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Original Article

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Elevated Plasma Level of Arginine and Its Metabolites at Labor Among Women With Preeclampsia: A Prospective Cohort Study

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BACKGROUND: Preeclampsia is associated with higher levels of asymmetric (ADMA) and symmetric (SDMA) dimethylarginines. Dimethylarginines are inhibitors of nitric oxide, a uterine smooth muscles relaxant. Women with preeclampsia experience a shorter labor duration compared with normotensive women. However, very little is known about the possible biochemical mechanisms behind these differences. We aimed to investigate if women with preeclampsia had higher levels of arginines (ADMA, SDMA, and L-arginine) at labor than controls and also investigate the association between arginines and labor duration.

METHODS: The study was based on data from the Swedish, Uppsala County population-based, prospective cohort BASIC, 2009–2018. Arginines were analyzed by ultra-high-performance liquid chromatography using plasma samples taken at labor from women with preeclampsia ($n = 47$) and normotensive pregnancy ($n = 90$). We also analyzed inflammation markers such as C-reactive protein, tumor necrosis factor (TNF)-R1, TNF-R2, and growth differentiation factor (GDF-15).

RESULTS: Women with preeclampsia had higher levels of ADMA ($P < 0.001$), SDMA ($P < 0.001$), L-arginine ($P < 0.001$), TNF-R1 ($P < 0.001$), TNF-R2 ($P = 0.03$), and GDF-15 ($P < 0.01$) compared with controls. Furthermore, ADMA and SDMA, not inflammation markers, were negatively correlated to labor duration in preeclampsia. No correlations were observed when comparing arginines and inflammation markers.

CONCLUSIONS: Among women with preeclampsia, our novel findings of higher level of arginines, negative correlation of arginines to labor duration, and absence of correlation of arginines to inflammation markers might support the theory that it is not inflammation but arginines which could be associated with shorter labor duration in preeclampsia.

Keywords: blood pressure; dimethylarginine; duration of labor; hypertension; inflammation marker; L-arginine; preeclampsia.

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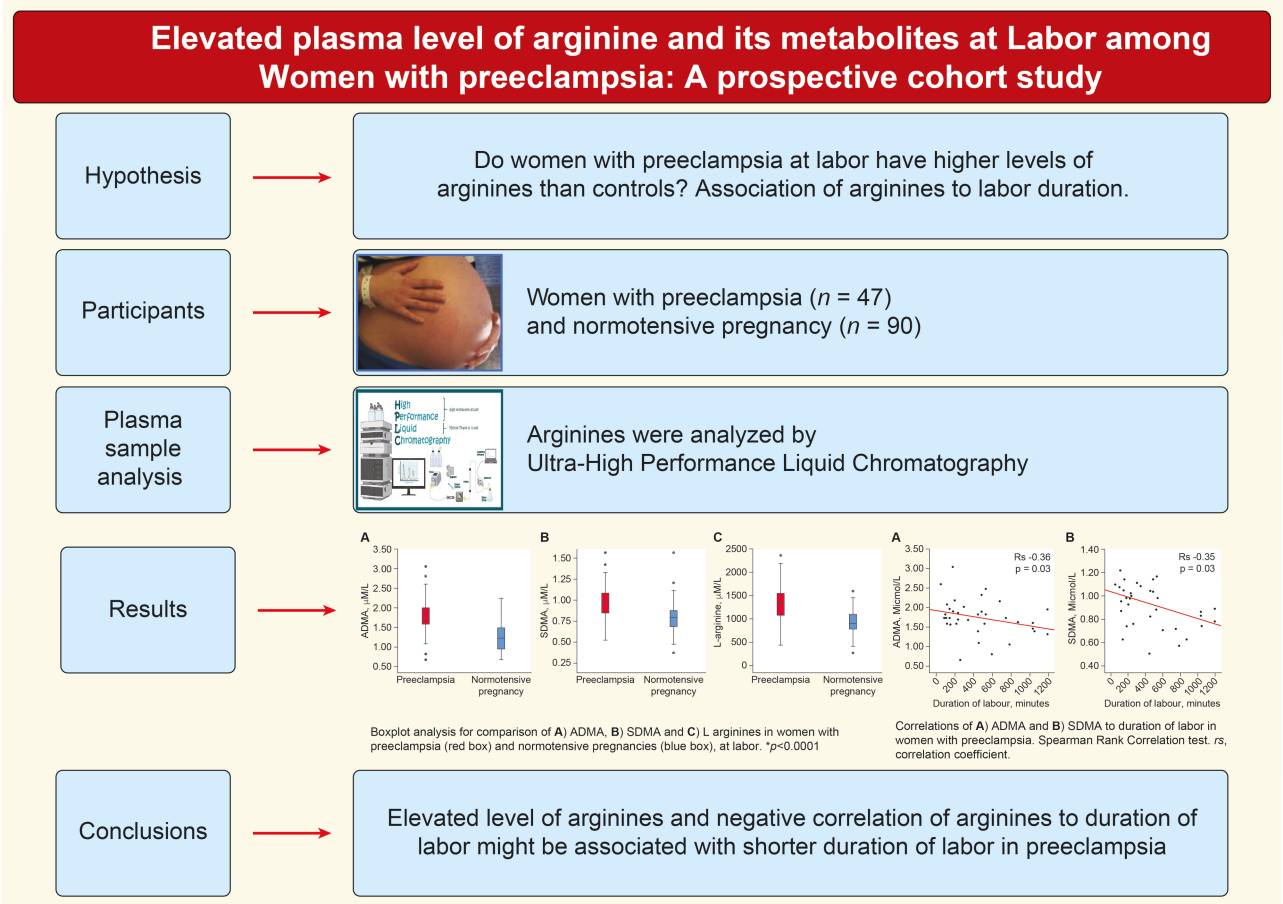
Graphical Abstract



Preeclampsia complicates about 3%–5% of all pregnancies.[1] Preeclampsia is associated with higher levels of dimethylarginines, e.g., asymmetric (ADMA) and symmetric (SDMA) compared with normotensive pregnancy.[2–4] L-Arginine is an amino acid and is the immediate precursor of nitric oxide (NO). L-Arginine can also be methylated by arginine methyltransferase to ADMA and SDMA.[5] NO is a potent relaxant of uterine smooth muscles and is important to keep the uterus relaxed until the onset of labor. Dimethylarginines (ADMA and SDMA) are direct endogenous inhibitors of nitric oxide synthase, which leads to decreased NO synthesis. Dimethylarginines may also reduce NO synthesis indirectly by inhibiting the cellular uptake of the NO precursor L-arginine.[5] Studies have shown that the L-arginine/ADMA ratio, rather than only ADMA levels, is the key determinant of nitric oxide synthase activity[6] and is a useful index for interpretation of effects of ADMA.[7]

Normal pregnancy is a state of inflammation,[8,9] and preeclampsia is associated with exaggerated inflammation[10] which increases at the initiation of labor.[11,12] Traditional inflammatory markers like C-reactive protein, tumor necrosis factor (TNF), and growth differentiation factor (GDF-15) at labor in normal pregnancy have been studied extensively with various results,[12–15] and their role for initiation and maintenance of labor including preterm labor and labor in women with preeclampsia is not conclusive. Studies about ADMA, SDMA, and L-arginine at labor are scarce, especially at labor in women preeclampsia. However, we have recently showed that women with vaginal birth had higher levels of dimethylarginines than women giving birth through elective cesarean section.[16] However, we could not show any difference in inflammatory markers between the groups.[16]

In a register-based cohort study from 2020, we showed that women with hypertensive disorders experienced a shorter duration of labor compared with normotensive women[17] as findings of a study by Bregand-White *et al.*[18] However, very little is known about the possible biochemical mechanism of shorter duration of labor in women preeclampsia.

The main aim of this study was to investigate the levels of L-arginine and its metabolites ADMA and SDMA at labor, among women with preeclampsia compared with women with normotensive pregnancies. As a comparator, we analyzed inflammation markers like C-reactive protein, TNF-R1, TNF-R2, and GDF-15 to study correlations between these markers and arginines. Our hypothesis was that plasma levels of L-arginine, ADMA, SDMA, and inflammation markers will be higher, and L-arginine/ADMA and L-arginine/SDMA will be lower in women with preeclampsia than in women with normotensive pregnancies. Another hypothesis was that dimethylarginines and inflammation markers might be negatively correlated to duration of labor and dimethylarginines might be positively correlated to inflammation markers.

METHODS

Study design and participants

This study was undertaken as a substudy based on the population-based, prospective cohort study BASIC (Biology, Affect,

Stress, Imaging and Cognition; https://www.basicstudie.se) at the Department of Obstetrics and Gynecology, Uppsala University Hospital, Sweden. The method of recruitment has been extensively described in a previous study of the cohort profile of the BASIC population.[19] In brief, all pregnant women in the county of Uppsala who were referred to Uppsala University Hospital for a routine ultrasound examination at gestational weeks 16–18 were offered written information and invited to the BASIC study. Exclusion criteria were age below 18 years, insufficient ability to read and understand Swedish, protected identity, known blood-borne infections, or nonviable fetus as diagnosed by ultrasound. Between September 2009 and November 2018, 31,687 pregnant women had been invited to the study of which 6,478 (20.4%) choose to participate. Participants were followed up at multiple points from baseline at gestational weeks 16–18 through 1-year postpartum. Biological samples were collected at several of these points and at childbirth.

In this study, we included both nulliparous and parous women. Exclusion criteria were multifetal pregnancy, labor dystocia (slow dilation of the cervix or descent of the fetus during the active phase of labor), and chorioamnionitis, as these conditions could potentially distort the levels of ADMA, SDMA, and inflammation markers. In Sweden, during the study period, preeclampsia was defined as new-onset hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg at 2 subsequent measurements) combined with proteinuria (>0.3 g/24 hours) after 20 gestational weeks.[20] After review of the BASIC database, women with preeclampsia ($n = 47$) and women with normotensive pregnancy ($n = 90$) were included after inclusion and exclusion criteria were met. Women in the normotensive group have been described in one of our previous publications[16] and comprised of women with spontaneous onset of labor ($n = 45$) and induced labor ($n = 45$) at term. Information about sociodemographic factors, medical history, pregnancy complications, labor characteristics (e.g., mode of onset, methods of induction, cervical dilatation at blood sampling, gestational length at birth, duration of labor, duration of the active second stage of labor, and postpartum bleeding), and neonatal information (e.g., gender, birth weight, and Apgar scores) were gathered.

Blood sample collection and processing

Peripheral blood samples were collected on admission at the delivery ward at start of labor. The process of collection, preparation, and storage of plasma samples was performed, as previously reported.[19] The samples were centrifuged within 2 hours at 1500 *g* for 10 minutes and stored at –70 °C until analysis.

Analysis of arginines by ultra-high-performance liquid chromatography

Plasma samples (100 µl) were prepared by spiking with 50 µl of an ADMA-d6, SDMA-d6, and L-arginine-13C6 mixture, followed by protein precipitation with 200 µl of ice-cold acetonitrile containing 0.1% formic acid.

L-Arginine, ADMA, and SDMA were quantitatively analyzed with ultra-high-performance liquid chromatography–tandem quadrupole mass spectrometry after addition of isotope-labeled internal standards and protein precipitation using the same method as in one of our previous publicatios[16] (Waters ACQUITY, Waters, Milford, MA) coupled to tandem quadrupole mass spectrometry (XEVO TQ-S, Waters). Chromatographic separation was achieved on a Premier HSS T3 column (150 mm, 3.0 mm, and 1.7 µm, Waters, Milford, MA) at 40 °C. Mobile phase A consisted of 20 mM ammonium formate in water, and mobile phase B consisted of methanol. Isocratic elution was performed with 10% of mobile phase B. The mass spectrometric detection was performed using positive ionization electrospray ionization. Quantification was performed using a selected reaction monitoring method with the following transitions: for ADMA *m/z* 203.1 > 116.1 (collision energy: 18 eV), ADMA-d6 *m/z* 209.2 > 52.2; for SDMA *m/z* 203.1 > 88.1 (collision energy: 20 eV), SDMA-d6 *m/z* 209.2 > 105.0 (collision energy: 17 eV); and for L-arginine *m/z* 175.1 > 70.0 (collision energy: 15 eV), L-arginine-13C6 *m/z* 181.0 > 121.1 (collision energy: 25 eV). The calibration ranges were 10–5,000 ng/ml for all analytes.

Analysis of inflammation markers

Analyses of high sensitivity C-reactive protein were performed using a Mindray BS380 chemistry analyzer (Mindray, Shenzhen, China) with reagents from Abbott Laboratories (6K26-41, Abbott Park, IL). Analyses of TNF-R1 (DY225) and TNF-R2 (DY726) and GDF-15 (DY957) were performed using commercial sandwich ELISA kits (R&D Systems, Minneapolis, MN).

Ethical approval

Apart from the original ethical application for the BASIC project (Reference number 2009/171, approved by the Regional Ethical Review Board in Uppsala), a supplementary application for this project was approved by the Swedish Ethical Review Authority (Reference number 2020-00633). A written informed consent was obtained from every participant in the context of the BASIC study.

Statistical analysis

We have estimated that 45 women in the preeclampsia group against 45 women in the control group will detect a 10% difference in main outcome variables (arginines) between the groups with a 90% power at 5% significance level. We chose to use non-parametric tests because the data were not normally distributed. The results are presented as medians with interquartile ranges for continuous variables or in numbers and percentages for categorical variables. The Spearman rank correlation test was used to test correlations of demographic data and clinical characteristics to arginines (Supplementary Table S1 online) to identify possible covariates and to test correlations of arginines and inflammation markers to labor characteristics. The Mann–Whitney *U* test was used when comparing the groups regarding continuous variables, and the Pearson chi-square test was used comparing categorical variables. Differences between groups were adjusted for maternal age, body mass index, mode of onset of labor, cervical dilatation at sampling, and gestational length at birth, by the univariate general linear model. Subgroup analyses of arginines and inflammation markers were performed in women with preeclampsia. In addition, we performed stratified analyses of arginines and inflammation markers according to the mode of onset of labor in both groups. The level of significance was set at $P < 0.05$. Statistical analysis was performed using the SPSS, version 27.0 (SPSS PASW statistics), for Windows software package.

RESULTS

Demographics and clinical characteristics

Demographic data and clinical characteristics of the study population are summarized in Table 1. There was no difference between the groups regarding demographic data except higher numbers of nulliparous women in the preeclampsia group

Table 1. Demographic and clinical characteristics of the study population

	Preeclampsia (*n* = 47)	Normotensive pregnancy (*n* = 90)	*P* value
Maternal age, y	31 (28, 33)	33 (29, 35)	0.10
BMI at 2nd trimester, kg/m^2	24 (22, 30)	23 (21, 26)	0.18
Parity			
Nulliparity	39 (83)	36 (40)	<0.001
Parous	8 (17)	54 (60)	
Education			
University	34 (72)	65 (72)	1.0
Below university	13 (28)	25 (28)	
Cohabitating	45 (98)	88 (98)	1.0
Country of birth			
Scandinavia	39 (85)	84 (93)	0.20
Others	7 (15)	5 (11.1)	
Employed	46 (98)	82 (91)	0.25
Smoking at 2nd trimester	0 (0)	3 (3)	0.52
Alcohol use at 2nd trimester	0 (0)	0 (0)	NA
Preterm preeclampsia	5 (11)		
Cervical dilatation at sampling, cm	3 (2, 4)	4 (3, 5)	0.13
Gestational length at birth, wk	40 (38, 41)	40 (39, 42)	0.06
Mode of onset of labor			
Spontaneous	16 (34)	45 (50)	0.11
Induction	31 (66)	45 (50)	
Methods of induction			
Prostaglandin/cervical catheter	20 (65)	20 (44)	0.18
Amniotomy/oxytocine	11 (36)	25 (56)	
Mode of childbirth			
Vaginal	35 (75)	90 (100)	<0.001
Cesarean section	12 (25)	0 (0)	
Duration of labor, min	299 (157, 532)	183 (100, 397)	0.06[a]
Active 2nd stage of labor, min	25 (15, 45)	20 (09, 39)	0.14
Stillbirth, *n* (%)	0 (0)	0 (0)	NA
Postpartum blood loss, ml	500 (350, 1,050)	375 (300, 500)	0.09[a]
Infant gender, girl	29 (62)	41 (46)	0.11
Infant birth weight, kg	3.5 (3.0, 3.8)	3.7 (3.4, 4,1)	<0.01
APGAR score <7 at 5 min	1 (2)	0 (0.0)	0.74
NICU care	7 (15)	0 (0.0)	<0.001

Values are presented as median (interquartile ranges) for continuous variable and number (percentage) for categorical variable.
Comparison between groups was made by the Mann–Whitney *U* test for continuous variables and the Pearson chi-square test for categorical variables.
Abbreviations: APGAR, appearance, pulse, grimace, activity, and respiration; BMI, body mass index; NICU, neonatal intensive care unit.
[a]Differences between groups are adjusted for parity and mode of onset of labor by univariate general linear model. Birth by cesarean section (*n* = 12) in the preeclampsia group is excluded.

Table 2. Differences in plasma levels of arginines and inflammation markers in the study population

Plasma levels	Preeclampsia (*n* = 47)	Normotensive pregnancy (*n* = 90)	[a]*P* value	[b]*P* value Adjusted
ADMA, µM/l	1.73 (1.56, 2.01)	1.19 (0.92, 1.47)	<0.001	<0.001
SDMA, µM/l	0.98 (0.83, 1.09)	0.79 (0.67, 0.87)	<0.001	<0.001
L-Arginine, µM/l	1,381 (1,072, 1,569)	902 (782, 1,110)	<0.001	<0.001
L-Arginine/ADMA	828 (536, 987)	838 (501, 1,062)	0.67	0.28
L-Arginine/SDMA	1,398 (1,088, 1,604)	1,182 (947, 1,494)	0.03	0.01
CRP, mg/l	3.60 (1.85, 8.78)	3.50 (2.19, 10.62)	0.72	0.39
TNF-R1, pg/ml	1,727 (1,206, 2,705)	923 (737, 1,156)	<0.001	<0.001
TNF-R2, pg/ml	4,870 (3,838, 7,083)	4,810 (4,063, 5,493)	0.19	0.03
GDF-15, pg/ml	101,501 (61,066, 126,274)	64,042 (46,147, 90,927)	<0.001	<0.01

Median med interquartile ranges.
Differences between groups are analyzed by the
[a]Mann–Whitney U test and
[b]adjusted for maternal age at birth, BMI at second trimester, mode of onset of labor, cervical dilatation at sampling, and gestational length at birth, by univariate general linear model.
Abbreviations: ADMA, asymmetric dimethylarginine; BMI, body mass index; CRP, C-reactive protein; GDF, growth differentiation factor; SDMA, symmetric dimethylarginine; TNF, tumor necrosis factor.

($P < 0.001$). Regarding clinical characteristics, there were differences between the groups: lower frequency of vaginal birth ($P < 0.001$), infant birth weight was lower ($P < 0.01$), and more infants ($P < 0.001$) were admitted to the neonatal intensive care unit in the preeclampsia group compared with the normotensive group.

Plasma levels of arginines

Plasma levels of arginine and its metabolites (ADMA and SDMA) and inflammation markers are summarized in Table 2. ADMA ($P < 0.001$), SDMA ($P < 0.001$), L-arginine ($P < 0.001$), and L-arginine/SDMA ($P = 0.01$) were higher in the preeclampsia group compared with the normotensive group, after adjustment for maternal age

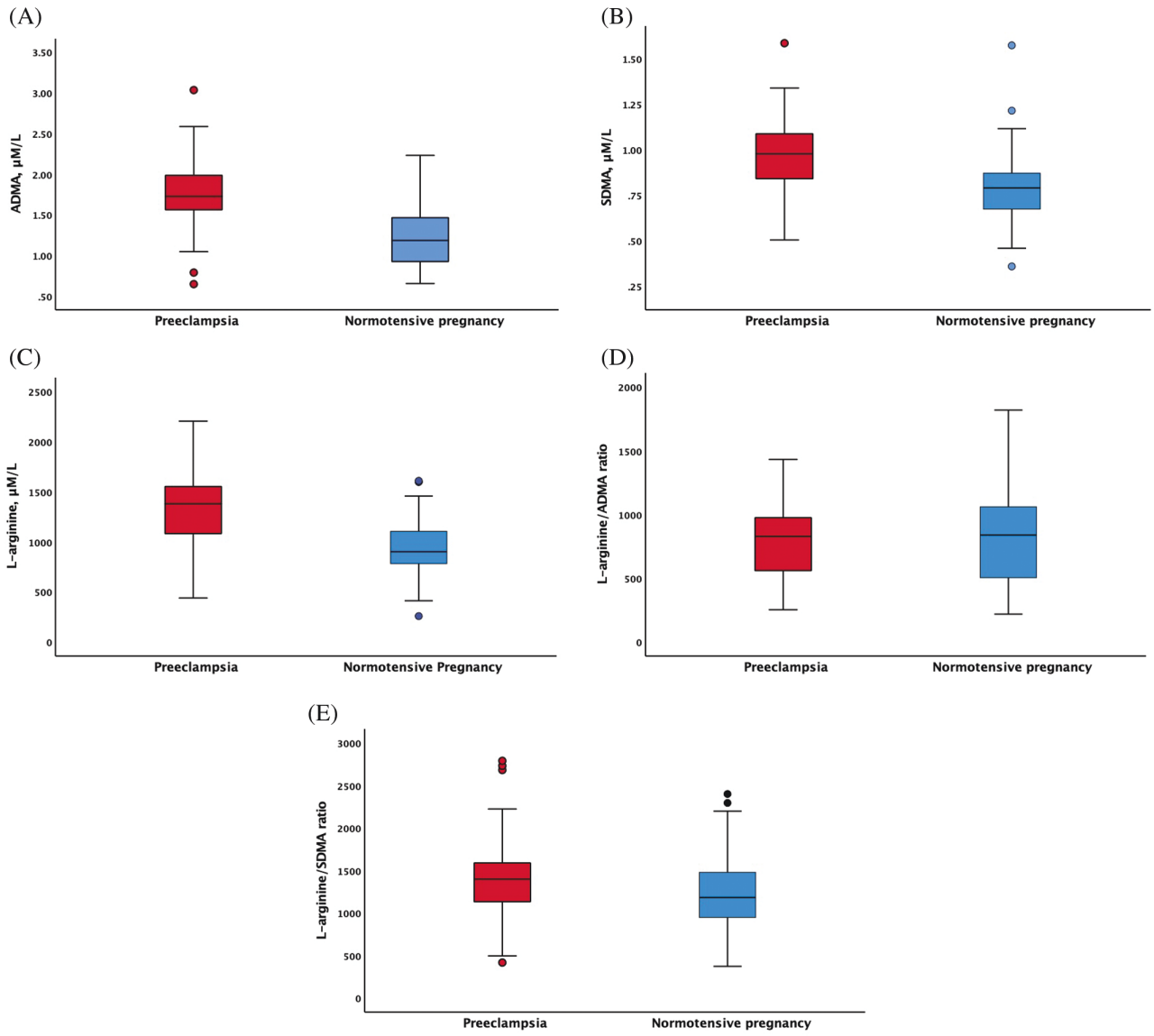


Figure 1. Boxplot analysis to show the differences in (**a**) ADMA, asymmetric dimethylarginine; (**b**) SDMA, symmetric dimethylarginine; (**c**) L-arginine; (**d**) L-arginine/ADMA; and (**e**) L-arginine/SDMA, at labor, in women with preeclampsia and normotensive pregnancy. The top and the bottom of the boxes represent the third and the first quartiles. The horizontal lines within the boxes represent the median values. The bars on the side of the boxes represent the highest and the lowest values. Red and blue filled circles represent extreme values. $P < 0.001$ for ADMA, SDMA, and L-arginine and $P = 0.02$ for L-arginine/SDMA between women with preeclampsia and normotensive pregnancy.

at birth, body mass index at second trimester, mode of onset of labor, cervical dilatation at sampling, and gestational length at birth (Figure 1). Because cervical dilatation at sampling was <6 cm in 44/47 women with preeclampsia and 69/90 women with normotensive pregnancy, we performed a subanalysis in these women and found that the results were similar as in analysis for both groups as a whole.

In subanalysis of women with preeclampsia, no differences in L-arginine, ADMA, and SDMA were found neither in comparison of preterm vs. term preeclampsia, spontaneous onset of labor vs. induction, nor in comparison of induction with prostaglandin or balloon catheter vs. induction with amniotomy or oxytocin (data not shown).

Plasma levels of inflammation markers

Significant differences were seen for the plasma levels of TNF-R1 ($P < 0.001$), TNF-R2 ($P = 0.03$), and GDF-15 ($P = 0.01$), after adjustment for maternal age at birth, body mass index at second trimester, mode of onset of labor, cervical dilatation at sampling, and gestational length at birth (Table 2). In subanalysis of women with preeclampsia, no differences in inflammation markers were found, neither in comparison of preterm vs. term preeclampsia, spontaneous onset of labor vs. induction, nor in comparison of induction with prostaglandin or balloon catheter vs. induction with amniotomy or oxytocin (data not shown).

Differences in arginines and inflammation markers, stratified according to mode of onset of labor

Among women with spontaneous onset of labor, women with preeclampsia had higher levels of ADMA ($P < 0.001$), SDMA ($P = 0.002$), L-arginine ($P = 0.01$), lower L-arginine/ADMA ($P = 0.02$), and higher TNF-R1 ($P < 0.001$) compared with normotensive group (Supplementary Table S2 online). Similarly, among women

with induction of labor, women with preeclampsia had higher levels of ADMA ($P = 0.02$), SDMA ($P = 0.03$), L-arginine ($P < 0.001$), higher L-arginine/SDMA ($P = 0.009$), and higher TNF-R1 ($P = 0.003$) and GDF-15 ($P = 0.04$) compared with the normotensive group (Supplementary Table S2 online).

Correlation of arginines and labor characteristics to inflammation markers in women with preeclampsia

In correlation analyses between L-arginine, ADMA, SDMA, and inflammation markers, no significant correlations have been found except SDMA vs. TNF-R2 (r_s 0.36, confidence interval 0.07, 0.59, $P < 0.05$) (Table 3). In correlation analyses between labor characteristics and arginines, duration of labor was negatively correlated with ADMA (r_s –0.36; confidence interval –0.63, –0.02; $P < 0.05$) and SDMA (r_s –0.35; confidence interval –0.62, –0.01; $P < 0.05$) (Table 4). No significant correlations have been found in correlation analyses between labor characteristics and inflammation markers (Table 4).

DISCUSSION

Main findings

To our knowledge, this is the first study assessing arginine levels around the time of labor in women with preeclampsia. In this prospective cohort study, we found that women with preeclampsia had higher plasma levels of ADMA, SDMA, and L-arginine at labor than women with normotensive pregnancy, despite many covariates taken into account. Regarding inflammation markers, TNF-R1, TNF-R2, and GDF-15 were higher in women with preeclampsia compared with women with normotensive pregnancy. However, there was no association between levels of L-arginine, ADMA, SDMA, and inflammation markers around the time of labor, except SDMA vs. TNF-R2 (r_s 0.36 and $P \leq 0.05$). Furthermore, ADMA and SDMA, not inflammation markers, were negatively correlated to the duration of labor in preeclampsia.

Interpretation of results

Novel findings of arginines in this study are a milestone in further understanding the possible biochemical mechanism of labor in women with preeclampsia and might support our previous findings of shorter duration of labor in women with hypertensive pregnancy than in women with normotensive pregnancy.[17] Findings of certain inflammation markers in our study confirm the previous findings of excessive inflammation in preeclampsia than normotensive pregnancy.[10,21,22] However, no association between ADMA, SDMA, and inflammation markers at labor in women with preeclampsia suggests that ADMA and SDMA are independent of inflammation markers. In addition, the negative correlation of arginines to the duration of labor further demonstrated that ADMA and SDMA could be a better marker of the duration of labor in women with preeclampsia.

Preeclamptic pregnancy is a state of exaggerated inflammation compared with normotensive pregnancy.[10,21,22] There are few studies about dimethylarginines in pregnancy complicated by preeclampsia,[3,4,23–27] and in one of them, similar to the findings of other studies, we showed that women with preeclampsia, at gestational

Table 3. Correlations between arginines and inflammation markers in women with preeclampsia ($n = 47$)

	CRP r_s (95% CI)	TNF-R1 r_s (95% CI)	TNF-R2 r_s (95% CI)	GDF-15 r_s (95% CI)
ADMA	0.02 (–0.27, 0.32)	–0.13 (–0.41, 0.17)	0.18 (–0.12, 0.45)	–0.05 (–0.34, 0.25)
SDMA	0.21 (–0.09, 0.47)	0.14 (–0.16, 0.42)	0.36 (0.07, 0.59)[a]	0.29 (0.13, 0.44)
L-Arginine	–0.08 (–0.37, 0.22)	–0.13 (–0.41, 0.17)	–0.01 (–0.31, 0.28)	0.16 (–0.14, 0.43)
L-Arginine/ADMA	–0.18 (–0.45, 0.12)	0.03 (–0.26, 0.33)	–0.10 (–0.38, 0.21)	0.21 (–0.09, 0.48)
L-Arginine/ADMA	–0.22 (–0.48, 0.08)	–0.14 (–0.42, 0.16)	–0.23 (–0.49, 0.07)	0.01 (–0.29, 0.30)

Spearman rank correlation test. r_s, correlation coefficient and CI, confidence interval.
Abbreviations: ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; GDF, growth differentiation factor; SDMA, symmetric dimethylarginine; TNF, tumor necrosis factor.
[a]$P < 0.05$.

Table 4. Correlations of labor characteristics with levels of arginines and inflammation markers in women with preeclampsia ($n = 47$)

	Gestational length r_s (95% CI)	Cervical dilatation at sampling r_s (95% CI)	Duration of labor r_s (95% CI)	Duration of active 2nd stage of labor r_s (95% CI)
ADMA	–0.18 (–0.45, 0.13)	–0.29 (–0.54, 0.03)	–0.36 (–0.63, –0.02)[a]	–0.18 (–0.50, 0.17)
SDMA	–0.13 (–0.41, 0.17)	0.01 (–0.31, 0.31)	–0.35 (–0.62, –0.01)[a]	–0.14 (–0.46, 0.21)
L-Arginine	0.07 (–0.23, 0.36)	–0.02 (–0.33, 0.29)	–0.11 (–0.44, 0.24)	–0.03 (–0.37, 0.32)
L-Arginine/ADMA	0.15 (–0.15, 0.43)	0.21 (–0.10, 0.49)	0.21 (–0.14, 0.52)	0.11 (–0.24, 0.44)
L-Arginine/SDMA	0.16 (–0.15, 0.43)	–0.08 (–0.38, 0.24)	0.10 (–0.25, 0.43)	0.12 (–0.24, 0.44)
CRP	0.11 (–0.19, 0.39)	–0.14 (–0.43, 0.18)	–0.23 (–0.53, 0.11)	–0.17 (–0.49, 0.18)
TNF-	–0.8 (–0.37, 0.22)	0.01 (–0.30, 0.32)	0.06 (–0.28, 0.39)	0.06 (–0.29, 0.39)
TNF-R2	–0.27 (–0.53, 0.03)	0.08 (–0.23, 0.38)	–0.06 (–0.39, 0.28)	–0.3 (–0.37, 0.32)
GDF-15	–0.18 (–0.45, 0.12)	0.16 (–0.16, 0.44)	0.19 (–0.16, 0.50)	0.13 (–0.22, 0.45)

Spearman rank correlation test. r_s, correlation coefficient and CI, confidence interval.
Missing values: cervical dilatation at sampling ($n = 4$), duration of labor ($n = 12$), and duration of active 2nd stage of ($n = 12$).
Abbreviations: ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; GDF, growth differentiation factor; SDMA, symmetric dimethylarginine; TNF, tumor necrosis factor.
[a]$P = 0.03$.

week 35, had higher plasma levels of dimethylarginines than normotensive pregnancy.[4] Normotensive pregnancy, at the beginning, is associated with lower levels of dimethylarginines than nonpregnant women, to facilitate NO`s effect on the cardiovascular system, e.g., decrease blood pressure through decrease resistance and dilatation of vessels.[26] In a longitudinal study of dimethylarginines, Lopez-Alarcon *et al.* showed that plasma levels of ADMA increased gradually throughout pregnancy in women who developed preeclampsia, and the highest increment was seen at sampling 1 month before diagnosis of preeclampsia.[28] Similarly, in another prediction study, Khalil *et al.* showed that at sampling in pregnancy weeks 11–13, ADMA levels were higher in women who later developed preeclampsia.[29] However, studies about arginines during labor, especially at labor in women with preeclampsia, are rare. We recently performed 2 studies about arginines at labor. In the first study, we showed that term pregnancy with vaginal birth had higher plasma levels of dimethylarginines at labor compared with birth by elective cesarean section.[16] In the other study, we showed that preterm birth at labor had higher levels of dimethylarginines than term birth.[30] Consequently, findings of higher levels of dimethylarginines at labor in women with preeclampsia in this study are in line with our previous studies and confirm the theory of anti-NO effect of dimethylarginines in relation to labor. Most importantly, findings of this study might provide a biochemical explanation of the findings of our previous study and other studies of shorter duration of labor in women with hypertensive disorders of pregnancy compared with normotensive women.[17,18] Higher levels of L-arginine in the preeclampsia group might indicate a compensatory mechanism in response to elevated concentrations of ADMA, to facilitate NO synthesis, since it also requires L-arginine as a substrate.[31] Moreover, the absence of differences in the L-arginine/ADMA ratio could suggest a relatively stable level of L-arginine compared with dimethylarginines. Despite these stable higher levels of L-arginine in women with preeclampsia, NO synthesis is indirectly lower by inhibitory effect of dimethylarginines on the cellular uptake of the NO precursor L-arginine. Unlike our previous study,[17] in this study, we found that, in the preeclampsia group, both spontaneous onset and induction of labor had higher levels of ADMA and SDMA than normotensive spontaneous onset and induction of labor groups, respectively. Furthermore, mostly nonsignificant correlation of ADMA and SDMA to inflammation markers and negative correlation of duration of labor to ADMA and SDMA proved that ADMA and SDMA are independent of inflammation markers and are a major and novel determinant of duration of labor in women with preeclampsia.

Lower level of ADMA and SDMA in pregnancy compared with nonpregnant women enhances activities of NO and maintains relaxation of uterine muscles.[26] Thus, it has been hypothesized that the initiation of labor could be associated with downregulation of NO through upregulation of ADMA and SDMA. Currently, there is no longitudinal study regarding arginines throughout pregnancy and at labor. However, Holden *et al.* and Lopez-Alarcon *et al.* showed a successive increase of ADMA during pregnancy with a maximum increment in the third trimester or 1 month before the clinical manifestation of preeclampsia.[26,28] These findings might support the theory that higher levels of ADMA and SDMA at labor are not an effect of labor; rather, women with preeclampsia enter labor with an already elevated level of ADMA and SDMA.

Strengths and limitations

This is the first study, to our knowledge, that explores the potential role of arginines during labor in women with preeclampsia and provides novel findings regarding arginines. Similarly, data about inflammation markers allowed the analysis for potential correlations with arginines to be considered. High-risk pregnancies which could be associated with higher levels of arginines were excluded which enhanced the results applicable to a low-risk population. Finally, both groups were relatively homogeneous with respect to their sociodemographic characteristics, reducing confounding factors. Recall bias was minimized as the information on maternal and neonatal health care was collected prospectively.

A major limitation was that arginines were not monitored continuously during pregnancy, at labor, and at some postpartum time points, which should be addressed in future studies. Additionally, differences in cervical dilatation at time of sampling was also a limitation. However, differences in arginine levels were still significant after adjustment and stratification for cervical dilatation. Furthermore, the preeclampsia group consisted of women with both vaginal birth (75%) and cesarean section (25%) compared with only vaginal birth in the normotensive group. However, in our opinion, it is not relevant because 11/12 cesarean sections were performed on an emergency basis and blood samples were taken at similar cervical dilatation as in vaginal birth. The study population comprised of both nulliparous and parous women to obtain a sufficiently large sample, especially in the preeclampsia group. For similar reason, we choose to include both preterm and term labor in the preeclampsia group. In the normotensive group, we used the material from one of our previous studies of arginines in spontaneous onset and induction of term labor with vaginal birth,[16] which explains the higher frequency of spontaneous onset of labor and vaginal birth in this group. Finally, the nonsignificant difference in the duration of labor in the 2 groups could be explained by the relatively small sample size, and the demonstration of differences between the groups was not an aim of the study.

In conclusion, among women with preeclampsia, our novel findings of higher levels of dimethylarginines, negative correlation of dimethylarginines to the duration of labor, and absence of correlation of dimethylarginines to inflammation markers might support the theory that it is not inflammation but dimethylarginines which could be associated with shorter duration of labor in preeclampsia. Our findings may provide additional guidance to clinicians in decision-making between induction of labor and cesarean section in women with preeclampsia. However, further studies with longitudinal measurement of arginines in pregnancy and at labor in women with preeclampsia are warranted.

SUPPLEMENTARY DATA

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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