The importance of the arginine/ADMA ratio on the bioavailability of nitric oxide and organ function in critically ill patients

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Chapter 3

Plasma ADMA concentrations at birth and mechanical ventilation in preterm infants: a prospective pilot study

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Abstract

Introduction
Nitric oxide (NO) produced in the lung is an important mediator of normal lung development, vascular smooth muscle relaxation, and ventilation perfusion matching. NO is synthesized from arginine by the action of NO-synthase (NOS). Asymmetric dimethylarginine (ADMA), an endogenous derivate of arginine, inhibits NOS and is thereby a determinant of NO synthesis. We compared ADMA and arginine levels in preterm infants requiring mechanical ventilation with preterm infants who did not require mechanical ventilation and determined the relation between ADMA and the length of mechanical ventilation in these infants.

Methods
Thirty preterm infants, mean (sd) gestational age 29.3 (1.7) weeks and birth weight 1340 (350) gram, of the Neonatal Intensive Care Unit of the VU University Medical Center were included. ADMA and arginine were measured in umbilical cord blood and the length of mechanical ventilation (days) was registered.

Results
Gestational age and birth weight were significantly smaller in infants requiring mechanical ventilation, but were not significantly correlated with plasma ADMA concentration after birth. Plasma ADMA concentrations were significantly higher in infants who required mechanical ventilation than in infants who did not require mechanical ventilation (1.53 ± 0.23 and 1.37 ± 0.14 μmol/L, respectively; p=0.036). ADMA concentration was significantly related to length of mechanical ventilation (B=3.4; 95%CI:1.1-5.6; p=0.006), also after adjustment for gestational age (B=2.3; 95%CI:0.4-4.2; p=0.024).

Conclusions
Preterm infants who require mechanical ventilation have increased ADMA levels compared to non-ventilated preterm infants. ADMA levels at birth are related to the length of mechanical ventilation. An increased ADMA concentration could reduce NO synthesis, which could lead to insufficient gas exchange and, consequently, a longer period of mechanical ventilation.

Introduction

Normal development and maturation of the lungs of preterm infants is mediated in part by nitric oxide (NO) (1-3). NO is synthesized from the amino acid arginine by the action of NO-synthases (NOS), a family of enzymes with endothelial, neuronal, and inducible isoforms (4). In addition, NO produced in the lung is an important mediator of vascular smooth muscle relaxation, ventilation perfusion matching, neurotransmission, host defence and bacteriostasis, mucociliary clearance and airway mucus secretion (5).
Since NO plays an essential role in lung development and maturation, studies were performed investigating the efficacy of inhaled NO.
In animal studies, inhaled NO reduced lung inflammation, improved surfactant function, attenuated hyperoxic lung injury and promoted lung growth (6-10).
In preterm infants with the respiratory distress syndrome, inhaled NO reduced the incidence of chronic lung disease and death (11). In addition, mechanical ventilation with NO decreased pulmonary hypertension, improved oxygenation and reduced the need for extracorporeal membrane oxygenation in term infants with persistent pulmonary hypertension (12). Initial reports showed that inhaled NO improved oxygenation in preterm infants with severe respiratory failure, and with developing or established bronchopulmonary dysplasia (BPD) and suggested that early treatment may decrease the risk of lung injury (13-15).
Although these are promising effects, currently there is insufficient evidence about the long-term safety and efficacy to recommend the routine use of inhaled NO (16;17).
Recent insights into NO metabolism have shown an important role of endogenously produced inhibitors of NOS, in particular asymmetric dimethylarginine (ADMA) (18). ADMA as well as symmetric dimethylarginine (SDMA), are synthesized when arginine residues in proteins are methylated by the action of protein arginine methyltransferases (PRMT) and are continuously released from the protein pool during the process of protein turnover (19). ADMA is an endogenous inhibitor of all isoforms of NOS, while SDMA is not.
ADMA is increased in both term and preterm infants compared to adults (20-22). In preterm infants the ADMA plasma concentration is even higher than in term infants (23;24). We hypothesize that preterm infants have elevated inhibition of NO synthesis, induced by increased ADMA levels, which could be associated with reduced pulmonary function and the need for mechanical ventilation.
The aims of this study were (1) to compare ADMA and arginine levels in preterm infants requiring mechanical ventilation after birth with preterm infants who do not require mechanical ventilation, and (2) to determine the relation between ADMA and arginine and the length of mechanical ventilation in these infants.

Methods

Study subjects

Thirty infants admitted between January 2002 and July 2003 to the level III Neonatal Intensive Care Unit (NICU) of the VU University Medical Center, Amsterdam, The Netherlands, entered the study. Inclusion criteria were gestational age of <32 weeks and/or birth weight of <1500 gram, appropriate for gestational age (birth weight >10^{th} percentile (25)), and written informed consent of their parents. Exclusion criteria were major congenital or chromosomal abnormalities. The study was approved by the institutional review board and the hospital ethics committee.

Study design

Directly after delivery, 0.3 ml venous umbilical cord blood was obtained.
During the admission at the NICU, the length of mechanical ventilation (in days) was registered. The need for mechanical ventilation was defined as mechanical ventilation for at least 24 hours, to exclude infants who were mechanically ventilated because of maternal medication.

Measurement of ADMA, arginine and SDMA

The umbilical cord blood samples were immediately placed on ice and centrifuged at 3000 rpm for 10 min at 4°C. Plasma was immediately put in liquid nitrogen, and stored at -80°C before analysis. The concentration of ADMA, arginine and SDMA were determined by high-performance liquid chromatography (HPLC) as described previously.(26) In brief, solid-phase extraction on polymeric cation-exchange columns was performed after addition of monomethylarginine as the internal standard. After derivatization with ortho-phtaldialdehyde reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection. For all analytes the intra- and inter-assay coefficients of variation were less than 1.2 and 3.0%, respectively. The arginine/ADMA molar ratio was calculated.

Statistical analysis

Data are presented as mean (±SD) or median (range) depending on the distribution. Distribution of the data was analysed by means of histograms and Q-Q plots.
Differences between baseline characteristics and plasma amino acid concentrations of preterm infants with and without mechanical ventilation were analyzed by Mann-Whitney U, chi-square or an independent t-test. To determine factors influencing the ADMA concentration, a stepwise linear regression analysis was performed, in which sex, gestational age and mechanical ventilation (yes/no) were considered as potential explanatory variables.

Linear regression analyses were performed to determine the relation between length of mechanical ventilation during the admission at the NICU (days) and plasma concentrations of ADMA, arginine, and the arginine/ADMA molar ratio. Since gestational age is highly associated with mechanical ventilation, we adjusted the linear regression analyses for gestational age. A two-tailed P-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 14.0 (SPSS Inc, Chicago, IL).

Results

Of the infants included in the study, 15 infants required mechanical ventilation after birth. Gestational age, birth weight, CRIB-score, administration of surfactant, IRDS, obstetric diagnosis, number of infections and length of stay at the NICU were significantly different between preterm infants with and without mechanical ventilation (Table 1).

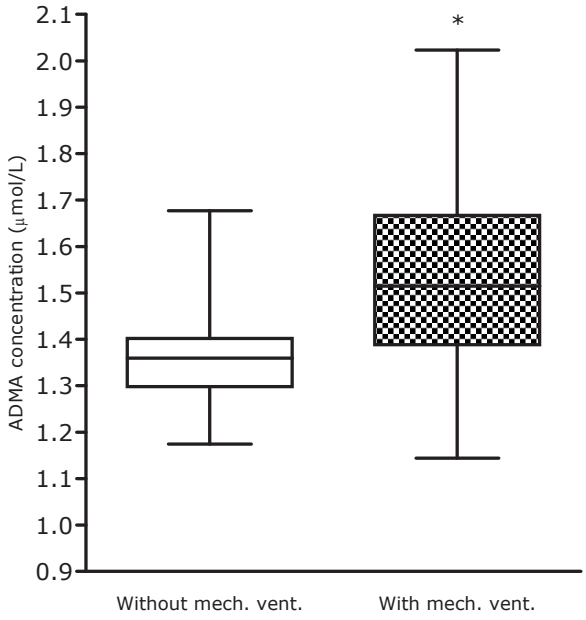
Table 1. Baseline characteristics of preterm infants without and with mechanical ventilation.

	Without mechanical ventilation (N=15)	With mechanical ventilation (N=15)	p-value
Male : Female	7 : 8	8 : 7	0.72
Gestational age (weeks) [1]	30 (26.7 – 31.7)	28 (25.4 - 31.2)	0.002
Birth weight (gram) [1]	1470 (1076 – 2255)	1164 (775 – 1755)	0.002
Head circumference (cm)	28.7 ± 1.7	27 ± 4.0	0.32
Antenatal corticosteroids [n (%)]	13 (87)	13 (87)	1.0
Apgar at 5 minutes <6 [n (%)]	2 (13)	-	0.14
pH umbilical artery <7.1 [n (%)]	1 (7)	-	0.31
CRIB*[1]	1 (0-1)	2 (1 – 10)	<0.001
Surfactant [n (%)]	-	11 (73)	<0.001
IRDS¶ [n (%)]			<0.001
No	10 (67)	1 (7)	
Grade I	5 (33)	2 (13)	
Grade II	-	10 (67)	
Grade III	-	2 (13)	
Obstetric diagnoses [n (%)]			0.02
Chorioamnionitis	6 (40)	7 (47)	
PE, E or HELLP†	-	5 (33)	
Other	9 (60)	3 (20)	
Death [n (%)]	-	2 (13)	0.14
≥ 1 serious infection [n (%)]	3 (20)	10 (67)	0.01
Length of mechanical ventilation (days)[1]	-	9 (2-17)	
Stay at the NICU (days)[1]	6 (1-48)	27 (4-66)	0.005

* Clinical Risk Index for Babies, ¶ Idiopathic Respiratory Distress Syndrome.
† Pre-eclampsia, Eclampsia and HELLP-syndrome, [1] Median and range.

The ADMA concentration was significantly higher in infants who required mechanical ventilation than in infants who did not require mechanical ventilation (1.53±0.23 vs. 1.37±0.14 µmol/L, respectively; p=0.036, table 2, figure 1). Arginine, SDMA and the arginine/ADMA molar ratio were not different in both groups (Table 2).

Figure 1. Umbilical cord plasma concentration of ADMA in infants with and without mechanical ventilation.



*p = 0.036

Table 2. Umbilical cord plasma concentrations of ADMA, arginine, SDMA and the arginine/ADMA molar ratio in infants without and with mechanical ventilation.

	Without mechanical ventilation (N=15)	*With mechanical ventilation (N=15)*	*p-value**
ADMA (µmol/L)	1.37 ± 0.14	1.53 ± 0.23	0.036
Arginine (µmol/L)	93.7 ± 27	84.7 ± 32	0.43
SDMA (µmol/L)	1.43 ± 0.47	1.67 ± 0.40	0.72
Arginine/ADMA ratio	70 ± 20	55 ± 18	0.082

Data are presented as mean ± SD. *p-value from t-test.

Gestational age, birth weight and sex were not correlated with ADMA concentration. In a stepwise multiple linear regression model, mechanical ventilation, but not gestational age or sex, proved to be an independent determinant of plasma ADMA concentration (data not shown).

In preterm infants requiring mechanical ventilation, the ADMA concentration was positively related to the length of mechanical ventilation (regression coefficient =3.4, p=0.006, table 3 and figure 2). This indicates that every 1 standard deviation increase of ADMA was associated with an increase of 3.4 days of mechanical ventilation. Arginine, SDMA and the arginine/ADMA molar ratio were not related to length of mechanical ventilation (Table 3).

Table 3. Univariate linear regression analyses of the relation between umbilical cord plasma concentrations of ADMA, arginine, SDMA and arginine/ADMA molar ratio, and length of mechanical ventilation in preterm infants (N=15).

	Crude analysis			*Adjusted analysis**		
Independent Variables	β¶	95% CI	p-value	β¶	95% CI	p-value
ADMA (µmol/L)	3.4	1.1 to 5.6	0.006#	2.3	0.4 to 4.2	0.024$
Arginine (µmol/L)	1.8	-1.0 to 4.5	0.18	1.7	-0.1 to 3.5	0.067
SDMA (µmol/L)	-1.2	-4.2 to 1.6	0.37	0.4	-2.0 to 2.8	0.74
Arginine/ADMA Ratio	0.4	-2.5 to 3.2	0.79	0.9	-1.2 to 3.1	0.36

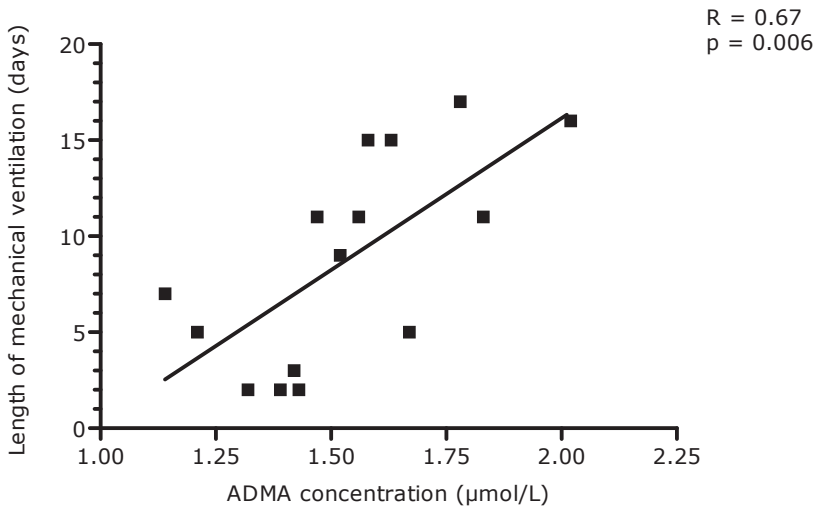
* Adjusted for gestational age (weeks).

β¶ Regression coefficients which are expressed as days of mechanical ventilation per 1 standard deviation increase of the independent variable.

R^2 (crude analysis) = 0.45.

$ R^2 (adjusted analysis) = 0.69.

Figure 2. Relation between ADMA concentration and length of mechanical ventilation in preterm infants (N=15).



After adjustment for gestational age, which is strongly associated with mechanical ventilation, ADMA concentration was still significantly related to length of mechanical ventilation (regression coefficient 2.3, p=0.024, table 3).
Two of the 30 infants died because of respiratory and circulatory insufficiency (Table 1). These two infants had plasma ADMA concentrations of 1.6 and 2.0 µmol/L (both in the highest quartile).

Discussion

This study shows that preterm infants who require mechanical ventilation directly after birth have higher umbilical cord plasma concentrations of ADMA compared to preterm infants who do not require mechanical ventilation. Furthermore, a positive relation was found between the umbilical cord plasma concentration of ADMA and the length of mechanical ventilation in these infants.
It is known that term born infants have substantially elevated plasma concentrations of ADMA (20-22) which decline with age (27). As shown by Mittermayer and co-workers, the plasma ADMA concentration in preterm male infants (but not in preterm female infants) is even higher compared to term infants (23). Indeed, also in our study, elevated ADMA plasma levels were found in preterms, but in contrast to the results of Mittermayer and co-workers, no significant difference in ADMA concentration was found between male and female preterms.
Currently, the origin of increased ADMA levels in preterm infants is not known. However, since ADMA is produced as a result of proteolysis of methylated proteins and is metabolized by dimethylarginine dimethylaminohydrolases (DDAH) enzymes which are expressed in the placenta and foetal tissue such as the liver (20;28-30), it can be hypothesized that increased ADMA levels may be accounted for by accelerated proteolysis and/or by reduced metabolization by DDAH (19;31).
The most likely mechanism by which ADMA is associated with mechanical ventilation is inhibition of NOS isozymes. NOS isozymes convert the amino acid arginine into NO and citrulline. NO has been recognized as an important mediator of normal lung development, pulmonary vascular resistance and ventilation perfusion matching (1-3). Since ADMA reduces NO synthesis by inhibiting all isoforms of NOS, increased ADMA levels could lead to an impaired lung development which could consequently lead to an increased requirement of mechanical ventilation as found in this study.
Recently, it was demonstrated that the lung expresses high amounts of PRMTs which correlated with enhanced ADMA concentrations (32). Furthermore, bronchoalveolar lavage fluid and serum exhibited almost identical levels of ADMA/SDMA, suggesting that methylarginine metabolism by the lung significantly contributes to circulating levels of ADMA. Therefore, apart from the

fact that the lung is a major source of NO, the lung seems also to be a major source of the NOS inhibitor ADMA (32;33).

Currently, no data are available on NO metabolism and its clinical consequences during intrauterine maturation in humans. However, the relation between NO metabolism and lung development during pregnancy is extensively studied in animal models (34-36). In these models, it was found that pulmonary NOS expression and NO production is increased during the early third trimester in the primate, which may enhance airway and parenchymal function in the immediate postnatal period. In preterm sheep, the expression of NOS in the lung was decreased (35). The consequences of decreased NOS expression was demonstrated in eNOS-deficient mice (36). These mice appeared to develop major defects in lung morphogenesis, resulting in respiratory distress and death within the first hours of life in the majority of animals. In addition, histological and molecular examination of preterm and newborn mutant lungs demonstrated marked thickening of saccular septae, with evidence of reduced surfactant production (36).

Despite the important role of NO in the development and maturation of the lungs as elucidated in animal models, there are conflicting results regarding the use of inhaled NO and lung function in preterm infants (16;37). The most promising indication for inhaled NO in preterm infants appears to be a 'prophylactic' treatment to prevent death and BPD (16). The results of the present study support the rationale for the prophylactic use of inhaled NO in preterm infants. Since preterm infants requiring mechanical ventilation have increased ADMA levels and presumably a concomitantly decreased NO production, early administration of inhaled NO may improve pulmonary function. However, further research is needed to confirm this hypothesis.

The limitations of our study need to be addressed. Firstly, the preterm infants who required mechanical ventilation were about two weeks younger compared to the infants who did not require mechanical ventilation. Ideally age-matched infants should be included in the study which was unfortunately not attainable since mechanical ventilation is highly associated with gestational age. Yet, in a stepwise regression analysis, mechanical ventilation, but not gestational age, proved to be an independent determinant of the plasma ADMA concentration.

Secondly, we did not measure NO or its oxidation products nitrite and nitrate. Due to the highly reactive properties of NO, its short half-life (< 0.1 second in human circulation) and because plasma levels of nitrite/nitrate are affected by many factors such as intake (food and water), excretion (faeces, urine, expired air) and clinical and therapeutic interventions, NO production may not be reliably assessed in preterm infants (38;39).

Taking the above-mentioned considerations into account, the present study showed that umbilical cord plasma concentrations of ADMA are associated with mechanical ventilation. These findings suggest that high plasma ADMA concentrations could be associated with decreased NO synthesis, which seems to

be important for the development of the lungs and could possibly lead to an increased period of mechanical ventilation in preterm infants.
Future studies should focus on the clinical consequences of increased ADMA concentrations and the effect on pulmonary function in preterm infants in more detail. We suggest that ADMA concentrations in preterm infants should be determined on several time points after birth and related to pulmonary function in a larger study.

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