

# Asymmetric Dimethylarginine in the Maternal and Fetal Circulation in Preeclampsia

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**ABSTRACT:** Preeclampsia is a leading cause of intrauterine growth restriction and preterm birth. Endothelial dysfunction is the common final pathway leading to clinical signs of preeclampsia including hypertension and proteinuria. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NOS and induces endothelial dysfunction by reversibly inhibiting NO production from L-arginine. The purpose of this study was to investigate maternal and fetal concentrations of ADMA, L-arginine, and symmetric dimethylarginine (SDMA). Women with preeclampsia ( $n = 47$ ) and controls ( $n = 51$ ) who gave birth by cesarean section were included in the study. We analyzed the maternal plasma and umbilical vein and artery plasma. We found that not only maternal concentrations of ADMA and SDMA but also L-arginine were significantly higher in women with preeclampsia than in controls. In fetal samples, only SDMA concentrations were higher in the preeclampsia group than in controls. The median ADMA concentration was three times higher in the fetal circulation than in the maternal circulation, but there was no difference between the preeclampsia group and the control group, and the veno-arterial gradient indicated that the placenta was the source of ADMA. (*Pediatr Res* 66: 411–415, 2009)

Preeclampsia is a common pregnancy complication associated with increased risk of preterm birth and fetal growth restriction (1). The exact etiology of preeclampsia is not known, and hence no definitive preventative measures or treatments are available.

Endothelial dysfunction is the common final pathway leading to the clinical signs of preeclampsia including hypertension and proteinuria. It is not known to what extent endothelial dysfunction also affects the infant. Increased concentrations of biomarkers for preeclampsia in the maternal circulation are usually not reflected in the infant. Previously, our group has shown that newborns from preeclamptic pregnancies did not differ from those born after uncomplicated pregnancies with respect to circulating biomarkers of inflammation (CRP and calprotectin) (2), and oxidative stress (3), but newborns from preeclamptic pregnancies had elevated concentrations of homocysteine (4).

In women with previous preeclampsia, there is an increased risk for cardiovascular disease later in life (5) and an increased risk of hypertension has also been found for their offspring (6,7).

It is not clear whether the increased risk in the offspring is caused by the intrauterine environment, shared genes, or impaired intrauterine growth. In epidemiologic and animal studies, low birth weight by itself is associated with an increased risk of cardiovascular disease and metabolic syndrome (8).

NO is essential for vascular tone and endothelial function, and elevated circulating concentrations of asymmetric dimethylarginine (ADMA) are associated with reduced vasodilation and endothelial dysfunction (9). ADMA is an endogenous inhibitor of NOS and induces endothelial dysfunction by reversibly inhibiting NO production from L-arginine. The ratio of L-arginine to ADMA is a key determinant of NOS activity and is a useful index for evaluating the effects of ADMA (10,11). Symmetric dimethylarginine (SDMA), an ADMA isomer, has no effect on NOS, but it is a competitor of L-arginine transport, and may therefore interfere with NO production and endothelial function (12). Inhibition of NO by ADMA has been proposed to be a unifying mechanism for endothelial dysfunction caused by elevated concentrations of homocysteine (13).

The fact that pregnant women have lower circulating ADMA concentrations than nonpregnant women suggests that ADMA may play a role in vascular dilatation and blood pressure regulation during pregnancy (14). There have been discrepant findings of elevated (15) and unchanged (16,17) ADMA concentrations in the maternal circulation in women with preeclampsia compared with women with uncomplicated pregnancies. Elevated maternal ADMA concentrations have been found preceding the clinical symptoms of preeclampsia (18,19) suggesting that ADMA could have a role in the pathogenesis of preeclampsia.

There are sparse data on fetal concentrations of ADMA and its associated molecules in pregnancy and preeclampsia (20). The aim of this study was to simultaneously explore maternal and fetal concentrations of L-arginine, ADMA, and SDMA in preeclampsia and possible associations of these measurements with gestational characteristics.

## METHODS

**Protocol.** In this study, we examined women with preeclampsia ( $n = 47$ ) and women with uncomplicated pregnancies (controls,  $n = 51$ ). All were

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**Abbreviations:** ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine

singleton pregnancies delivered by cesarean section, providing maternal and fetal (umbilical cord) blood. At the time of blood sampling, all women were fasting, they were not in active labor, and none had rupture of membranes or signs of infection. Cesarean section was performed in the preeclamptic women because vaginal delivery was not considered appropriate because of disease progression and/or unfavourable cervical ripening. Controls were healthy, normotensive women undergoing cesarean section because of breech presentation, previous cesarean section, or for psychological reasons. This study was part of an ongoing biobank collection at Ullevål University Hospital. Findings for homocysteine and creatinine levels in this cohort have been reported in a previous publication (4).

Preeclampsia was defined as a rise in blood pressure above 140/90 after gestational week 20 on at least two occasions 6 h apart in a previously normotensive woman, combined with proteinuria. Proteinuria was defined as a protein dipstick reading equal to or higher than 1+ on more than one midstream urine sample 6 h apart or a 24-h urine excretion of  $\geq 0.3$  g protein in the absence of urinary infection. Severe preeclampsia included patients with systolic blood pressure  $\geq 160$  mm Hg (21).

Pregnancy duration was calculated on the basis of routine ultrasonographic screening between gestational weeks 17 and 20. Medical history and obstetrical information were obtained from medical records and from the patients' national pregnancy charts, as well as by interviewing the patients. The infants' weight percentiles were estimated from weight curves based on longitudinal ultrasonographic measurements of a population of healthy pregnant Norwegian women (22). Infants below the 10th percentile were defined as small for gestational age (SGA). The study protocol was approved by the Regional Committee of Medical Research Ethics in Eastern Norway, and informed written consent was obtained from each woman.

**Biologic samples.** Maternal blood samples were obtained before cesarean section. The umbilical cord was double clamped near the placenta and near the infant; then, immediately after delivery of the placenta, blood from the umbilical arteries and blood from the umbilical vein were sampled separately into EDTA-containing vials. Samples were kept on ice for a maximum of 30 min and centrifuged at 4°C for 10 min at 2000  $\times$  g, and plasma was stored in aliquots at -76°C until analysis. Laboratory personnel were blinded to clinical information.

**Biochemical analyses.** L-Arginine, ADMA, and SDMA were measured in EDTA plasma using normal-phase liquid chromatography and tandem mass spectrometry (LC-MS/MS) (23). The within- and between-day imprecision CVs for all three metabolites were between 4 and 6%. Serum uric acid was measured as part of the routine screen only in preeclamptic patients; the results were obtained from the women's clinical records.

**Statistics.** As not all the values were normally distributed, medians are reported and the Mann-Whitney *U* test was used for testing differences between groups, and the Wilcoxon Signed Rank Test was used for related samples (comparing concentrations in the umbilical vein and artery). Statistical analyses were performed using the Statistical Package for the Social Sciences (version 14.0; SPSS Inc., Chicago, IL). A probability level of  $<0.05$  was considered statistically significant.

## RESULTS

Clinical characteristics are presented in Table 1. Median gestational age, birth weight, and infant birth weight percentiles

**Table 1.** Characteristics of the study population

	Preeclampsia (n = 47)	Controls (n = 51)	<i>p</i>
Maternal age, y	31.5 (19–42)	32.5 (21–44)	0.76
BMI before pregnancy, kg/m <sup>2</sup>	24.1 (18.9–41.1)	22.5 (17.4–29.4)	0.01*
Gestational age, wk	32.9 (24.9–38.7)	38.7 (34.4–41.7)	$<0.001^*$
BP at delivery, systolic	160 (145–220)	120 (90–140)	$<0.001^*$
BP at delivery, diastolic	102 (90–119)	70 (60–92)	$<0.001^*$
Birth weight, kg	1.72 (0.87–3.86)	3.47 (2.80–4.68)	$<0.001^*$
Birth weight percentile	7 (1–92)	69 (6–99)	$<0.001^*$
Percent SGA	60	6	$<0.001^*$

Values are presented as medians and range (minimum and maximum values), except for percent SGA.

\*  $p < 0.05$ .

were lower for the preeclampsia group compared with the uncomplicated pregnancy group, whereas maternal BMI at start of pregnancy was higher in the preeclampsia group.

Because of the technical problems in sampling not all analyses were done in both the fetal and maternal blood samples; the exact numbers are given in Table 2. Samples from all three compartments were obtained for 47 cases (22 preeclampsia cases and 25 controls). In this sample set, the results were essentially the same as for the total number of samples.

Concentrations of L-arginine, ADMA, SDMA, and relevant ratios for all three compartments (maternal and fetal, both umbilical vein and umbilical artery) are shown in Table 2.

**Metabolites in maternal circulation.** Median maternal concentrations of L-arginine, ADMA, and SDMA were higher (16, 13, and 30%, respectively), whereas the maternal ADMA/SDMA ratio was 12% lower in the preeclampsia group compared with the uncomplicated pregnancy group. There was no statistically significant difference in the maternal L-arginine/ADMA ratio between preeclampsia patients and controls.

Maternal L-arginine, ADMA, and SDMA concentrations were highly correlated with each other in the preeclampsia group, but not in controls (Table 3).

**Metabolites in fetal circulation.** There was no difference in L-arginine or ADMA between infants in the preeclampsia and control groups in either the umbilical vein or the umbilical artery samples (Table 2).

Concentrations of L-arginine and ADMA were significantly higher in the umbilical vein than in the umbilical artery (both  $p < 0.001$ ), for both the preeclampsia and control groups, and there was a strong positive correlation between umbilical vein and artery concentrations for both L-arginine ( $r = 0.88$ ,  $p < 0.001$ ) and ADMA ( $r = 0.63$ ,  $p < 0.001$ ). Also, a higher median SDMA concentration was found in the umbilical vein than in the umbilical artery ( $p = 0.001$ ) in the newborns that provided paired artery and vein samples (controls  $n = 31$ , preeclampsia  $n = 22$ ). Also, SDMA levels in the umbilical vein and artery were highly correlated ( $r = 0.87$ ,  $p < 0.001$ ).

**Relation between metabolites in the maternal and fetal circulation.** The median ADMA concentration was almost three times higher in umbilical vein plasma than in maternal plasma. As with most amino acids, the L-arginine concentration was higher in the fetal circulation than in the maternal circulation, but the significantly higher maternal L-arginine concentration found in preeclampsia patients relative to controls was not reflected on the fetal side (in neither the umbilical vein nor the umbilical artery samples). In controls, the median concentration of L-arginine was twofold higher in the umbilical vein than in the maternal plasma. This ratio was significantly lower for the preeclampsia group ( $p < 0.001$ ). The L-arginine/ADMA ratio in the umbilical vein was 15% lower in the preeclampsia group compared with the control group.

Fetal and maternal concentrations were positively correlated for ADMA, L-arginine, and SDMA ( $r = 0.5$ – $0.7$ ,  $p < 0.001$  for all). This applied to both the preeclampsia and control groups, except for ADMA in the control group for which there was no correlation between the maternal and umbilical artery concentrations ( $r = 0.09$ ,  $p = 0.68$ ). Because

**Table 2.** Median values and interquartile ranges for the measured variables and relevant ratios. Creatinine is included

Variable	Maternal			Umbilical vein			Umbilical artery		
	Preeclampsia (n = 43)	Controls (n = 40)	p	Preeclampsia (n = 38)	Controls (n = 45)	p	Preeclampsia (n = 25)	Controls (n = 31)	p
L-arginine (μmol/L)	45.4 (37.7–54.4)	39.1 (34.6–45.2)	0.006*	77.3 (61.3–86.9)	85.3 (69.7–95.0)	0.13	65.7 (51.8–84.0)	69.3 (63.0–88.7)	0.08
ADMA (μmol/L)	0.44 (0.38–0.50)	0.39 (0.33–0.43)	0.003*	1.27 (1.15–1.38)	1.28 (1.18–1.38)	0.67	1.18 (1.07–1.36)	1.21 (1.10–1.31)	0.93
SDMA (μmol/L)	0.69 (0.59–0.75)	0.53 (0.46–0.59)	<0.001*	1.98 (1.80–2.40)	1.69 (1.48–2.01)	<0.001*	2.14 (1.78–2.32)	1.61 (1.39–1.84)	0.001*
L-arginine/ ADMA	105.8 (91–126)	106.5 (90–121)	0.78	57.4 (50.8–68.3)	66.0 (56.3–72.9)	0.03*	55.1 (48.1–64.0)	62.1 (54.3–72.6)	0.07
ADMA/ SDMA	0.64 (0.54–0.77)	0.73 (0.59–0.87)	0.026*	0.63 (0.51–0.75)	0.74 (0.65–0.86)	0.002*	0.62 (0.49–0.71)	0.77 (0.64–0.86)	<0.001*
Creatinine (μmol/L)	59.9 (52.2–69.8)	51.4 (45.9–58.6)	<0.001*	59.7 (53.0–66.7)	51.1 (47.6–57.4)	0.001*	60.6 (52.1–69.7)	51.0 (47.3–56.9)	0.005*

\* p < 0.05.

**Table 3.** Correlations between maternal plasma concentrations of L-arginine, ADMA, SDMA, homocysteine, and creatinine

	L-arginine	ADMA	SDMA	Homocysteine
Controls				
ADMA	0.21			
SDMA	0.003	0.21		
Homocysteine	0.10	0.18	0.24	
Creatinine	0.22	0.18	0.40*	0.41*
Preeclampsia				
ADMA	0.66*			
SDMA	0.35*	0.38*		
Homocysteine	0.09	0.25	0.31*	
Creatinine	0.26	0.19	0.59*	0.15

\* p < 0.05.

of the high correlation between maternal and fetal concentrations, only correlations for maternal values are presented in Table 3.

**Metabolites and clinical outcomes.** No correlation was found between maternal ADMA (or SDMA and L-arginine) and maternal clinical characteristics such as prepregnancy BMI, maternal age at delivery, or maximum systolic or diastolic blood pressure, either in the preeclampsia group or in the control group (data not shown).

We did not find any significant difference in the concentrations of maternal or fetal L-arginine, ADMA, or SDMA between patients with severe (n = 34) and nonsevere (n = 10) preeclampsia. Also, there was not a statistically significant difference in the median concentrations of maternal or fetal L-arginine, ADMA, or SDMA between the preeclampsia groups delivered before or after week 34 (data not shown).

We found an inverse correlation between the maternal L-arginine/ADMA ratio and birth weight percentile (r = -0.39, p = 0.01), whereas no association was found between infant weight percentile and ADMA or any of the other metabolites presented in Table 2.

Maternal SDMA was correlated with creatinine both in the preeclampsia and control groups, whereas no correlation was found between maternal ADMA and creatinine (Table 3). The median maternal uric acid concentration was above normal for the preeclampsia group (median 400 μM, interquartile range 337–445 μM). Uric acid is often elevated in preeclamptic women, and in our study, it was positively correlated with

ADMA (r = 0.32, p = 0.04) and SDMA (r = 0.39, p = 0.01), but not with L-arginine (r = 0.19, p = 0.24) in the preeclampsia group (uric acid was not measured in the control group).

### DISCUSSION

In this study, we found that ADMA concentrations were slightly elevated in maternal, but not in fetal (umbilical vein and artery), plasma in preeclampsia patients compared with controls, whereas SDMA was significantly elevated in all three compartments in preeclampsia. We found a positive correlation between maternal concentrations of creatinine and SDMA, but not between creatinine and ADMA. This is consistent with SDMA being eliminated strictly by renal excretion, whereas ADMA is mainly metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) (24), and it has been hypothesized that ADMA concentration is dependent on the activity of DDAH (25). Although maternal creatinine concentrations for the preeclampsia group were well within the normal range, median plasma creatinine was elevated in the preeclampsia group compared with the control group (Table 2), which is indicative of generalized endothelial dysfunction in preeclampsia, with a typical histologic finding of “glomerular endotheliosis” in the kidneys (26). The elevated concentrations of SDMA throughout all compartments in preeclampsia could be regarded as a marker of mild renal dysfunction in preeclampsia. We did not address the question of whether this elevated SDMA concentration influences NO through competition for the arginine y-transporter system in this study.

Circulating SDMA concentrations in preeclampsia have previously been explored in a few studies (17,27). Ellis *et al.* reported elevated maternal SDMA in preeclampsia but, unlike our findings, they found that SDMA was correlated with arterial blood pressure and disease severity (27). In a study of Colombian women, Maas *et al.* (17) did not find that maternal ADMA or SDMA was elevated in preeclampsia. In contrast to our study, the majority of their participants had moderate preeclampsia, whereas ADMA levels seemed to be higher in their severe preeclampsia group (19 of 67), but it was not stated whether this was a significant difference (17).

Elevated maternal L-arginine concentrations have been previously found in preeclampsia (18), as we found in our study,



but other studies have shown unchanged (15,17,19) or lower concentrations (16) compared with controls. It has been argued that the L-arginine/ADMA ratio, rather than ADMA levels, is the key determinant of NOS activity (10), which is regarded as a useful index for interpretation of ADMA's effects (11). The L-arginine/ADMA ratio may be more important than ADMA levels, but in our study, there was no difference in the maternal plasma L-arginine/ADMA ratio between preeclampsia patients and controls. Therefore, an inhibitory effect of elevated ADMA on NOS in preeclampsia may be blunted because of concomitant elevation of the NO precursor L-arginine.

We found an unexpected negative correlation between the maternal L-arginine/ADMA ratio and birth weight percentile, suggesting that the offspring of preeclamptic pregnancies with more severe disease including placental insufficiency and low birth weight percentile, have the most "beneficial" L-arginine/ADMA ratio. Elevated concentrations of maternal plasma amino acids have previously been reported in preeclampsia (28), and the higher L-arginine/ADMA ratio in preeclampsia could be a proxy for disease severity.

Both animal and human studies have suggested that ADMA could be a link between elevated homocysteine concentrations and endothelial dysfunction as a cardiovascular risk factor (13). We have previously shown in the same biobank cohort that homocysteine is elevated in preeclampsia both in the maternal and fetal circulation (4). However, we found no correlation between ADMA and homocysteine in our preeclampsia or control group (Table 3), which is in accordance with a study by Herrmann *et al.* (29).

The role of ADMA in normal pregnancies has been explored in a few studies. Maeda *et al.* (30) found maternal concentrations of ADMA to be lower in normal pregnancies than in nonpregnant women and suggested that ADMA is important for vasodilation in pregnancy. Recently, Saarelainen *et al.* (31) have expressed doubt regarding the role of ADMA as a key regulator of blood pressure in normal pregnancy, because their study demonstrated only subtle alterations of ADMA and no correlation between endothelium-dependent vasodilation markers and maternal serum ADMA or L-arginine concentrations.

The role of NO inhibition in preeclampsia is not entirely clear, and the evidence that endothelial dysfunction is induced by ADMA inhibition or low L-arginine is not unequivocal. Despite this, L-arginine supplementation to prevent preeclampsia has been tried in several studies. Oral L-arginine supplementation was investigated by our group; the intervention did not alter blood pressure in preeclampsia (32). A recent review found that evidence is still insufficient to draw reliable conclusions as to whether L-arginine can prevent preeclampsia or its complications (33). Interestingly, a study involving dietary supplementation with 3 g/d L-arginine found no difference in biochemical markers, but supplementation significantly decreased maternal blood pressure and improved fetal outcome (34,35). Conceivably, in studies evaluating L-arginine and other nutritional supplements (*i.e.*, calcium, antioxidants), the outcomes depend on the general nutritional status of the group. In some populations where insufficient

nutrition is more prevalent, it is more likely that the incidence of preeclampsia could be reduced by supplementing the deficient nutrients (36).

In our study, fetal ADMA concentrations (in the umbilical vein and artery) did not differ between preeclampsia patients and controls, and this is consistent with a recently published study (20). This is also in accordance with our previous findings that in preeclampsia, elevated maternal levels of markers of inflammation and oxidative stress are generally not reflected in the fetal circulation (2,3).

For ADMA and L-arginine concentrations, there were consistent veno-arterial differences, with higher levels in the umbilical vein compared with the umbilical artery, suggesting that these metabolites are mainly placentally derived, not fetally, which is consistent with a study performed in normal infants (37). ADMA and SDMA may be derived from the placenta, but may also be concentrated from the maternal plasma; it is not possible to discern between these possibilities from this study. The very high concentration of ADMA in the fetal circulation is an enigma, and it has been hypothesized that it is a compensatory mechanism to stabilize very high NO concentrations in the fetoplacental unit (30,37).

A strength of this study is that the included subjects are clinically well defined. As our preeclampsia group was delivered by cesarean section, they clinically had more severe preeclampsia than if vaginal deliveries were also included. Our preeclampsia group is therefore not representative of all women with preeclampsia, but is possibly the most interesting and important group to study, as severe preeclampsia and premature delivery have serious consequences for the infant. Our model, with concomitant sampling from all three compartments (maternal, placental, and fetal), allows us to speculate on the origin of metabolites and their correlations.

It is possible that some of the discrepant findings in studies on ADMA are because of the differences in laboratory methods. In this study, a well-described and validated LC-MS/MS method was used (23), which has the advantage of differentiating between ADMA and SDMA with high specificity (24).

One limitation of our study is that the median gestational age was different in preeclampsia patients and controls. It is difficult to match for gestational age when sampling fetal blood between the often prematurely delivered preeclamptic patients and women with uncomplicated deliveries, because cesarean section is not performed preterm on healthy women. However, in a longitudinal study of ADMA in normal pregnancies (14), maternal concentrations of ADMA increased slightly with increasing gestational age, which in our study means that the difference in ADMA concentration between preeclampsia patients and controls could be underestimated.

In summary, ADMA is increased in the maternal circulation in preeclampsia, but so is L-arginine, possibly attenuating the effects on NOS. Elevated SDMA levels are possibly because of slightly reduced renal clearance. ADMA is three times higher in the fetal circulation than in the maternal circulation, but with no difference between preeclampsia patients and controls, and the veno-arterial gradient indicates that the placenta is the source of ADMA. Our study, further, blurs the previously suggested picture of ADMA as an important de-

terminant of endothelial function in preeclampsia, but does not exclude local or intracellular effects of ADMA and related molecules in the placenta and in the maternal endothelium.

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