causes an increase in intracellular cGMP and activation of cGMP-dependent protein kinases. This facilitates the actions of NO, such as relaxation of vascular smooth muscle (Meher and Duley 2007).

Unfortunately, these studies did not find any significant benefit. The Cochrane review in 2007 (Meher and Duley 2007) concluded that there were insufficient data to assess the value of interventions designed to optimize NO bioavailability. Moreover, the review noted the high frequency of side effects (as with other nitrate therapy) such as headaches and dizziness, which calls into question the acceptability of such an intervention.

However, since that review, further evidence has been accumulating on this topic. A study from Mexico (Vadillo-Ortega et al. 2011) evaluated the effect of supplementation with L-arginine and antioxidant vitamins (Vitamins C, E, B6, B12 and niacin) in at-risk pregnancies. This study included 672 pregnant women, divided into three equal groups: control, antioxidant vitamin supplementation, and L-arginine plus antioxidant vitamin supplementation. Those given a combination of L-arginine and antioxidant vitamins had significantly reduced rates of PE as compared to both the control (absolute risk reduction 0.17; 95 % CI 0.12–0.21) and the antioxidant vitamin supplementation alone group (absolute risk reduction 0.09; 95 % CI 0.05–0.14). This is the largest study to date and its size strongly supports the benefit of this intervention. It should be noted, however, that it was conducted not in a normal population, but in an at-risk group. Moreover, this study currently stands alone in showing a clear reduction in rates of PE.

Kalidindi and colleagues (2012) systematically reviewed the current literature on studies using NO donors and L-arginine that assessed both uteroplacental blood

flow and prevention of PE. The review found that there was good evidence that supplementation aids uteroplacental blood flow and reduces gestational hypertension. Neri et al. (2006) also demonstrated a substantial reduction in blood pressure after intravenous infusion of L-arginine. Dorniak-Wall et al. (2013) examined the role of L-arginine in the prevention and treatment of PE. They identified eight randomized controlled trials including 884 cases. In women at increased risk of PE, L-arginine significantly reduced the risk of developing the disease and of being delivered preterm. Data show that physiological plasma L-arginine concentrations are in a range that enables full activity of NOS in the presence of physiologically low ADMA levels (Tsikas et al. 2000b). However, in the presence of elevated levels of ADMA, an inhibitor of NOS, the conversion of L-arginine to NO is impaired, resulting in decreased biological actions of NO (Tsikas et al. 2000b). Under such circumstances, increasing L-arginine concentration secondary to dietary supplementation may result in restoring NO production to near-normal levels (Böger et al. 2010). Yet, it should be considered that ADMA is a weak inhibitor of eNOS (Kielstein et al. 2007), and the NOS isoform is believed to be of particular importance in pregnancy.

Conclusions and future perspectives

The role played by NO, arginine, ADMA and homoarginine in normal and pathological pregnancy is gradually being elucidated. Thus far, the majority of investigations in this area have focused on ADMA as an inhibitor of eNOS. Yet, given that ADMA has very weak inhibitory potency toward eNOS, it may exert additional biological activities that remain to be delineated. It is only very recently that homoarginine has attracted the interest of investigators and its biological activities are still largely unrevealed. Homoarginine may serve as an NO precursor and contribute to NO-related effects. However, homoarginine is present at much lower concentrations than arginine and has a much lower affinity to eNOS. Analogous to ADMA, homoarginine is likely to exert biological effects that are not primarily related to NO. Recent studies suggest that ADMA and homoarginine may act antagonistically in the renal and cardiovascular systems. Thus, it is clear that much more work on ADMA and homoarginine is needed. Therapeutic interventions that manipulate arginine, homoarginine and ADMA, their interactions and underlying pathways hold real promise for the management of pregnancies complicated by PE and/or IUGR and the results of ongoing studies are eagerly awaited.

Conflict of interest None of the authors has any conflict of interest.

Ethical standard All studies reported here were approved by the local ethics committees.

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