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Plasma levels of arginines at term pregnancy in relation to mode of onset of labor and mode of childbirth

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Abstract

Problem: The exact biochemical mechanisms that initiate labor are not yet fully understood. Nitric oxide is a potent relaxant of uterine smooth muscles until labor starts, and its precursor is L-arginine. Asymmetric (ADMA) and symmetric (SDMA) dimethylarginines, are potent NO-inhibitors. However, arginines (dimethylarginines and L-arginine) are scarcely studied in relation to labor and childbirth. We aimed to investigate arginines in women with spontaneous (SLVB) and induced (ILVB) term labor with vaginal birth and in women undergoing elective caesarean section (ECS).

Method of Study: Women at gestational week 16–18 were recruited to the population-based prospective cohort study BASIC at the Uppsala University Hospital, Sweden. Plasma samples taken at start of labor were analyzed for arginines, from SLVB ($n = 45$), ILVB ($n = 45$), and ECS ($n = 45$), using Ultra-High Performance Liquid Chromatography. Between-group differences were assessed using Kruskal–Wallis and Mann–Whitney U-test.

Results: Women with SLVB and ILVB had higher levels of ADMA ($p < .0001$), SDMA ($p < .05$) and lower L-arginines ($p < .01$), L-arginine/ADMA ($p < .0001$), and L-arginine/SDMA ($p < .01$, respectively $<.001$) compared to ECS. However, ILVB had higher ADMA ($p < .0001$) and lower L-arginine ($p < .01$), L-arginine/ADMA ($p < .0001$), and L-arginine/SDMA ($p < .01$) compared to SLVB. Results are adjusted for gestational length at birth and cervical dilatation at sampling.

Conclusion: Our novel findings of higher levels of dimethylarginines in term vaginal births compared to ECS give insights into the biochemical mechanisms of labor. These findings might also serve as a basis for further studies of arginines in complicated pregnancies and labor.

KEYWORDS
arginines, dimethylarginines, mode of childbirth, mode of onset of labor, term pregnancy

1 | INTRODUCTION

About 85%–95% of all births occur at term.[1] Term birth is defined as birth on gestational weeks 37 + 0 to 41 + 6.[2] According to the latest report from The Swedish National Board of Social Affairs and Health, about 75% of all vaginal births start spontaneously and the remaining 25% after induction of labor.[3] Further, about 18% of all births occur by cesarean section and 8% of all births are by elective cesarean section (ECS).[3] Labor involves increased uterine contractility, cervical dilation and rupture of the chorioamniotic membranes,[4] initiated by a physiological process of progesterone withdrawal, oxytocin initiation, and decidual activation.[4] Uterine contractility is the most recognizable sign of labor, and is accompanied by a shift in signaling from anti-inflammatory to pro-inflammatory pathways.[5] However, the exact biochemical mechanisms that initiate spontaneous labor are not yet fully understood.

Nitric oxide (NO) is a potent relaxant of uterine smooth muscles and is important to keep the uterus relaxed until onset of labor. L-arginine is the NO precursor. Dimethylarginines, asymmetric (ADMA), and symmetric (SDMA) are naturally occurring amino acids in plasma and generated by degradation of proteins methylated by arginine methyltransferase.[6] Dimethylarginines are direct endogenous inhibitors of nitric oxide synthase (NOS), causing decreased NO synthesis.[6] Dimethylarginines may also reduce NO synthesis indirectly by inhibiting the cellular uptake of the NO precursor L-arginine.[6] Thus, elevated levels of dimethylarginines are associated with decreased levels of NO and L-Arginine. Studies have shown that the L-arginine/dimethylarginines ratio, rather than only dimethylarginine levels is the key determinant of NOS activity,[7] and is a useful index for interpretation of effects of dimethylarginines.[8]

Plasma levels of dimethylarginines are increased in patients with cardiovascular disease[9,10] and preeclampsia.[11–13] Holden et al. showed that plasma levels of ADMA are lower in normotensive pregnancies compared to non-pregnant women and preeclamptic pregnancies.[14–16] However, dimethylarginines are scarcely studied in relation to maintaining human pregnancy and its possible role in the initiation of labor; existing evidence focused mainly on animal studies.[17] Thus, there is a growing need to examine arginine's role in human labor. Normal pregnancy is a state of tightly regulated inflammation, which shifts to predominantly pro-inflammatory state at the initiation of labor. Systemic inflammation markers have been studied extensively for their role and levels during initiation and maintenance of labor with varying and inconclusive results.[5,18–26]

The main aim of this study was to investigate arginine (ADMA, SDMA, and L-arginine) levels at labor, among women at term pregnancy with either spontaneous start of term labor resulting in vaginal birth (SLVB), induced labor resulting in vaginal birth (ILVB) or ECS. As a complement, we analyzed few conventional inflammation markers like C-reactive protein (CRP), tumor necrosis factor (TNF)-R1, TNF-R2, and growth differentiation factor (GDF)-15 to study correlations between these markers and arginines. We hypothesized that plasma levels of ADMA, SDMA, and inflammation markers will be higher and L-arginine, L-arginine/ADMA, and L-arginine/SDMA will be lower in women with SLVB and ILVB than in birth with ECS. Further, we hypothesized that, SLVB might be associated with higher levels of ADMA, SDMA and inflammation markers than ILVB. Another hypothesis was that inflammation markers might be positively correlated to ADMA and SDMA.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This study was undertaken as a sub-study based on the populations-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 cohort profile of the BASIC population.[27] Shortly, all pregnant women in the county of Uppsala are referred to Uppsala University Hospital for a routine ultrasound examination at gestational week 16—18, whereby they were offered written information and invited to the BASIC study. Participants gave their written consent separately for every modality (including blood sampling) they wish to take part in. Exclusion criteria were age below 18 years, insufficient ability to read and understand Swedish, protected identity, known bloodborne infections and/or non-viable pregnancy as diagnosed by routine ultrasound. Between September 2009 and November 2018, 31 687 pregnant women had been invited to the study of which 6478 (20.4%) choose to participate. Participants were followed-up at multiple points from baseline at gestational week 16—18 through 1-year postpartum. Biological samples were collected at several of these points and at childbirth. A parallel track of recruitment involved women that attended their preoperative appointment before giving birth via ECS. Participants in the study gave their consent to a review of their medical records. Information about sociodemographics, medical history concerning obstetrics/gynecology, current medication, pregnancy complications, labor characteristics (e.g., mode of onset, induction methods, cervical dilatation at blood sampling, gestational length at birth, duration of labor, expulsion time, mode of childbirth, and intrapartal bleeding) and neonatal information (e.g., infant gender, birth weight, and Apgar scores) were gathered prospectively. In order to be able to adjust for cervical dilation at blood sampling, which is a proxy for the relative timepoint in the childbirth process in which blood was collected, all participants in the ECS group were considered as having a cervical dilation of one cm.

Inclusion criteria for the present study were term birth and both nulliparous and parous women were included. Exclusion criteria were multifetal pregnancy, labor dystocia, chorioamnionitis, and hypertensive disorders of pregnancy as these conditions could potentially distort the levels of arginines and inflammation markers in the physiological birth process. Participants in the study were randomly selected as women with SLVB (n = 45), ILVB (n = 45), and ECS (n = 45).

2.2 | Blood sample collection and processing

Peripheral blood samples were collected upon the admission at the delivery ward at start of labor or before ECS. The process of collection, preparation and storage of plasma samples were performed as previously reported.[27] The samples were centrifuged within 2 h at 1500 g for 10 min and stored at −70°C until analysis.

2.3 | Analysis of arginines by ultra-high performance liquid chromatography

Plasma samples (100 μL) were prepared by spiking with 50 μL of a 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. The samples were protected against oxidation by the addition of 0.05 mg/mL butylated hydroxytoluene to the extraction solvent. The samples were vortexed for 10 min and centrifuged for 10 min at 13 000 g and the supernatant was evaporated under a steam of nitrogen and reconstituted in mobile phase A (see below in the next paragraph).

L-arginine, ADMA, and SDMA were analyzed with Ultra-High Performance Liquid Chromatography (UHPLC-MS/MS, Waters ACQUITY®, Waters Inc., Milford, MA) coupled to tandem quadruple mass spectrometry (XEVO® TQ-S, Waters Inc.). Chromatographic separation was achieved on a Premier HSS T3® column (150 mm, 3.0 mm, 1.7 μm, Waters, Milford, MA) at 40°C. Mobile phase A consisted of 20 mM ammonium formate in water and mobile phase B was methanol. Isocratic elution was performed with 10% of mobile phase B. The total analytical run time was less than two minutes. The mass spectrometric detection was performed using positive ionization electrospray ionization, with nitrogen and argon serving as desolvation and collision gas, respectively. Quantification was carried out 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). Calibration curves were constructed by linear regression of the peak area analyte/peak area internal standard as a function of analyte concentration. The calibration ranges were 10–5000 ng/mL for all analytes. All data were acquired, analyzed, and processed using the MassLynx™ 4.1 software (Waters, Milford, MA).

2.4 | Analysis of inflammation markers

Analyses of high sensitivity C-reactive protein (CRP) 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).

2.5 | Ethical approval

The original application was made in the context of the BASIC study, and was approved by the Regional Ethical Review Board in Uppsala (Reference number 2009/171). A supplementary application for the present study has been later approved by the Swedish Ethical Review Authority (Reference number 2020-00633).

2.6 | Statistical analysis

We have estimated that 45 women in SLVB and ILVB against 45 women in ECS will detect a 10% difference in main outcome variables (arginines) between the groups with a 90% power at 5% significance level. The results are presented as medians with interquartile ranges for continuous variables or in numbers and percentages for categorical variables. Spearman rank correlation test was used to test correlations of demographic data and clinical characteristics to arginines to identify covariates and to test correlations of arginines to inflammation markers. Kruskal–Wallis test or Mann–Whitney U-test were used when comparing the groups regarding continuous variables and Pearson Chi-square test were used comparing categorical variables. Differences between groups were adjusted for gestational length at birth and cervical dilatation at sampling by Univariate General Linear Model. In order to be able to adjust for cervical dilation at blood sampling, which is a proxy for the relative timepoint in the childbirth process in which blood was collected, all participants in the ECS group were considered as having a cervical dilation of 1 cm. The level of significance was set at $p < .05$. Statistical analysis was performed using the SPSS, version 27.0 (SPSS Inc. PASW statistics) for Windows software package.

3 | RESULTS

3.1 | Demographics and clinical characteristics

Demographic data and clinical characteristics of the study population are summarized in Table 1. No differences were found between the three groups regarding demographic data. There were differences between the three groups regarding clinical characteristics: gestational length at birth ($p < .001$), intrapartal bleeding ($p < .05$), and fetal birth weight ($p < .05$) (Table 1). Further, cervical dilatation at sampling was more advanced ($p < .0001$) and expulsion time was shorter ($p < .001$) in SLVB group than in ILVB. Five newborns in the ECS group compared to none in the SLVB and ILVB groups were admitted to the neonatal intensive care unit.

TABLE 1 Demographic and clinical characteristics of the study population.

	Spontaneous start of labor with vaginal birth ($n = 45$)	Induction of labor with vaginal birth ($n = 45$)	Elective cesarean section ($n = 45$)	(p-value)
Maternal Age, years	32 (28, 34)	33 (30, 35)	33 (31, 36)	.10
BMI at 2nd trimester, kg/m^2	24 (21, 25)	24 (22, 27)	24 (23, 26)	.37
Nulliparity	17 (37.8)	19 (42.2)	18 (40)	.91
Education				
Below university	14 (31.1)	9 (20)	18 (40)	.15
University	31 (68.9)	34 (75.6)	23 (51.1)	
Cohabitating	41 (91.1)	44 (97.8)	42 (93.3)	.62
Country of birth				
Scandinavia	42 (93.3)	42 (88.9)	37 (82.2)	.84
Others	3 (6.7)	5 (11.1)	4 (8.9)	
Employed	40 (88.9)	42 (93.3)	39 (86.7)	.53
Smoking at 2nd trimester	0 (0.0)	3 (6.7)	5 (11.1)	.06
Alcohol use at 2nd trimester	0 (0.0)	0 (0.0)	0 (0.0)	NA
Cervical dilatation at sampling, cm	5 (4, 8)	3 (2, 3)	NA	<.001
Gestational length at birth, weeks	40 (39, 41)	41 (40, 42)	39 (38, 40)	<.001
Mode of birth				
Spontaneous vaginal	42 (93.3)	37 (82.2)	NA	.11
Vacuum extraction	3 (6.7)	8 (17.8)	NA	
Duration of labor, minutes	158 (83, 365)	253 (114, 423)	NA	.14
Expulsion time, minutes	16 (9, 40)	22 (10, 40)	NA	<.001
Intrapartal bleedning, mL	350 (275, 400)	400 (300, 600)	425 (300, 700)	<.05
Intrapartal bleeding > 1000 mL	1 (2.2)	3 (6.7)	2 (4.4)	.69
Stillbirth, n (%)	0 (0)	0 (0)	0 (0)	NA
Infant gender, girl	21 (46.7)	20 (44.4)	16 (35.6)	.53
Infant birth weight, kg	3.6 (3.4, 3.9)	3.9 (3.4, 4,2)	3,7 (3.3, 4.0)	<.05
APGAR score <7 at 5 min	0 (0.0)	0 (0.0)	0 (0)	NA
NICU care	0 (0.0)	0 (0.0)	5 (11.1)	NA

Note: Values are presented as median (interquartile ranges) for continuous variable and number (percentage) for categorical variable.

Abbreviations: APGAR, appearance, pulse, grimace, activity and respiration; BMI, body mass index; NICU, neonatal intensive care unit.

*Comparison between groups were done by Kruskal–Wallis test (when comparing all three groups) or Mann–Whitney U test (when comparing two groups) for continuous variables and Pearson Chi-square test for categorical variables.

3.2 | Plasma levels of arginines in SLVB, ILVB, and ECS and differences between the groups

Plasma levels of arginines and inflammation markers are summarized in Table 2. Statistically significant differences were observed among the three study groups regarding levels of ADMA ($p < .001$), SDMA ($p < .05$), L-arginine ($p < .001$), L-arginine/ADMA ($p < .001$), and L-arginine/SDMA ($p < .001$), after adjustment for gestational length at birth and cervical dilatation at sampling. Regarding inflammation markers, significant differences were seen for TNF-R1 ($p < .05$) and GDF-15 ($p < .01$) (Table 2 and Table S1).

Further, differences in plasma levels of arginines according to mode of onset of labor and mode of childbirth were analyzed (Table S2 and Figure 1). In women with vaginal birth, both SLVB and ILVB, the levels of ADMA and SDMA were higher and L-arginine, L-arginine/ADMA, and L-arginine/SDMA were lower, compared to women with ECS. Further, women with ILVB had higher ADMA ($p < .0001$) and lower L-arginine ($p < .01$), L-arginine/ADMA ($p < .0001$), and L-arginine/SDMA ($p < .01$) ratios compared to women with SLVB. All comparisons were adjusted for gestational length at birth and cervical dilatation at sampling.

In a sub-analysis of women with ILVB, no differences in arginines were found in comparison of induction with prostaglandin to other induction methods like balloon catheter, amniotomy and oxytocin (data not presented). Because cervical dilatation at sampling was <6 cm in 25/45 women with SLVB compared to 44/45 women with ILVB, we performed a sub-analysis among all women with cervical dilatation <6 cm at blood sampling and found that women with ILVB still had higher

TABLE 2 Plasma levels of arginines and inflammation markers during childbirth/before elective cesarean section in the study population.

| Plasma levels of	Spontaneous start of labor with vaginal birth (n = 45)	Induction of labor with vaginal birth (n = 45)	Elective cesarean section (n = 45)	[a]p-value	[b]p-value
ADMA, μM/L	0.95 (0.83, 1.13)	1.42 (1.24, 1.74)	0.75 (0.69, 0.85)	<.0001	<.001
SDMA, μM/L	0.76 (0.66, 0.84)	0.82 (0.70, 0.94)	0.56 (0.46, 0.94)	<.01	<.05
L-arginine, μM/L	1030 (892, 1137)	784 (646, 1023)	1233 (1034, 1496)	<.001	<.001
L-arginine/ADMA	1039 (886, 1247)	504 (421, 779)	1620 (1368, 1946)	<.0001	<.001
L-arginine/SDMA	1318 (1100, 1614)	999 (803, 1248)	1907 (1419, 2887)	<.001	<.001
CRP, mg/L	4.59 (2.36, 11.43)	3.14 (2.12, 9.43)	3.16 (1.72, 5.18)	.26	.29
TNF-R1, pg/mL	834 (725, 1051)	1027 (762, 1250)	1105 (938, 1373)	<.001	<.05
TNF-R2, pg/mL	4832 (4083, 5536)	4722 (4037, 5502)	5042 (4118, 6266)	.36	.28
GDF-15, pg/mL	61 386 (41 669, 81 064)	70 446 (54 077, 100 970)	82 576 (49 749, 112 726)	<.01	<.01

Values are presented as median with interquartile ranges.

Abbreviations: ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; GDF, growth differentiation factor; SDMA, symmetric dimethylarginine; TNF, tumor necrosis factor.

[a]Differences between groups are analyzed by Kruskal–Wallis test.

[b]Differences are adjusted for gestational length at birth and cervical dilatation at sampling by Univariate General Linear Model.

FIGURE 1 Boxplot analysis to show the differences in (A) ADMA, asymmetric dimethylarginine (red box) and SDMA, symmetric dimethylarginine (blue box) and (B) L-arginine/ADMA (red box) and L-arginine/SDMA (blue box) in women with SLVB, spontaneous start of labor with vaginal birth; ILVB, induction of labor with vaginal birth and ES, elective caesarean section. 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. Black filled circles represent extreme values. (A) $p < .0001$, for comparison between a and e; between c and e and between a and c. $p < .05$ for comparison between b and f and between d and f; $p = .26$ between b and d. (B) $p < .0001$, for comparison between a and e; between c and e and between a and c. $p < .01$ for comparison between b and f; $p < .001$ between d and f and $p < .01$ between b and d.
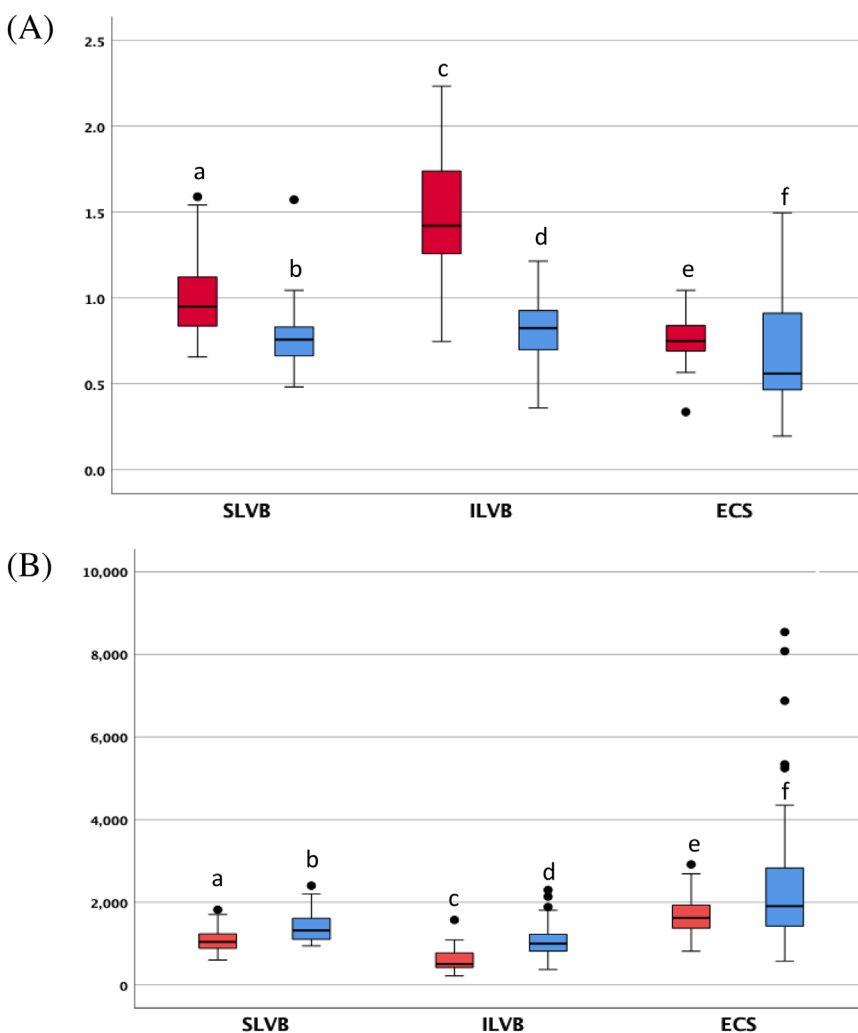
TABLE 3 Correlations between arginines and inflammation markers in women with spontaneous start of labor and vaginal birth.

	CRP r_s (95% CI)	TNF-R1 r_s (95% CI)	TNF-R2 r_s (95% CI)	GDF-15 r_s (95% CI)
ADMA	−0.15 (−0.43, 0.16)	0.04 (−0.27, 0.34)	0.13 (−0.18, 0.42)	0.20 (−0.11, 0.47)
SDMA	−0.29 (−0.55, 0.02)	0.14 (−0.17, 0.42)	0.11 (−0.20, 0.40)	0.19 (−0.12, 0.46)
L-Arginine	0.03 (−0.28, 0.33)	−0.03 (−0.44, 0.28)	0.10 (−0.21, 0.39)	−0.01 (−0.31, 0.30)
L-Arginine/ADMA	0.07 (−0.24, 0.36)	−0.17 (−0.45, 0.14)	−0.13 (−0.41, 0.18)	−0.12 (−0.40, 0.19)
L-Arginine/ADMA	0.17 (−0.14, 0.45)	−0.21 (−0.48, 0.10)	−0.07 (−0.36, 0.24)	−0.18 (−0.45, 0.13)

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.

ADMA ($p < .0001$) and lower L-arginine ($p < .001$), L-arginine/ADMA, ($p < .001$), and L-arginine/SDMA ($p < .001$) ratios than SLVB.

3.3 | Plasma levels of inflammation markers and their correlation to arginines in SLVB, ILVB, and ECS

Regarding inflammation markers, the only differences were that TNF-R1 ($p < .001$) and GDF-15 ($p < .05$) were lower in SLVB than in women with ECS (Table S2)

In the correlation analyses between arginines and inflammation markers, no significant correlations have been detected in any of the three groups (Table 3, correlation analyses only in women with SLVB are presented).

4 | DISCUSSION

To our knowledge, this is the first study assessing arginine levels around the time of human labor at term pregnancy. We found that vaginal birth, both SLVB and ILVB, was associated with higher plasma levels of ADMA and SDMA and lower L-arginine, L-arginine/ADMA, and L-arginine/SDMA ratios compared to ECS, even taking into account gestational length at birth and cervical dilation at sampling. Furthermore, levels of arginines were not correlated to inflammation markers around the time of labor. Hence, this study provides helpful information to further understand the biochemical mechanism of human labor.

Accumulating evidence suggests that human labor is a complex mechanism involving, among others, inflammatory pathways. However, the exact biochemical mechanisms that initiate and maintain labor are not yet fully understood. Uterine quiescence during pregnancy, until start of labor, is maintained mainly by NO, which is a potent relaxant of uterine smooth muscles. It has been hypothesized that lower level of dimethylarginines in pregnancy compared to non-pregnant women facilitate NO and maintain uterine quiescence.[14] Thus, it can be hypothesized that the initiation of labor is associated with upregulation of dimethylarginines that in turn downregulate L-arginine and NO to initiate uterine smooth muscles contraction. It has been shown that inducible NOS expression in myocytes from human samples fell by 75% from midgestation to term and was barely detectable in term labor.[17,28] These findings supports the findings of Holden et al that plasma lev-

els of dimethylarginines in uncomplicated pregnancy increased from first trimester to second trimester with a further increase in third trimester. Further, Pettersson et al. showed that plasma levels of ADMA increased significantly from gestational week 36 to about three days postpartum.[16] However, there is a big knowledge gap about what happened to arginines in human labor. Thus, the result of our study is a novel addition in understanding the mechanism of labor further, especially in different types term labor, that is, spontaneous- or induced labor with vaginal birth compared to ECS. All births by ECS in our study was done before the onset of labor. Hence, our findings of higher levels of ADMA and SDMA and lower L-arginine, L-arginine/ADMA, and L-arginine/SDMA ratios in SLVB and ILVB compared to ECS is completely logical. However, findings of arginines in ILVB compared to SLVB were different from our hypothesis and could be explained by the theory that, to succeed with vaginal birth after induction of labour, L-arginie and NO must be downregulated more by higher levels of ADMA and SDMA. However, to understand this theory better it is of interest to study arginines at term labor in women with induction of labor resulting in childbirth by emergency caesarean section compared to ILVB and SLVB.

Normal pregnancy is a state of tightly regulated inflammation that shifts to predominantly pro-inflammatory state at the initiation of labor. Systemic inflammation markers have been studied extensively for their role and levels during initiation and maintenance of labor with varying and inconclusive results.[5,18–26] In a study of prediction of spontaneous onset labor Stelzer et al. showed that certain aspects of inflammatory reaction are toned down before labor starts to prepare the mother's immune system for the next phase of the labor.[19] Our results of no difference and sometimes even lower levels of analyzed traditional inflammatory markers in SLVB and ILVB compared to birth by ECS is in line with the findings of above study and confirms it again that these markers are not so useful in explaining the biochemical mechanism of labor. In a study of spontaneous onset contra induction of term labor Cierny et al. showed that excess inflammation can be detrimental to efficient labor progress and conclude that relationship between inflammatory markers and term labor performance is complex.[29] Studies about inflammation at induced term labor are very scarce. Our results of no difference in inflammatory markers in women with ILVB compared to SLVB, although we had limited our analyses to participants with similar cervical dilatation at sampling is a new addition in this field. However, plasma levels of arginines were still higher

in the above comparison. Further, non-significant and sometimes even non-logical correlations between arginines and inflammation markers indicate that findings of arginines are independent of inflammation at term labor, regarding mode of onset and mode of childbirth.

4.1 | Strengths and limitations

To our knowledge, this is the first study on arginines levels around the time of human labor. The generalizability to a low-risk population was enhanced as risk pregnancies, with suspected higher levels of arginines, were excluded. Another strength was that the information on maternal and neonatal health care was collected prospectively, limiting recall bias. A major limitation was that arginines were not monitored continuously during pregnancy, at labor and at some postpartum time-point, which should be addressed in future studies. Another limitation was the differences in cervical dilatation at sampling. However, differences in arginine levels were still significant after adjustment and stratification for cervical dilatation at sampling.

5 | CONCLUSION

Our novel findings of differences in the plasma levels of arginines at term labor, according to onset and mode of childbirth after uncomplicated pregnancy, help us to further understand the biochemical mechanism of human labor that should be reconsidered. Further, it seems that arginines at labor are independent of several inflammatory markers. These findings might serve as a basis for further studies of arginines at labor in complicated pregnancies.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The dataset for this study is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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