The Journal of Maternal-Fetal and Neonatal Medicine, 2012; 25(8): 1311–1315

ISSN 1476-7058 print/ISSN 1476-4954 online
DOI: 10.3109/14767058.2011.632037

informa
healthcare

Levels of asymmetric dimethylarginine throughout normal pregnancy and in pregnancies complicated with preeclampsia or had a small for gestational age baby

Demetrios Rizos[1], Makarios Eleftheriades[2], Emmanuel Batakis[1], Myrto Rizou[1], Alexander Haliassos[3], Demetrios Hassiakos[4] & Demetrios Botsis[4]

[1]Hormone Laboratory, 2nd Dept of Obstetrics & Gynecology, Medical School, University of Athens, Aretaieio Hospital, Athens, Greece, [2]EmbryoCare, Fetal Medicine Unit, Athens, Greece, [3]Diamedica, Clinical Chemistry Laboratory, Athens, Greece, and [4]2nd Dept of Obstetrics & Gynecology, Medical School, University of Athens, Aretaieio Hospital, Athens, Greece

***Objective*: The aim of this study was to investigate maternal asymmetric dimethylarginine (ADMA) concentrations at the three trimesters of pregnancy in uncomplicated pregnancies and in women who developed preeclampsia or had small for gestational age infants (SGA) without preeclampsia. *Methods*: ADMA concentrations were retrospectively determined in the first, second and third trimester of pregnancy in 41 uncomplicated pregnancies, 10 pregnancies complicated with preeclampsia and 14 pregnancies that delivered a SGA baby. ADMA was measured with an ELISA kit. *Results*: Mean (±SD) concentrations of ADMA (μmol/L) in uncomplicated l pregnancies were: 0.51 ± 0.14; 0.52 ± 0.13; 0.58 ± 0.16 in the three trimesters, respectively. ADMA concentrations in SGA pregnancies were significantly lower in each trimester compared to uncomplicated pregnancies: (0.40 ± 0.10, $p = 0.005$ 1st trim; 0.42 ± 0.10, $p = 0.007$ 2nd trim; 0.45 ± 0.10, $p = 0.007$ 3rd trim). Although pregnancies that developed preeclampsia had higher ADMA concentration in all trimesters compared to uncomplicated pregnancies (0.58 ± 0.10; 0.63 ± 0.14; 0.68 ± 0.11), the difference was statistically significant only in the 2nd trimester ($p = 0.02$). *Conclusions*: Maternal serum ADMA concentration tends to increase during normal pregnancy. Pregnancies with SGA infants had significantly lower ADMA levels in all trimesters of pregnancy. ADMA concentrations in the 2nd trimester was significantly elevated in pregnancies that later developed preeclampsia.**

Keywords: ADMA, pregnancy, preeclampsia, SGA

Introduction

Preeclampsia is a systematic disease of pregnancy characterized by hypertension and proteinouria developing after 20th week of pregnancy. The term "preeclampsia" comes from the Hellenic word "έκλαμψη-eklampsi" that means "lightning" or "sudden flashing" and it is thought that the term refers to the bright flashes that women sometimes see during their preeclamptic seizures. It is estimated that preeclampsia affects 3–5% of all pregnancies worldwide and is one of the most frequently encountered medical complications of pregnancy [1]. In Greece, the estimated incidence of preeclampsia (2.8%) is comparable to the worldwide incidence [2].

The pathogenesis of preeclampsia is still not fully elucidated despite the intense research on the field [3]. Abnormal cytotrophoblastic invasion of spiral arterioles, reduced or insufficient placental perfusion and the subsequent maternal endothelial dysfunction seems to be central to the pathophysiology of the syndrome [4,5]. Studies in the early 1990s started to explore the role of nitric oxide and the endogenous inhibitors of nitric oxide synthase (NOS). Asymmetric dimethylarginine (ADMA) and its regioisomer, symmetric dimethylarginine (SDMA), are generated by the degradation of methylated proteins due to protein arginine methyltransferase-1 activity and subsequent physiological protein turnover. ADMA, but not SDMA, is an endogenous inhibitor of endothelial NOS [6]. ADMA is eliminated from the circulation either by renal excretion or by degradation to dimethylamine and citrulline by the enzyme dimethylarginine dimethylaminohydrolase (DDAH [7]). ADMA inhibits endothelial NOS by competitive displacement of the physiological substrate, L-arginine, from the enzyme [8]. The inhibition leads to decreased NO production in the endothelium of vessel walls and thus, when ADMA levels are elevated, endothelial dysfunction may result [9].

In several studies, maternal serum ADMA concentration in the 3rd trimester of pregnancy has been found to be higher in women with preeclampsia [10–13]. Other studies reported elevated ADMA concentration in the 2nd trimester of pregnancy [14,15] but not in the 1st trimester [16] in women who later developed preeclampsia. In the study of Speer et al. [15], ADMA concentration was not different from uncomplicated pregnancies in women with small for gestational age babies (SGA), either in the 3rd or in the 2nd trimester. However, in the study of Speer et al., both babies with symmetric growth profile and intrauterine growth restriction (IUGR) were included in the SGA group. In a recent study, ADMA was found to be elevated in the 3rd trimester not only in preeclamptic pregnancies but also in normotensive pregnancies complicated by isolated IUGR [17].

The aim of this study was to investigate maternal ADMA concentrations at all three trimesters of pregnancy in normal uncomplicated pregnancies, in women who developed preeclampsia and in women who gave birth to SGA infants without preeclampsia.

Correspondence: Prof. Demetrios Rizos, PhD, EurClinChem, Assist. Prof. of Clinical Chemistry, Hormone Laboratory, 2nd Department of Obstetrics & Gynecology, Medical School, University of Athens, Aretaieio Hospital, 76, Vas. Sofias ave, 115 28, Athens, Greece. Tel/Fax: +30 210 7286229. E-mail: drizos@aretaieio.uoa.gr

Methods

The pregnant women included in the present study were selected from a pool of pregnancies that are recruited for a wider ongoing investigation project on biochemical and ultrasound markers for the prognosis of adverse pregnancy outcomes. Pregnancies are recruited in the study in the first trimester of pregnancy as they appear for the routine prenatal screening for chromosomal abnormalities (ultrasonographic measurement of nuchal translucency and maternal serum determination of PAPP-A and Fb-hCG), in the 2nd Dept of Obstetrics & Gynecology of Medical School of Athens University in Aretaieio Hospital and in a private setting of obstetric care (EmbryoCare, Fetal Medicine Unit, Athens). All women gave their informed consent for their participation in the study and Hospital's ethics committee approved the protocol.

The study included 41 uncomplicated pregnancies, 10 pregnancies that developed preeclampsia and 14 pregnancies that gave birth to SGA infants. Pregnancies of all three groups were singleton. Pregnancy complications, outcomes, as well as characteristics of the newborns, were judged and copied from medical records.

Preeclampsia was defined as hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg) and proteinouria (protein concentration ≥0.3 g per 24-hour urine collection) developed after 20th week of pregnancy. Blood pressures measured in the 3rd trimester were averages of two or more measurements during the weeks before delivery. Out of the 10 pregnancies with preeclampsia, 4 delivered before 35th week of gestation and the remaining 6 after 35th week of gestation. Clinical characteristics of the women who developed preeclampsia and their infants are shown in Table I.

SGA infants were defined by infant birth weight ≤10th percentile of the corresponding curves after adjustment for gestational age and gender [18]. The SGA infants were born from pregnancies with no sighs of pathology (hypertension, preeclampsia, diabetes). Their intrauterine development was recorded at the same percentile since 2nd trimester and they had normal amniotic fluid and umbilical artery Doopler in the 3rd trimester. Demographic and clinical characteristics of the studied pregnancies as well as their newborns are shown in Table II.

Blood samples were collected prospectively in the 1st (11th to 14th week), 2nd (20th to 24th week) and 3rd (28th to 35th week) trimester of pregnancy as the women were coming for their appointments for the routine ultrasound in each trimester. Serum samples were aliquoted and stored at −35°C until analysis. Samples were collected from December 2007 to April 2009. Ultrasonographic and Doppler data were also collected in each trimester.

Table I. Demographic and clinical characteristics of the 10 pregnancies complicated with preeclampsia and their newborns.

No	Age (years)	BMI at 1st trimester	Mode of delivery	Gestational age at delivery (week + days)	Sex	Birth weight (g)	Birth weight percentile (%)	Uterine artery Doppler PI			Average blood pressure at 3rd trimester
								1st trimester	2nd trimester	3rd trimester	
1	28.2	35.7	CS	32 + 3	Boy	1665	14.7	1.72	1.14	–	150/100
2	27.6	22.8	CS	37 + 2	Boy	2010	0.3	–	1.75	–	140/90
3	38.6	32.0	CS	38 + 4	Girl	3800	90.6	1.47	0.88	0.62	140/84
4	32.0	23.1	CS	38 + 5	Boy	3240	40.8	1.44	0.84	0.86	161/72
5	34.2	33.3	CS	31 + 1	Boy	1040	1.7	1.21	0.95	1.12	140/82
6	36.6	23.1	VD	38 + 2	Girl	2260	0.8	2.09	1.98	1.11	131/84
7	38.1	23.9	CS	37 + 4	Girl	2760	23.8	1.11	0.99	0.62	168/87
8	34.7	28.5	VD	38 + 2	Girl	3270	53.8	1.6	0.96	0.86	133/97
9	34.2	30.0	CS	35 + 0	Girl	1315	0.3	1.81	1.14	1.33	142/89
10	35.8	–	CS	30 + 2	Boy	930	2.3	2.55	2.0	2.04	142/78

BMI, body mass index; CS, cesarean section; VD, vaginal delivery; PI, pulsatility index.

Table II. Demographic and clinical characteristics of the studied pregnancies and their newborns.

Characteristics	Uncomplicated pregnancies	Pregnancies complicated with preeclampsia	Pregnancies with SGA infants
N	41	10	14
Maternal age at 1st trimester (y)	32.6 ± 3.9	34.0 ± 3.7	30.7 ± 4.7
Parity (% of nulliparus)	44	80	50
Maternal BMI at 1st trimester (Kg/m^2)	24.2 ± 4.0	28.0 ± 5.0[a]	24.6 ± 4.7
Doppler PI of uterine artery			
1st trimester	1.58 ± 0.37	1.66 ± 0.45	1.72 ± 0.54
2nd trimester	0.92 ± 0.27	1.26 ± 0.46[b]	1.0 ± 0.29
3rd trimester	0.75 ± 0.14	1.07 ± 0.46[b]	0.80 ± 0.14
Mean blood pressure at 3rd trimester			
Systolic (mm Hg)	111 ± 9	145 ± 12[c]	105 ± 9
Diastolic (mm Hg)	68 ± 7	87 ± 8[c]	65 ± 7
Gestational age at delivery (weeks)	39.2 ± 1.0	35.7 ± 3.3	39.1 ± 0.8
Birthweight (g)	3340 ± 334	2220 ± 1008[d]	2663 ± 223[d]
Birthweight percentile (median, range)	40 (9–97)	9 (1–91)	6 (1–10)[d]

[a] $p < 0.02$ compared to uncomplicated pregnancies.
[b] $p < 0.005$ compared to uncomplicated pregnancies.
[c] $p < 0.001$ compared to uncomplicated pregnancies and SGA pregnancies.
[d] $p < 0.001$ compared to uncomplicated pregnancies.
BMI, body mass index; PI, pulsatility index.

ADMA was measured with the commercial kit: ADMA-ELISA (DLD, Hamburg, Germany). The method includes a step in which ADMA in samples is acylated, before the standard competitive ELISA procedure. The sensitivity of the method is 0.05 μmol/L and the total CV (%) ranges from 5.7 to 10.3 according to ADMA concentration [19].

The statistical software IBM SPSS statistics version 19 (IBM Corporation, Somers, NY 10589, USA) was used for data analysis. ADMA concentrations of uncomplicated pregnancies in the three trimesters were found to be normally distributed using Shapiro–Wilk test. Comparisons of ADMA concentrations between both, trimesters and patients groups, were done by means of analysis of variance (ANOVA) and Bonferroni test was used for multiple comparisons. Comparisons of quantitative data were done by *t*-test and correlations were evaluated using Pearson correlation coefficient. Comparisons of birth weight percentiles were done by non-parametric Mann–Whitney test. Data are presented as mean ± SD and range in parenthesis. A probability level of less or equal to 0.05 was considered significant.

Results

There was no statistically significant difference in maternal age between the studied groups (Table II). However, maternal body mass index (BMI), calculated in 1st trimester, was found greater in women who developed preeclampsia ($p < 0.02$) compared to women with uncomplicated pregnancies. Mean pulsatility index (PI) estimated by uterine artery Doppler ultrasound was found significantly elevated in women with preeclampsia compared to uncomplicated pregnancies both in 2nd ($p < 0.005$) and in 3rd trimester ($p < 0.001$) but not in the 1st trimester. PI didn't differ between uncomplicated pregnancies and pregnancies with SGA fetuses in either trimester. Women with preeclampsia had significantly higher average blood pressure in 3rd trimester ($p < 0.001$), as it is predictable, compared to women of the two other groups. The mean gestational age at delivery didn't differ between uncomplicated pregnancies and pregnancies with SGA infants, but it was more than 3 weeks earlier in pregnancies with preeclampsia. Average birth weight of the babies from women who developed preeclampsia as well as from women with SGA was significantly lower ($p < 0.001$) than that of the babies from uncomplicated pregnancies. Median birth weight percentile of SGA babies was, by definition, significantly lower ($p < 0.001$; Mann–Whitney test) than the median birth weight percentile of babies from uncomplicated pregnancies. The median birth weight percentile of babies born by preeclamptic women was also lower ($p = 0.05$; Mann–Whitney test) from that of uncomplicated pregnancies.

ADMA concentrations (μmol/L) in uncomplicated pregnancies, pregnancies that developed preeclampsia and pregnancies with SGA infants are presented in Table III. There was no significant difference in gestational age at the time of sampling between the three studied groups in either trimester.

Mean ADMA concentration in uncomplicated pregnancies increased from 0.51 ± 0.1 μmol/L in the 1st trimester to 0.52 ± 0.13 μmol/L in the 2nd trimester and 0.58 ± 0.16 μmol/L in the 3rd trimester. The difference between 1st and 3rd trimester was statistically significant ($p = 0.04$).

In the 1st trimester, ADMA concentration was significantly lower in pregnancies with SGA fetuses (0.40 ± 0.10 μmol/L) than that in uncomplicated pregnancies ($p = 0.005$) and in pregnancies with preeclampsia (0.58 ± 0.10 μmol/L; $p < 0.001$). The difference between uncomplicated pregnancies and pregnancies with preeclampsia was not found statistically significant.

In the 2nd trimester, ADMA was found significantly higher in pregnancy with preeclampsia (0.63 ± 0.14 μmol/L; $p = 0.02$) and significantly lower in pregnancies with SGA infants (0.42 ± 0.10 μmol/L; $p = 0.007$) compared to uncomplicated pregnancies.

In the 3rd trimester, ADMA concentration was again significantly lower in pregnancies with SGA infants (0.45 ± 0.10 μmol/L) than that in uncomplicated pregnancies ($p = 0.007$). The difference between uncomplicated pregnancies and pregnancies with preeclampsia (0.68 ± 0.11 μmol/L) was not found to be statistically significant ($p = 0.12$).

ADMA levels showed the same pattern of increase throughout pregnancy in both uncomplicated pregnancies and pregnancies with SGA infants. (Two-way ANOVA; $p = 0.9$).

In uncomplicated pregnancies, birth weight of the newborns was marginally positively correlated only with ADMA concentration of the 2nd trimester ($r = 0.29$; $p = 0.068$). When the uncomplicated and the SGA pregnancies were merged ($n = 55$), birth weight was significantly correlated with ADMA concentration of the 1st trimester ($r = 0.33$; $p = 0.014$) and 2nd trimester ($r = 0.38$; $p = 0.004$) but not of the 3rd trimester ($r = 0.22$; $p = 0.12$).

In uncomplicated pregnancies, a strong correlation was found between ADMA concentrations of the 1st and the 2nd trimester ($r = 0.73$; $p < 0.001$), 1st and 3rd trimester ($r = 0.64$; $p < 0.001$) and 2nd and 3rd trimester ($r = 0.71$; $p < 0.001$). The correlations were even stronger when we included pregnancies with SGA ($n = 55$) (1st–2nd trimester: $r = 0.76$; 1st–3rd trimester: $r = 0.71$; 2nd–3rd trimester: $r = 0.74$).

Table III. Mean (±SD, range) ADMA concentration (μmol/L) of the three studied groups in the three trimesters of pregnancy.

	Uncomplicated pregnancies	Pregnancies complicated with preeclampsia	Pregnancies with SGA infants
N	41	10	14
1st trimester 1			
GA at sampling (weeks)	12.6 ± 0.5 (11.7–13.9)	12.4 ± 0.3 (12.0–13.0)	2.7 ± 0.5 (12.0–13.7)
ADMA	0.51 ± 0.14 (0.23–0.84)	0.58 ± 0.10 (0.42–0.78)	0.40 ± 0.10 (0.25–0.57)[a]
2nd trimester			
GA at sampling (weeks)	21.8 ± 1.6 (19.7–25.9)	21.6 ± 1.1 (20.3–23.4)	22.0 ± 1.4 (21.0–25.9)
ADMA	0.52 ± 0.13 (0.32–0.74)	0.63 ± 0.14 (0.51–0.99)[b]	0.42 ± 0.10 (0.32–0.65)[c]
3rd trimester			
GA at sampling (weeks)	31.8 ± 1.4 (28.1–36.1)	31.8 ± 2.1 (31.6–34.0)	31.7 ± 1.0 (30.3–33.0)
ADMA	0.58 ± 0.16 (0.27–0.86)[d]	0.68 ± 0.11 (0.53–0.84)	0.45 ± 0.10 (0.33–0.62)[c]

[a] $p < 0.005$ compared to uncomplicated pregnancies.
[b] $p < 0.02$ compared to uncomplicated pregnancies.
[c] $p < 0.007$ compared to uncomplicated pregnancies.
[d] $p < 0.04$ compared to 1st trimester.
GA, gestational age.

Discussion

In the present study, we measured ADMA concentration in the 1st (11th to 14th week), 2nd (20th to 24th week) and 3rd (28th to 35th week) trimester of pregnancy in uncomplicated pregnancies as well as in pregnancies complicated with preeclampsia and in pregnancies who gave birth to SGA infants.

We found an increase trend of ADMA concentration in uncomplicated pregnancies along with gestational age. The difference between 1st and 3rd trimester was statistically significant. Our data are very similar to that of Holden et al. [11] who also found an increase trend of ADMA concentration from the 1st (0.40 ± 0.15 μmol/L) to the 2nd (0.52 ± 0.20 μmol/L) and the 3rd trimester (0.56 ± 23 μmol/L) in normotensive pregnancies. The authors reported a statistically significant difference between 1st and 2nd trimester. In contrast, Maeda et al. [20] didn't notice differences in ADMA between 1st and 3rd trimester. The observed increase of ADMA concentration as the pregnancy advances may be a physiological contribution to preparation of the uterine muscle fibers to higher contractile activity that is necessary during delivery by antagonizing NO induced uterine relaxation [9]. On the other hand, the well documented reduction of ADMA during early pregnancy compared to non pregnant women [7,21], and the consequent increase in NO may be one of the possible mechanisms leading to undisturbed placental perfusion and allowing normal growth of the fetus.

Our results, regarding the elevated ADMA concentration during 2nd trimester of pregnancies that later developed preeclampsia compared to uncomplicated pregnancies, are in agreement with several other studies [14,15]. As in the study of Savvidou et al., we also found elevated Doppler PI, together with elevated ADMA concentration in pregnancies that later developed preeclampsia. Using a logistic regression model, both Doppler PI and ADMA concentration in the 2nd trimester were statistically significant independent predictors of pregnancy outcome (uncomplicated or preeclampsia) and together with BMI, they could predict preeclampsia with 67% sensitivity for 93% specificity. These performance characteristics of the combination of ADMA with Doppler PI and BMI for the prediction of preeclampsia are comparable with the characteristics of other combinations of biochemical and ultrasound markers in the 2nd trimester [22]. May be ADMA has a role in the panel of the most potent biochemical markers in the 2nd trimester which, in combination with ultrasound markers could offer an efficient early prediction of preeclampsia.

In our study, we didn't find a statistically significant increase of ADMA concentration during 3rd trimester, in pregnancies with preeclampsia. These findings are in disagreement with many studies [11–13,15,17] that have found elevated ADMA values in preeclamptic pregnancies in the 3rd trimester. In contrast, data from Colombian women [23] didn't show significant difference between normal and preeclamptic pregnancies. A likely explanation of our findings could be the small number of samples from preeclamptic women in the 3rd trimester (7 instead of 10 in the first two trimesters). Moreover, two of the three missing samples in the 3rd trimester are from pregnancies that delivered very early (Table I, cases 1 and 5) and it's known [24] that pregnancies with severe preeclampsia have more clear elevated ADMA concentrations.

In the present study, we found significantly reduced ADMA concentrations in pregnancies with SGA infants compared to uncomplicated pregnancies in all trimesters. To our knowledge, this is a novel finding and in contrast to previous results. In the study of Speer [15], ADMA concentration in pregnancies with SGA infants was not found significantly different from the uncomplicated pregnancies in the 2nd and in the 3rd trimester. However, Speer and coworkers in their study, in the group of "SGA" pregnancies included 8 pregnancies with IUGR infants and only four pregnancies with normal SGA fetuses. In our study, all 14 SGA newborns were normal with an apparently uncomplicated pregnancy follow-up. Their ultrasound assessment was normal in all three trimesters, their growth pattern was stable and their low birth weight could rather be attributed to genetic factors. Since it was recently reported that pregnancies with IUGR and without signs of preeclampsia had elevated ADMA concentrations [17], the ADMA concentration in the "SGA" group of Speer's study could be an average of high concentrations in IUGR pregnancies and low concentrations in pregnancies with normal SGA infants giving a compromise mean near that of uncomplicated pregnancies. Interestingly, in very recently published study [25] ADMA levels were found reduced in young adults born preterm at extremely low birth weight compared to healthy adults born at term. Moreover, in our study ADMA concentrations in 1st and in the 2nd trimester showed a well positive correlation with birth weight in uncomplicated together with SGA pregnancies. This finding may imply that factors involved in prenatal growth acquisition and birth weight determination may also affect ADMA concentration and is in accordance with our study's finding for lower ADMA levels in SGA pregnancies. It's likely that as the pregnancy proceeds, more than one factor or mechanisms with different potency in each trimester influence ADMA concentrations. Since, as we observed in the present study, the rate of increase of ADMA levels with the progress of gestation in uncomplicated and SGA pregnancies seems to be the same, the SGA pregnancies could be considered as uncomplicated pregnancies rather than pathological ones. If this is true, the inclusion of SGA pregnancies with the uncomplicated ones decreases the mean ADMA levels in that population affecting their difference with preeclamptic pregnancies. Thus, the mean ADMA concentration of this unified group ($n = 55$) in each trimester is 0.48 ± 0.14 μmol/L in the 1st; 0.50 ± 0.13 μmol/L in the 2nd and 0.54 ± 0.16 μmol/L in the 3rd trimester, respectively. If we use these concentrations for comparison with the corresponding concentrations of preeclamptic pregnancies in each trimester, then ADMA in preeclamptic pregnancies is significantly higher not only in the 1st ($p = 0.02$) and the 2nd ($p = 0.004$) trimester but also in the 3rd trimester ($p = 0.03$), in agreement with the majority of the studies mentioned above.

The findings of our study may indicate a complicated mechanism of ADMA influence on preeclampsia, different in each trimester. In the 1st and 2nd trimester of a preeclamptic pregnancy, oxidative stress caused by the ischemic placenta, the result of the ongoing impaired pseudovasculogenesis, could be responsible for the elevated ADMA levels through the reduction of DDAH activity [7] which is known to be very sensitive to oxidative stress [26]. In the 3rd trimester, the impaired renal function due to established endothelial damages may represent an additional mechanism which also contributes to the elevated ADMA levels in preeclamptic pregnancies [27].

Our study has a number of limitations. These include (1) the small number of pregnancies with preeclampsia, and especially the small number of samples from preeclamptic pregnancies in the 3rd trimester of pregnancy (2) missing data on pregnancies with IUGR infants without preeclampsia. These data could be helpful to clarify the difference in ADMA concentration between IUGR and normal SGA infants (3) missing data on women's BMI

in the 2nd and the 3rd trimester of pregnancy which would help to reveal the relation between ADMA and BMI and their impact on the development of preeclampsia.

In conclusion, maternal serum ADMA concentration tends to increase from the 1st to the 3rd trimester of normal pregnancy. We have found significantly elevated ADMA concentrations in the 2nd trimester, in pregnancies that later developed preeclampsia. ADMA measurements in the 2nd trimester of pregnancy could be used as an early marker for the prediction of preeclampsia together with Doppler ultrasound and maternal BMI. An important finding of our study is that pregnancies that gave birth to SGA infants had significantly lower ADMA levels in the three trimesters of pregnancy. ADMA concentration in 1st and in the 2nd trimester correlates positively with birth weight in uncomplicated together with SGA pregnancies. ADMA concentration in preeclamptic pregnancies is significantly higher in all trimesters compared to the concentration in uncomplicated together with SGA pregnancies.

***Declaration of Interest*:** Authors declare no conflict of interest.

References

1. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. *Int J Gynaecol Obstet* 2002;77:67–75.
2. Roussos D, Panidis D, Tsimas V, Kourtis A, Mamopoulos M. Preeclampsia-eclampsia in Greece. *Hellen Obstet Gynacol* 2003; 15:37–43.
3. Solomon CG, Seely EW. Preeclampsia – searching for the cause. *N Engl J Med* 2004;350:641–642.
4. Roberts JM. Objective evidence of endothelial dysfunction in preeclampsia. *Am J Kidney Dis* 1999;33:992–997.
5. Davison JM, Homuth V, Jeyabalan A, Conrad KP, Karumanchi SA, Quaggin S, Dechend R, Luft FC. New aspects in the pathophysiology of preeclampsia. *J Am Soc Nephrol* 2004;15:2440–2448.
6. Böger RH. Association of asymmetric dimethylarginine and endothelial dysfunction. *Clin Chem Lab Med* 2003;41:1467–1472.
7. Slaghekke F, Dekker G, Jeffries B. Endogenous inhibitors of nitric oxide and preeclampsia: A review. *J Matern Fetal Neonatal Med* 2006;19:447–452.
8. Böger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. *J Nutr* 2004;134:2842–2847.
9. Böger RH, Diemert A, Schwedhelm E, Lüneburg N, Maas R, Hecher K. The role of nitric oxide synthase inhibition by asymmetric dimethylarginine in the pathophysiology of preeclampsia. *Gynecol Obstet Invest* 2010;69:1–13.
10. 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.
11. 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. *Am J Obstet Gynecol* 1998;178:551–556.
12. Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet Gynecol Scand* 1998;77:808–813.
13. Ellis J, Wennerholm UB, Bengtsson A, Lilja H, Pettersson A, Sultan B, Wennergren M, Hagberg H. Levels of dimethylarginines and cytokines in mild and severe preeclampsia. *Acta Obstet Gynecol Scand* 2001;80:602–608.
14. Savvidou MD, Hingorani AD, Tsikas D, Frölich 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.
15. Speer PD, Powers RW, Frank MP, Harger G, Markovic N, Roberts JM. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants. *Am J Obstet Gynecol* 2008;198:112–117.
16. Prefumo F, Thilaganathan B, Whitley GS. First-trimester uterine artery resistance and maternal serum concentration of asymmetric dimethylarginine. *Ultrasound Obstet Gynecol* 2008;31:153–157.
17. Laskowska M, Laskowska K, Leszczynska-Gorzelak B, Oleszczuk J. Asymmetric dimethylarginine in normotensive pregnant women with isolated fetal intrauterine growth restriction: A comparison with preeclamptic women with and without intrauterine growth restriction. *J Matern Fetal Neonatal Med* 2011;24:936–942.
18. Bonellie S, Chalmers J, Gray R, Greer I, Jarvis S, Williams C. Centile charts for birthweight for gestational age for Scottish singleton births. *BMC Pregnancy Childbirth* 2008;8:5.
19. Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, Böger RH. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. *Clin Chem Lab Med* 2004;42:1377–1383.
20. Maeda T, Yoshimura T, Okamura H. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, in maternal and fetal circulation. *J Soc Gynecol Investig* 2003;10:2–4.
21. Valtonen P, Punnonen K, Saarelainen H, Heiskanen N, Raitakari OT, Viikari JS, Lyyra-Laitinen T, et al. Maternal serum ADMA is not associated with proinflammatory cytokines or C-reactive protein during normal pregnancy. *Cytokine* 2009;46:216–221.
22. Giguère Y, Charland M, Bujold E, Bernard N, Grenier S, Rousseau F, Lafond J, et al. Combining biochemical and ultrasonographic markers in predicting preeclampsia: A systematic review. *Clin Chem* 2010;56:361–375.
23. Maas R, Böger RH, Schwedhelm E, Casas JP, López-Jaramillo P, Serrano N, Díaz LA. Plasma concentrations of asymmetric dimethylarginine (ADMA) in Colombian women with pre-eclampsia. *JAMA* 2004;291: 823–824.
24. Mao D, Che J, Li K, Han S, Yue Q, Zhu L, Zhang W, Li L. Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia. *Arch Gynecol Obstet* 2010;282:371–375.
25. Bassareo PP, Puddu M, Flore G, Deidda M, Manconi E, Melis A, Fanos V, Mercuro G. Could ADMA levels in young adults born preterm predict an early endothelial dysfunction? *Int J Cardiol* 2011; (in press).
26. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, Tsao PS, et al. Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: Genetic and physiological evidence. *Circulation* 2003;108:3042–3047.
27. Siroen MP, Teerlink T, Bolte AC, van Elburg RM, Richir MC, Nijveldt RJ, van der Hoven B, van Leeuwen PA. No compensatory upregulation of placental dimethylarginine dimethylaminohydrolase activity in preeclampsia. *Gynecol Obstet Invest* 2006;62:7–13.