Published in final edited form as:

Am J Obstet Gynecol. 2008 January ; 198(1): 112.e1–112.e7. doi:10.1016/j.ajog.2007.05.052.

ELEVATED ASYMMETRIC DIMETHYLARGININE CONCENTRATIONS PRECEDE CLINICAL PREECLAMPSIA BUT NOT PREGNACIES WITH SMALL FOR GESTATIONAL AGE INFANTS

Robert W. Powers, PhD^{*,1,2}, Paul D. Speer, MD^{*,1}, Michael P. Frank, BS², Gail Harger, MS², Nina Markovic, PhD^{2,3}, and James M. Roberts, MD^{1,2,3}

¹Department of Obstetrics, Gynecology, Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

²Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

³Department of Epidemiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Abstract

Objective—The purpose of this study was to investigate maternal plasma concentrations of asymmetric dimethylarginine (ADMA) in mid-pregnancy and at the time of disease in women who develop preeclampsia compared to women with uncomplicated pregnancies and women with SGA infants.

Study design—Plasma samples were collected at mid-pregnancy and at the time of delivery from 31 women with uncomplicated pregnancies, 12 women with SGA infants and 15 women with preeclampsia. ADMA and L-arginine were measured by high-pressure liquid chromatography.

Results—Maternal ADMA concentrations were elevated at mid-pregnancy and remained elevated at delivery in women who later developed preeclampsia [$0.45 \pm 0.09 \mu\text{mol/L}$] compared to women with uncomplicated pregnancies [$0.34 \pm 0.08 \mu\text{mol/L}$, $p < 0.01$] and women with SGA infants [$0.33 \pm 0.06 \mu\text{mol/L}$, $p < 0.01$].

Conclusions—Maternal ADMA concentrations are higher in mid-pregnancy in women who develop preeclampsia compared to uncomplicated controls and SGA. Elevated ADMA prior to clinical onset of preeclampsia suggests a role of this NOS inhibitor in the pathophysiology of preeclampsia.

Correspondence: Robert W. Powers, Ph.D., Magee-Womens Research Institute, 204 Craft Ave, Room 620, Pittsburgh, PA 15213, (412) 641-6005 phone, (412) 641-1503 fax, E-mail: rsirwp@mwri.magee.edu.

*These authors contributed equally to this work.

Presented at the 14th World Congress of the International Society for Study of Hypertension in Pregnancy, Vienna, Austria, November 14–17, 2004.

Condensation: ADMA is elevated in mid-pregnancy in women who later develop preeclampsia and during disease, but not in women who deliver only SGA infants.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Preeclampsia; pregnancy; small for gestational age; asymmetric dimethylarginine; arginine

INTRODUCTION

The pregnancy syndrome preeclampsia complicates approximately 3–7% of all pregnancies and continues to be a major contributor to maternal and neonatal morbidity and mortality.¹ The pathogenesis of preeclampsia is unclear, reduced placental perfusion and endothelial dysfunction appear to be central to the pathophysiology of the syndrome.^{2, 3}

Nitric oxide (NO), produced by endothelial cells, is proposed to contribute to vasodilation and the resulting decrease in vascular resistance observed during normal pregnancy.⁴ Reduced nitric oxide production/availability has been postulated to contribute to the increased blood pressure, systemic vascular resistance and sensitivity to pressors noted in preeclampsia.^{4, 5} Naturally occurring methylated arginine analogues are endogenous inhibitors of the three isoforms of nitric oxide synthase (NOS).^{6, 7} Asymmetric dimethylarginine (ADMA) and monomethylarginine (L-NMMA) compete with L-arginine, the substrate for NOS. However, plasma ADMA concentrations are more than ten fold higher than NMMA, and are therefore considered more relevant biologically. In contrast, the methylated arginine analogue symmetric dimethylarginine (SDMA) does not compete with L-arginine.

Maternal plasma ADMA has been reported to be higher in women with preeclampsia.^{8–10} In addition, Savvidou et al. reported that elevated ADMA concentrations preceded preeclampsia. ADMA was higher at 23–25 weeks in women who later developed preeclampsia, and interestingly ADMA was also elevated in other women with abnormal uterine artery Doppler measurements including women who later had a pregnancy with a growth restricted infant in the absence of preeclampsia.¹¹ These data suggest that elevated plasma ADMA may not be limited to preeclampsia and may contribute to the pathophysiology of other pregnancy disorders with reduced placental perfusion. The objective of this study was to investigate maternal plasma ADMA concentrations at both mid-pregnancy and at delivery in women who later developed preeclampsia and in women with SGA infants but without preeclampsia compared to uncomplicated pregnancies.

Materials and Methods

Study population

This was a nested case-control study from an ongoing investigation of preeclampsia at the University of Pittsburgh, Magee-Womens Hospital and Magee-Womens Research Institute. The study was approved by the institutional review board and informed consent was obtained from all subjects.

Subjects were recruited to the study at the time of presentation to Magee-Womens Hospital for prenatal care. All subjects, both cases and controls, were nulliparous healthy women without known medical complications. Subjects were matched for parity and maternal age. The medical records of subjects were juried regarding pregnancy outcome by a group of physician and research investigators, and consisted of uncomplicated control pregnancies (N=31), subjects with SGA infants and without preeclampsia (N=12) and subjects with preeclampsia (N=15). Preeclampsia was diagnosed by the presence of gestational hypertension, proteinuria, and hyperuricemia beginning after the 20th week of pregnancy with resolution of blood pressure and proteinuria postpartum. Gestational hypertension was defined as an absolute blood pressure ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic after 20 weeks of gestation.

Proteinuria was defined as ≥ 300 mg per 24-hour urine collection, $\geq 2+$ protein on voided urine sample, $\geq 1+$ protein on catheterized urine specimen, or a protein-creatinine ratio of ≥ 0.3 . Hyperuricemia was defined as plasma uric acid concentration ≥ 1 SD above reference values at the gestational age the sample was obtained (e.g. term, > 5.5 mg/dL).

Small for gestational age (SGA) infants were defined by infant birthweight $\leq 10^{\text{th}}$ centile, after adjusting for gestational age, race, and gender, in an otherwise uncomplicated pregnancy. SGA infants with clinical or pathological evidence of chronic intrauterine infection or chromosomal abnormalities were excluded from the study. Because birth weight centile alone cannot distinguish constitutional smallness from failure to reach genetic growth potential (definition of IUGR), we attempted to identify IUGR infants as a subset of SGA infants with additional clinical evidence of fetal growth restriction including: birth weight centile $< 5^{\text{th}}$ centile, asymmetric fetal growth profile, abnormal umbilical artery Doppler waveform where available, oligohydramnios, and/or elevated cord blood erythropoietin. The diagnosis of SGA and the subset of IUGR were determined at delivery. The umbilical artery systolic/diastolic ratio (S/D) was considered abnormal if it was elevated above the 95th percentile for gestational age or if diastolic flow was either absent or reversed. Erythropoietin was measured in venous cord blood EDTA plasma samples by ELISA in duplicate (cat # DEP00, R&D Systems, Minneapolis, MN), range 2.5–200 mIU/ml and the inter-assay coefficient of variation was between 3.5 and 4.6%. Cord blood erythropoietin concentrations were considered to be elevated if $\geq 95^{\text{th}}$ percentile for gestational age. Babies were considered to have asymmetric growth when the growth percentiles for baby weight were significantly less than for baby length and/or head circumference. SGA infants were considered IUGR if at least two of these five criteria were met. As shown in Table 3, eight of the twelve SGA infants had clinical characteristics consistent with IUGR.

Blood samples

Maternal venous EDTA plasma samples were collected between 9–21 weeks gestation during a routine prenatal visit and again at time of admission for delivery. Samples were stored at -70°C for later analysis. These samples are part of a collection accumulated longitudinally over the last 10 years. With a frequency of preeclampsia of 3% by strict criteria there are approximately 50 subjects with preeclampsia and 150 subjects with SGA. Power analysis based on previous published data,¹¹ indicated we would require at least 10 subjects with preeclampsia or SGA and 20 uncomplicated controls to observe a 35% increase in ADMA with an alpha of 0.05 and 80% power. Samples were chosen for analysis based on availability. Samples were stored on average 5.2 ± 1.1 years before ADMA quantitation, and this duration was not different in the different diagnostic groups. Storage time has not been found to significantly affect the measurement of ADMA in properly stored samples.

L-ADMA and L-Arginine quantitation

Maternal plasma ADMA and L-arginine concentrations were determined by high-pressure liquid chromatography (HPLC) as described by Teerlink et al.¹² In brief, samples were processed by solid phase extraction using Oasis MCX cation exchange SPE columns (30mg, 1 ml) (Waters, Milford, MA). N-mono-methyl L-arginine (NMMA) was used as an internal standard; 50 μL of plasma from patients were spiked with 50 μL of 1 $\mu\text{mol/L}$ NMMA, and 900 μL of phosphate buffered saline, pH 7.4. Extracted plasma samples were injected onto a prepared HPLC system and the amino acids were derivitized online using a Waters 717 plus autosampler. Separation of the amino acids was performed using a Symmetry C18 column (3.9×150 mm, 5 μm particle size) with a Sentry Guard column (3.9×20 mm, 5 μm particle size). Standard curves were generated using known quantities of ADMA, SDMA, L-arginine, and NMMA spiked into pooled control plasma. All samples were measured in duplicate and the

average was used for statistical analysis. The lower limit of detection is 0.01 $\mu\text{mol/L}$, with an inter-assay variability of < 5%.

Statistical Analysis

Results are presented as the mean $\pm$ SD. Statview 5.0.1 software (Cary, NC) was used to analyze the data. The values of ADMA, L-arginine and the L-arginine/ADMA ratio were found to be normally distributed using the K-S Normality test. Data was analyzed by ANOVA with Bonferroni/Dunn post-hoc testing and statistical significance was accepted at $p < 0.05$. Differences in ADMA, L-arginine and the L-arginine/ADMA ratio between mid-pregnancy and delivery in individual subjects were analyzed by paired t-test and statistical significance was accepted at $p < 0.05$. Self reported smoking status was lower in the women who developed preeclampsia (13%) compared to the uncomplicated controls (20%) and the women with SGA infants (25%). There was no difference in ADMA concentration between smokers and nonsmokers in the uncomplicated controls and SGA cases ($p = 0.65$). Similarly, 13% of the women with preeclampsia, 33% of the SGA women and 45% of the uncomplicated controls were in labor at the time of sample collection in the third trimester. There was no difference in ADMA concentration between women in labor and women not in labor in the uncomplicated controls and SGA cases ($p = 0.25$).

Results

Demographic characteristics of the three groups of study subjects are shown in Table 1. There was no significant difference in maternal age between the study subjects. However, maternal pre-pregnancy body-mass index was greater in women with preeclampsia and women with SGA infants compared to the uncomplicated controls. The average systolic blood pressure before 20 weeks gestation was higher in subjects who later developed preeclampsia compared to the other two study groups. By definition, women with preeclampsia had significantly higher systolic and diastolic blood pressures at delivery when compared to the other study groups. The average gestational age at delivery was earlier in the women with preeclampsia compared to the other study groups. Infant birth weight was lower in the preeclampsia and SGA without preeclampsia groups compared to the infants of control pregnancies, however the average infant birth weight centile was statistically lower only in the SGA infants of women without preeclampsia compared to the infants of control pregnancies and infants of women with preeclampsia. There was no difference between groups in the sample collection time at mid-gestation (Table 2).

In the samples collected near mid-pregnancy, mean maternal plasma ADMA concentrations were significantly higher in women who later developed preeclampsia compared to control pregnant women ($p < 0.01$) and the women with SGA infants ($p < 0.01$) (Table 2, Figure). In addition, plasma L-arginine concentrations were significantly lower in women who later developed preeclampsia compared to the control pregnant women ($p < 0.05$), but not women with SGA infants (Table 2), while L-arginine/ADMA ratio was significantly lower in the women who later developed preeclampsia compared to both groups (all $p < 0.01$) (Table 2).

At delivery, plasma ADMA concentrations were still significantly higher in women with preeclampsia compared to control pregnant women ($p < 0.05$) and the women with SGA infants ($p < 0.05$) (Table 2, Figure). The concentration of ADMA was not different from controls in women with SGA pregnancies. In contrast to samples obtained at mid-pregnancy, plasma L-arginine concentrations and L-arginine/ADMA ratios at delivery were similar in all study groups in the later samples (Table 2). In a paired analysis, there was a significant increase in plasma ADMA concentrations and a significant decrease in the L-arginine/ADMA ratios between the mid-pregnancy and delivery samples among all three study groups (all $p < 0.05$). However, there was no significant difference in the change in ADMA between the mid-

pregnancy and delivery samples among all the groups (controls= 0.0072 ± 0.0047 ; SGA pregnancies= 0.0056 ± 0.0034 ; and preeclampsia= $0.0057 \pm 0.0047 \mu\text{mol/L ADMA/week}$, $p=0.47$).

Similar to the entire group of women with SGA infants, plasma ADMA, L-arginine and the L-arginine/ADMA ratio in samples from mothers of infants with additional characteristics of IUGR (Table 3) were not different compared to values in control pregnant women at either mid-pregnancy or at delivery.

As noted above, BMI was greater in the women with preeclampsia and the women with SGA infants. However, ADMA concentrations were only higher in the women who developed preeclampsia. Furthermore, there was no significant difference in ADMA concentrations at mid-pregnancy between normal weight, BMI < 25 ($0.46 \pm 0.09 \mu\text{mol/L}$, $n=6$), and overweight and obese, BMI ≥ 25 , ($0.45 \pm 0.09 \mu\text{mol/L}$, $n=9$), preeclamptic women in this study ($p=0.76$).

Comment

In this study we have observed that the concentration of maternal plasma ADMA is significantly higher (all $p < 0.01$) in mid-pregnancy in women who later develop preeclampsia compared to women with an uncomplicated pregnancy or with SGA infants, and this difference persists at admission for delivery. Several studies reported elevated maternal plasma ADMA in women with preeclampsia.^{8–10, 13} Ellis et al. also found higher ADMA concentrations in preeclamptic women with manifest preeclampsia at 24 and 32 weeks gestation.¹³ The study by Savvidou et al. also reported higher maternal plasma ADMA at 23–25 weeks gestation in women with abnormal Dopplers who later developed preeclampsia, as well as higher ADMA values in women with abnormal Dopplers who later delivered growth restricted infants in the absence of preeclampsia and women with abnormal Dopplers with uncomplicated pregnancies. In contrast to our study, ADMA was not measured later in pregnancy in the study by Savvidou. One important difference and limitation of our study compared to the study by Savvidou is that we lack Doppler data on the majority of our subjects, and therefore we cannot make a direct comparison of these studies. The growth restricted infants in the study by Savvidou are likely more severely affected since these infants did evidence abnormal uterine artery Dopplers. Therefore, we have limited our discussion primarily to SGA infants. However, despite this limitation many of the SGA infants in our study had other evidence consistent with growth restriction, especially asymmetric growth. There may also be methodological differences in ADMA determinations between these two studies. The concentration of plasma ADMA in the Savvidou study was much higher in all groups than in most other published work (example: average ADMA = $0.81 \mu\text{mol/L}$ [0.45–1.08 IQR] among the subjects with normal outcomes and normal Dopplers compared to $0.49 \pm 0.08 \mu\text{mol/L}$ in control subjects in our and other studies).

Endothelial-dependent vascular dysfunction is a central pathophysiological feature of preeclampsia.² Endothelial dysfunction could be the result of impaired nitric oxide synthesis. Increasing evidence suggests that elevated concentrations of ADMA act as an endogenous inhibitor of NOS to contribute to endothelial-dependent vascular dysfunction in diabetes, hypertension, hypercholesterolemia, and obesity.^{7, 14, 15} In addition to preventing NO production by competitive inhibition of NOS, ADMA may promote the uncoupling of NOS enzymatic activity and convert NOS to a superoxide generator. This uncoupling activity of ADMA is proposed to further contribute to vascular dysfunction and may play a role in the pathophysiology of preeclampsia by serving as a source of vascular oxidative stress. Recently, there has been an increased interest in the role of angiogenic growth factors (VEGF and PlGF) and their soluble receptors (sFlt1, antiangiogenic) in the pathophysiology of preeclampsia.¹⁶ ADMA is another important molecule capable of affecting angiogenesis in pregnancy and preeclampsia. Specifically, angiogenesis is negatively affected by high ADMA concentrations

6, 17, and the angiogenic activity of growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and basic fibroblast growth factor (bFGF) are mediated through a nitric oxide dependent mechanism that is inhibited by ADMA.^{18, 19} ADMA is also reported to decrease the expression of VEGF in cultured endothelial cells, and therefore may affect not only growth factor activity but its production.²⁰ Therefore, it is interesting to speculate that there may be interactions between pro-angiogenic growth factors (VEGF and PIGF) and antiangiogenic factors such as ADMA as well as sFlt1 in early pregnancy that could influence the risk of preeclampsia.

The mechanisms responsible for high plasma concentrations of ADMA in women who later develop preeclampsia is not known. ADMA is derived from the catabolism of proteins that contain methylated arginine residues. When methylated proteins undergo catabolism, their methylated arginine residues are released into the maternal circulation. The major degradation pathway for ADMA is by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). DDAH has two isoforms: DDAH I and DDAH II. DDAH II is the primary isoform in endothelial cells and the placenta, and decreases in DDAH activity are thought to be a primary mechanism by which ADMA is increased. Importantly, oxidative stress is proposed to be a significant contributor to the pathophysiology of preeclampsia,²¹ and inhibits DDAH activity.²² Furthermore, ADMA concentrations, similar to other amino acids, are affected by renal function and ADMA concentrations decrease during pregnancy compared to nonpregnant women.⁹ Therefore, among women who develop preeclampsia, ADMA might be increased secondary to over-production of methylated arginine residues on proteins, the metabolism of ADMA by DDAH may be decreased, or high ADMA may be the result of underlying alterations in renal function.

Limitations of this study include the inability to definitively document IUGR, and therefore the results and discussion have primarily been limited to SGA. However, the documentation of IUGR is not easy, and it is quite clear that not all SGA infants are IUGR (and vice versa). To reduce confounding we have removed infants with evidence of intrauterine infection or chromosomal abnormalities. The gold standard for the determination of IUGR would likely be serial sonographic evidence of reduced fetal growth. In many studies umbilical artery Doppler is considered the sine qua non of IUGR. Despite this, in a study of infants >5th centile, more than half of infants with oligohydramnios, asymmetric head to abdominal growth, redistribution of blood flow to the middle cerebral artery and fetal intolerance of labor necessitating C-section did not evidence abnormal umbilical artery velocimetry.²³ Therefore, excluding IUGR infants based on the absence abnormal Doppler data may systematically exclude a relevant population of growth restricted infants, despite additional valid evidence of abnormal fetal growth. There are other limitations to our study including: lack of SDMA data, relatively small sample size, differences in gestational age at delivery, and differences in maternal BMI. It would have been useful to have SDMA data in this analysis since a greater ADMA/SDMA ratio may indicate impaired DDAH activity and this might have given us further insight into the cause of elevated ADMA in the women with preeclampsia. Despite the relatively small sample size, we still observed higher maternal plasma ADMA concentrations in mid-pregnancy in women who later developed preeclampsia. Although the gestational age at delivery in women with manifest preeclampsia was less than that of other groups, the increase in ADMA with advancing gestation makes this an unlikely explanation for the higher values, and of course is not relevant to higher concentrations in mid-pregnancy. Lastly, average BMI was elevated in preeclamptic women and women with SGA infants, and ADMA is higher in obese individuals.²⁴ However, there was no significant difference in ADMA concentrations between normal weight, and overweight and obese preeclamptic women in this study; and despite a higher BMI there was no difference in ADMA between the women with SGA infants who did not have preeclampsia and the control pregnant women. This is an interesting finding and may indicate that lean women who later develop preeclampsia are more similar to obese

women and obese women who develop preeclampsia. Given that 30% of the adult women in the US are clinically obese, this similarity may indicate one mechanism by which obesity contributes to the increased risk of preeclampsia.

In conclusion, we have observed that maternal plasma ADMA is elevated in mid-pregnancy and remains elevated at the time of delivery in women who develop preeclampsia compared to women with uncomplicated pregnancies and women with SGA infants without preeclampsia. ADMA plays a critical role in vascular function including: endothelial-dependent vasodilatation, angiogenesis and arteriogenesis; and these activities are all adversely affected in preeclampsia. In addition, several disease states and conditions are associated with high ADMA and vascular dysfunction including: hypertension, renal dysfunction, inflammation, diabetes, hypercholesterolemia, oxidative stress, and obesity; and all of these conditions are associated with an increased risk of preeclampsia. These data suggest that elevated ADMA may be a possible biological convergence of conditions that increase the risk of preeclampsia, and therefore increased ADMA may play an important role in the pathophysiology of preeclampsia. However, important questions remain to be answered including: what is the source of increased ADMA in preeclampsia and what is the relationship between ADMA and epidemiologic risk factors for preeclampsia? Lastly, although a few small studies of L-arginine therapy in pregnancy suggest benefit,²⁵ can pregnancy outcome be improved by L-arginine administration to overcome the competitive effects of ADMA upon NOS?

Acknowledgements

This project was supported by National Institutes of Health grant number P01-HD30367 and by funds received from the NIH/NCRR/GCRC Grant M01-RR00056

References

1. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists number 33, January 2002. Diagnosis and Management of Preeclampsia and Eclampsia. *Obstet Gynecol* 2002;99:159–161. [PubMed: 16175681]
2. Roberts JM. Objective evidence of endothelial dysfunction in preeclampsia. *American Journal of Kidney Diseases* 1999;33:992–994. [PubMed: 10328745]
3. Kingdom JCP, Kaufmann P. Oxygen and placental vascular development. *Hypoxia* 1999;vol 474
4. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. *American Journal of Physiology* 1997;272:R441–R463. [PubMed: 9124465]
5. Khalil RA, Granger JP. Vascular mechanisms of increased arterial pressure in preeclampsia: lessons from animal models. *American Journal of Physiology Regulatory Integrative & Comparative Physiology* 2002;283
6. Cooke JP. Does ADMA cause endothelial dysfunction? *Arteriosclerosis, Thrombosis & Vascular Biology* 2000;20:2032–2037.
7. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? *Circulation* 2004;109:1813–1818. [PubMed: 15096461]
8. Fickling SA, Williams D, Vallance P, Nussey SS, Whitley GS. Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and pre-eclampsia. *Lancet* 1993;342:242–243. [PubMed: 8100963]
9. Holden DP, Fickling SA, Whitley GS, Nussey SS. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *American Journal of Obstetrics & Gynecology* 1998;178:551–556. [PubMed: 9539525]
10. Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstetrica et Gynecologica Scandinavica* 1998;77:808–813. [PubMed: 9776593]

11. Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet* 2003;361:1511–1517. [PubMed: 12737861]
12. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Analytical Biochemistry* 2002;303:131–137. [PubMed: 11950212]
13. Ellis J, Wennerholm UB, Bengtsson A, Lilja H, Pettersson A, Sultan B, et al. Levels of dimethylarginines and cytokines in mild and severe preeclampsia. *Acta Obstetrica et Gynecologica Scandinavica* 2001;80:602–608. [PubMed: 11437716]
14. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arteriosclerosis, Thrombosis & Vascular Biology* 2003;23:1455–1459.
15. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation* 1998;98:1842–1847. [PubMed: 9799202]
16. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *New England Journal of Medicine* 2004;350:672–683. [PubMed: 14764923]
17. Cooke JP. NO and angiogenesis. *Atherosclerosis Supplements* 2003;4:53–60. [PubMed: 14664903]
18. Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, et al. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:2604–2609. [PubMed: 11226286]
19. Papapetropoulos A, Garcia-Cardena G, Madri JA, Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *Journal of Clinical Investigation* 1997;100:3131–3139. [PubMed: 9399960]
20. Smith CL, Birdsey GM, Anthony S, Arrigoni FI, Leiper JM, Vallance P. Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. *Biochemical & Biophysical Research Communications* 2003;308:984–989. [PubMed: 12927816]
21. Hubel CA. Oxidative stress and preeclampsia. *Fetal and Maternal Medicine Review* 1997;9:73–101.
22. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 1999;99:3092–3095. [PubMed: 10377069]
23. Hershkovitz R, Kingdom JC, Geary M, Rodeck CH. Fetal cerebral blood flow redistribution in late gestation: identification of compromise in small fetuses with normal umbilical artery Doppler. *Ultrasound in Obstetrics & Gynecology* 2000;15:209–212. [PubMed: 10846776]
24. Krzyzanowska K, Mittermayer F, Kopp HP, Wolzt M, Schernthaner G. Weight loss reduces circulating asymmetrical dimethylarginine concentrations in morbidly obese women. *Journal of Clinical Endocrinology & Metabolism* 2004;89:6277–6281. [PubMed: 15579789]
25. Rytlewski K, Olszanecki R, Korbut R, Zdebski Z. Effects of prolonged oral supplementation with l-arginine on blood pressure and nitric oxide synthesis in preeclampsia. *European Journal of Clinical Investigation* 2005;35:32–37. [PubMed: 15638817]

Figure.

Maternal plasma ADMA is greater at mid-pregnancy and at delivery in women who develop preeclampsia compared to control pregnant women and women with SGA infants. A. Scatter plot of maternal plasma ADMA average at mid-pregnancy. B. Scatter plot of pre-delivery maternal plasma ADMA. *: $p < 0.05$ and **: $p < 0.01$ compared to control pregnant women and women with SGA infants.

Table 1**Maternal and Newborn Demographics**

	Control pregnancy (n=31)	Pregnant women with SGA infants and without preeclampsia (n=12)	Preeclampsia (n=15)
Maternal age (years)	21.9±4.7	22.9±4.7	25.1±5.8
Maternal prepregnancy body mass index (kg/m ²)	21.9±4.2	25.5±6.1 [*]	28.0±5.9 [†]
Average blood pressure before 20 weeks gestation (mmHg)	108.8±8.1/67.1±6.1	109.1±9.3/67.3±5.8	119.3±8.7 ^{†§} /71.3±5.6
Average blood pressure at delivery (mmHg)	118.7±9.5/70.4±7.0	118.3±8.6/71.8±8.3	150.6±15.9 ^{†§} /90.2±6.2 ^{†§}
Maternal plasma uric acid at delivery (mg/dl)	4.8±1.2	4.8±0.7	6.8±1.2 ^{†§}
Gestational age at delivery (weeks) [range]	39.5±1.4 [36.7–41.7]	39.7±1.3 [37.0–42.1]	35.9±3.8 ^{†§} [26.6–39.0]
Infant birth weight (grams)	3255.2±486.4	2472.5±222.5 [†]	2478.6±853.7 [†]
Infant birth weight centile (%)	44.6±29.3	3.9±2.9 [†]	29.5±20.8

Data are mean±SD

^{*}
p<0.05[†]
p<0.01 compared to control pregnancy[‡]
p<0.05[§]
p<0.01 compared to women with SGA infants.

Table 2 ADMA concentrations are higher in mid-pregnancy and at delivery among women who later develop preeclampsia.

	Control pregnancy (n=31)		Pregnant women with SGA infants and without preeclampsia (n=12)		Preeclampsia (n=15)	
Gestational age at sampling (weeks) [range]	18.3±1.6 [14.1–21.1]	39.5±1.4 [36.7–41.7]	18.1±1.8 [14.3–20.6]	39.7±1.3 [37.0–42.1]	16.9±3.2 [8.7–21.0] [∅]	35.5±4.2 ^{‡§} [26.6–39.0]
Plasma ADMA (μmol/L)	0.34±0.08	0.49±0.08	0.33±0.06	0.45±0.08	0.45±0.09 ^{‡§}	0.55±0.07 ^{*‡}
Plasma L-Arginine (μmol/L)	49.5±17.2	36.3±17.9	45.5±13.3	35.2±14.8	37.7±10.9 [*]	35.4±13.9
L-Arginine/ADMA ratio	155.8±62.4	74.2±31.7	141.1±36.3	82.1±44.4	84.7±24.3 ^{‡§}	64.6±24.5

Data are mean±SD

^{*} p<0.05

[‡] p<0.01 compared to control pregnancy.

[#] p<0.05

[§] p<0.01 compared to women with SGA infants.

[∅] note only 2 samples collected before 14 weeks gestation and exclusion does not change the result.

Table 3

Clinical characteristics of SGA infants.

Subject number	Oligohydramnios	Umbilical Doppler	Cord blood erythropoietin	Birth weight centile (%)	Growth profile	Research classification
1	no	ND	normal	2.1	symmetric	SGA
2	no	normal	normal	<1.0	asymmetric	IUGR
3	no	normal	normal	2.6	symmetric	SGA
4	no	ND	elevated	7.5	symmetric	SGA
5	ND	ND	normal	2.9	asymmetric	IUGR
6	no	ND	normal	4.5	asymmetric	IUGR
7	no	ND	normal	<1.0	asymmetric	IUGR
8	no	ND	elevated	6.4	asymmetric	IUGR
9	no	abnormal	normal	2.3	asymmetric	IUGR
10	no	normal	normal	<1.0	asymmetric	IUGR
11	no	normal	normal	9.7	symmetric	SGA
12	yes	ND	elevated	<1.0	asymmetric	IUGR

ND = not determined