



Original Article

Birth by cesarean section is associated with elevated neonatal plasma levels of dimethylarginines

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Abstract **Background:** This study was undertaken to compare the effects of vaginal delivery and cesarean section on the L-arginine-nitric oxide system by measuring levels of L-arginine, an endogenous nitric oxide synthase antagonist asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) in the cord blood and postnatally. **Methods:** Plasma samples were obtained from the umbilical vein and artery at birth and from peripheral venous blood on the second postnatal day in 30 full-term newborn infants: 10 born vaginally and 20 born by cesarean section. **Results:** After vaginal delivery, ADMA concentration was higher in the umbilical vein than in the umbilical artery (mean 1.06 vs 0.90 $\mu\text{mol/L}$ [$P = 0.027$]); and ADMA level fell after birth to 0.66 $\mu\text{mol/L}$ on the second postnatal day ($P = 0.007$ vs umbilical artery). Newborns born by cesarean section had similar ADMA levels in umbilical arterial and venous blood, 1.19 and 1.18 $\mu\text{mol/L}$, and the ADMA level fell to 0.84 $\mu\text{mol/L}$ by the second postnatal day ($P < 0.001$). Vaginal birth induced neither significant umbilical venoarterial difference nor a postnatal fall in SDMA. After cesarean section, SDMA was essentially the same in umbilical vein, umbilical artery and postnatal peripheral vein samples. At 2 days of age, both ADMA and SDMA levels stayed higher in infants born by cesarean section than in vaginally born infants. **Conclusions:** ADMA level falls after both vaginal and cesarean birth, whereas SDMA level does not. The higher ADMA level after cesarean birth compared with vaginal birth may contribute to decreased nitric oxide production and bioavailability in neonatal vascular beds.

Key words cesarean section, dimethylarginine, neonate, vaginal delivery.

Cesarean section after near term or term gestation is frequently associated with neonatal respiratory morbidities, including transient tachypnea of the newborn (TTN), neonatal respiratory distress syndrome (RDS) and primary pulmonary hypertension (PPH).¹ The stress experienced by the fetus and neonate during the normal vaginal birth results in a surge of endogenous steroids and catecholamines that plays a critical role in lung fluid clearance and surfactant production.² Newborn infants born by elective cesarean section are deprived of these hormonal changes. In these infants, lung fluid clearance may be delayed, and the production and release of surfactant may be reduced, making them prone to develop TTN or RDS. The reason for increased risk of PPH after cesarean section is less apparent.^{3,4} Evidence indicates that the endothelium-derived relaxing factor, nitric oxide (NO), may be implicated in maintaining pulmonary circulation after birth.⁵

The present study was designed to assess the effects of mode of delivery on the L-arginine-NO system by determining L-arginine, asymmetric dimethylarginine (ADMA) and symmetric dimethyl-

arginine (SDMA) plasma concentrations in blood samples obtained from the umbilical artery and vein at birth and from the peripheral blood of the infant on the second postnatal day.

Methods

Two groups of healthy full-term newborn infants born after uncomplicated, singleton pregnancies with cephalic presentation were included in the study. Group I consisted of 10 infants whose mother underwent spontaneous vaginal delivery with epidural analgesia, whereas Group II comprised 20 infants delivered by cesarean section because of cephalopelvic disproportion ($n = 11$) or previous cesarean section ($n = 9$). The mothers were on normal diets and were not receiving diuretic therapy. None had a history of renal disease, hypertension, diabetes mellitus, pre-eclampsia, infection, labor induction or premature rupture of fetal membranes.

Maternal age, gestational age, birthweight, sex and 1- and 5-min Apgar scores did not differ between the two groups (Table 1). None of the newborns had clinical or laboratory evidence of perinatal asphyxia, infection, or respiratory distress syndrome. All of them received routine neonatal care.

Blood was taken from the umbilical artery and vein following clamping of the cord but prior to the infant's first breath. Blood

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Table 1 Clinical characteristics of the studied neonates

	Normal vaginal birth	Cesarean section
Cases	10	20
Gestational age (weeks) mean (SD)	39.4 ± 0.97	39.5 ± 1.5
Birthweight (g) mean (SD)	3330 ± 405	3526 ± 516
1-min Apgar score, range	7–9	8–9
5-min Apgar score, range	9–10	9–10
Female/male	3/7	9/11
Maternal age (years) mean (SD)	28.6 ± 4.35	31.4 ± 3.88

samples were also obtained by peripheral venipuncture on the second day of life. After the samples were drawn into syringes they were immediately injected into ethylenediaminetetraacetic acid tubes in ice water. Plasma was separated in a refrigerated centrifuge and stored at -20°C until analysis.

Plasma concentrations of L-arginine, ADMA and SDMA were measured by liquid chromatography-electrospray tandem mass spectrometry.⁶ The samples were prepared by protein precipitation. Separation was done by liquid chromatography on a $150 \times 3\text{-mm}$ silica column with an isocratic mobile phase consisting of water-acetonitrile-trifluoroacetic acid-propionic acid (100:900:0.25:10 by volume). The chromatographic run time was 7 min. The inter- and intra-assay variation was less than 8%.⁶

Statistical evaluation was done by using two-tailed, paired Student's *t*-test after confirming the normal distribution of the data using the Kolmogoro–Smirnov test.

Written informed consent was obtained from the mothers for blood sampling, and the study was approved by the Institutional Ethics Committee.

Results

After spontaneous vaginal birth, plasma L-arginine level was higher in the umbilical vein than in the umbilical artery (73.9 vs 63.0 $\mu\text{mol/L}$ [$P = 0.004$]) (Table 2 and Fig. 1). Postnatally, L-arginine concentration rapidly fell to 34.8 $\mu\text{mol/L}$ by the second day of life ($P = 0.002$). Infants delivered by cesarean section also had elevated L-arginine in the cord blood, but no discernible difference could be detected between its level in umbilical vein and artery. The mode of delivery had no apparent influence on the postnatal fall in L-arginine. L-arginine values in umbilical artery, umbilical vein, and peripheral blood were higher after cesarean birth than after vaginal birth.

Table 2 Effects of the mode of delivery on the plasma L-arginine, ADMA, and SDMA concentrations

	Umbilical artery (A1) [†]	Umbilical vein (V1) [†]	Postnatal vein (V2) [†]	AVD [‡] (<i>P</i>)		Postnatal fall [§] (<i>P</i>)	
Vaginal delivery							
Arginine	63.03 ± 13.14	73.89 ± 13.83	34.78 ± 13.66	-10.86	0.005	28.25	(0.002)
ADMA	0.90 ± 0.28	1.06 ± 0.24	0.66 ± 0.16	-0.16	0.027	0.25	(0.007)
SDMA	1.31 ± 0.32	1.45 ± 0.25	1.32 ± 0.22	-0.13	0.034	-0.01	(0.932) NS
Cesarean section							
Arginine	85.06 ± 26.85	84.69 ± 24.24	46.88 ± 19.24	0.37	0.929 NS	38.18	(<0.001)
ADMA	1.19 ± 0.20	1.18 ± 0.17	0.84 ± 0.14	0.01	0.770 NS	0.35	(<0.001)
SDMA	2.06 ± 0.47	2.05 ± 0.42	2.03 ± 0.42	0.01	0.844 NS	0.03	(0.778) NS

[†]Mean ($\mu\text{mol/L}$) ± SD. [‡]AVD = A1 - V1. [§]Postnatal fall = A1 - V2. ADMA, asymmetric dimethylarginine; AVD, arteriovenous differences; NS, not significant; SDMA, symmetric dimethylarginine.

The response pattern of ADMA to vaginal birth proved to be quite similar to L-arginine. After spontaneous vaginal birth, ADMA concentration was higher in the umbilical vein than in the umbilical artery (mean 1.06 vs 0.90 $\mu\text{mol/L}$ [$P = 0.027$]); ADMA level fell after birth to 0.66 $\mu\text{mol/L}$ on the second postnatal day ($P = 0.007$ compared with umbilical vein). Newborns born by cesarean section had similar ADMA levels in umbilical arterial and venous blood, 1.19 and 1.18 $\mu\text{mol/L}$, and the ADMA level fell to 0.84 $\mu\text{mol/L}$ by the second postnatal day ($P < 0.001$). The ADMA level on postnatal day 2 was higher in the cesarean section infants than in the vaginally born infants ($P = 0.003$). Both umbilical artery and umbilical vein ADMA levels were higher after cesarean birth than after vaginal birth.

Vaginal birth induced neither significant venoarterial difference in the umbilical vessels nor a postnatal fall in SDMA. After cesarean section, SDMA was essentially the same in umbilical vein, umbilical artery and postnatal peripheral vein samples. On postnatal day 2, SDMA level was higher among infants born by cesarean section than among those born vaginally ($P < 0.001$).

The different trend in ADMA levels after vaginal versus cesarean birth is further emphasized by the clear association of ADMA levels in umbilical artery with those in postnatal venous plasma after vaginal delivery ($P < 0.05$), which could not be observed following cesarean section.

Vaginal birth induced no significant venoarterial differences in cord blood SDMA levels and they remained unchanged postnatally. In response to cesarean section, essentially the same SDMA pattern was seen. However, SDMA levels from all sources and at all times were higher after cesarean delivery than they were after vaginal birth.

Discussion

The present and the former study demonstrated that the elements of L-arginine-NO system ADMA and SDMA are markedly elevated in the fetoplacental circulation after term gestation irrespective of the mode of delivery. Vaginal birth, however, generated a significant venoarterial gradient of L-arginine and ADMA in umbilical vessels and there was an early, rapid fall by the second postnatal day.⁷ Delivery by cesarean section failed to establish such a gradient, L-arginine and ADMA levels remained elevated in the umbilical artery, and their postnatal fall proved to be less pronounced. Interestingly, elective cesarean section

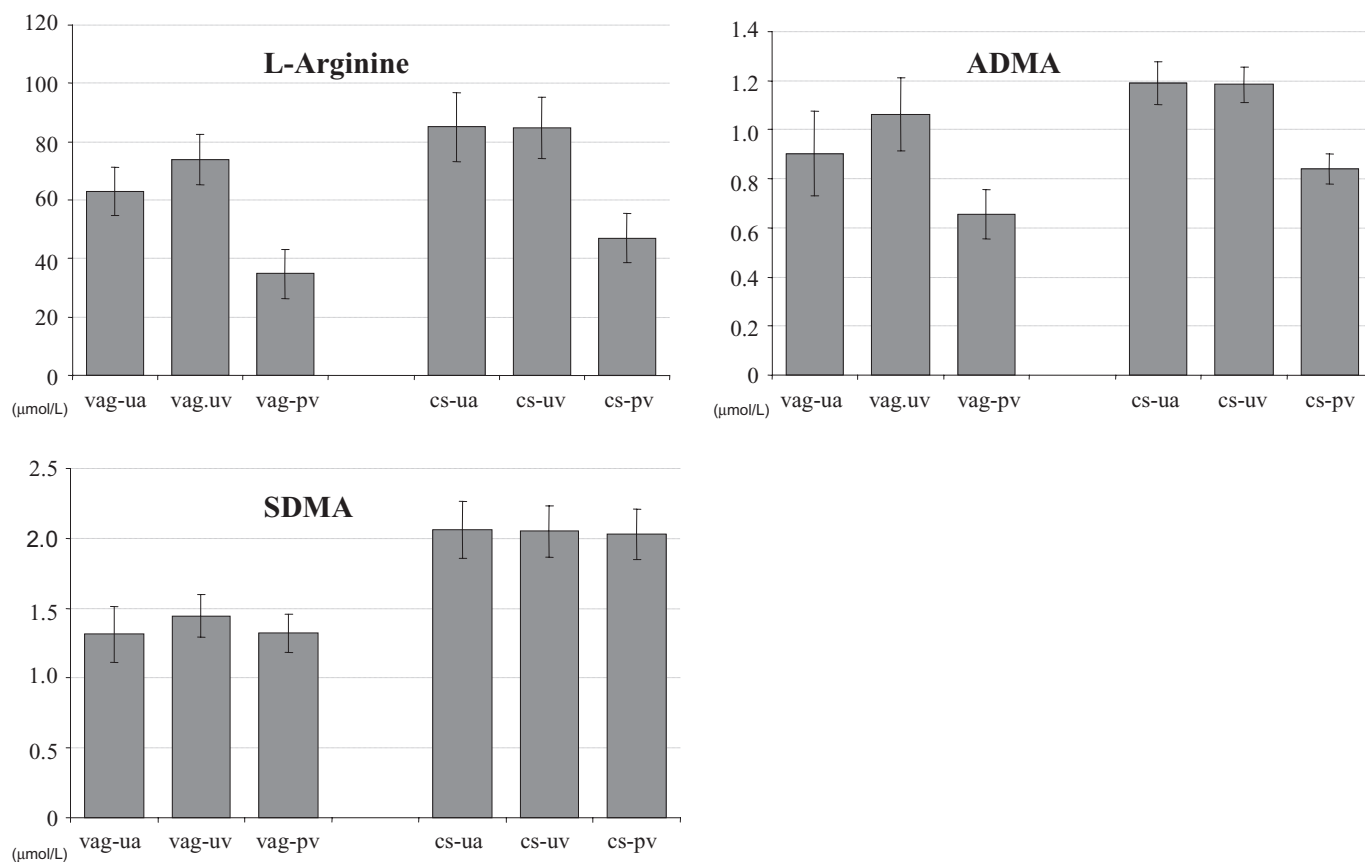


Fig. 1 Plasma L-arginine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) concentrations (mean with 95% confidence interval in $\mu\text{mol/L}$) in the umbilical vein and artery and in the peripheral vein on the second postnatal day in the mature neonate (vaginal delivery vs cesarean section). cs, mature neonates with cesarean section; pv, peripheral venous plasma on second day of life; ua, umbilical artery plasma; uv, umbilical venous plasma; vag, mature neonates with vaginal delivery.

resulted in higher SDMA levels in cord vessels than vaginal birth, and this difference could still be seen on the second day of life.

Our observations support the concept that the l-arginine-NO system is excessively activated in the fetoplacental unit and the increased NO generation contributes to the low vascular resistance and high blood flow characteristic of this system.^{8,9} The reason for and the role of increased ADMA levels in the fetoplacental circulation is not clearly established. ADMA is formed by the methylation of l-arginine residues in proteins and is released during proteolysis.¹⁰ Most ADMA is metabolized by the specific enzyme dimethylarginine dimethylaminohydrolase (DDAH) into l-citrulline and dimethylamine, and only about 10% of ADMA is excreted by the kidney.¹¹ It has been shown to act as an endogenous competitive inhibitor of NO synthase (NOS), which enables it to control endothelial NO production and to maintain vascular homeostasis.¹² It has been claimed that the high rate of protein turnover and the subsequent accelerated proteolysis of proteins containing methylated arginine residues in the uteroplacental unit may account for the increased ADMA levels in the umbilical vein.¹³ It is also to be considered that NO overproduction itself may regulate ADMA levels through a negative feedback loop by inhibiting the activity of DDAH.¹⁴ Furthermore, labor and delivery have been documented to be associated with

release of vasoactive hormones, including prostaglandins,¹⁵ endothelin,¹⁶ kallikrein-bradykinin¹⁷ and angiotensin,¹⁸ all known to stimulate NO production.

Labor-induced increased activity of the renin-angiotensin system may also be implicated in the ADMA accumulation in umbilical vein. In support of this contention, treatment with angiotensin converting enzyme inhibitor has been shown to reduce ADMA concentration in various clinical and experimental conditions.^{19,20}

The major finding of this study is that after cesarean section, ADMA levels in umbilical artery remained elevated and they fell postnatally at a slower rate than after vaginal birth. It seems to be unlikely that fetal/neonatal ADMA production is enhanced by cesarean section, but rather the higher ADMA levels may be attributed to decreased elimination by DDAH. In fact, there are reports that labor provides protection for the fetus against oxidative stress in early life and this protection is lost after elective cesarean delivery.²¹ Furthermore, some proinflammatory cytokines have been found to increase in the cord blood of the neonate born by elective cesarean section.²²

The effects of the mode of delivery on the perinatal changes in plasma SDMA levels were also addressed in this study. SDMA is an inactive structural isomer of ADMA that does not interfere

directly with NOS activity and is exclusively eliminated by renal excretion. We found no venoarterial differences or postnatal fall in SDMA either after cesarean section or vaginal delivery following labor. However, SDMA was significantly higher when the full-term infants were born by elective cesarean section rather than vaginally. We suggest that the clear dissociation of SDMA from ADMA may not be accounted for by the differences in their production rate but rather it may be attributed to differences in their elimination.²³ DDAH is expressed in the placenta and fetal tissues, and its protein level and activity have been shown to increase in the early postnatal period providing effective enzymatic breakdown of ADMA.^{24–26} In contrast, renal elimination of SDMA by the immature kidney is markedly reduced, and the excretory capacity of fetal/neonatal kidney is further compromised by the circulatory adaptation to elective cesarean section.

Conclusion

Our results provide evidence that cesarean section is associated with delayed perinatal decline of plasma ADMA levels, which may result in decreased NO bioavailability in fetal/neonatal vascular bed and may contribute to the disturbances of adaptation. Interestingly, the mode of birth does not seem to affect methylarginine concentrations among premature infants.^{27,28} Further studies are needed to provide more information regarding the pathophysiological and clinical significance of the ADMA-related endothelial dysfunction in mature neonates.

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