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Maternal and fetal nitric oxide synthesis is decreased in pregnancies with small for gestational age infants

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Our purpose was to evaluate whether maternal and fetal nitric oxide synthesis in pregnancies with small for gestational age (SGA) infants are different from those in pregnancies with appropriate for gestational age (AGA) infants. Maternal and fetal circulating nitrate and nitrite concentrations were compared between 30 pregnancies with AGA and 10 pregnancies with SGA at birth. End-products of nitric oxide synthesis were measured in maternal and cord venous blood samples using a fluorometric assay. Umbilical artery blood pH and PO_2 were also measured. Maternal circulating nitrite and nitrate concentrations (6.91 ± 1.27 μM) in pregnancies with SGA were significantly lower than those (11.69 ± 1.33 μM) in pregnancies with AGA (P = 0.015). Fetal circulating nitrite and nitrate concentrations (7.54 ± 1.09 μM) in pregnancies with SGA were also significantly lower than those (11.24 ± 1.08 μM) in pregnancies with AGA (P = 0.024). There were no significant differences in umbilical artery blood pH and PO_2 between the two groups. These results suggest that maternal and fetal nitric oxide synthesis are decreased in pregnancies with SGA infants.

Key words: appropriate for gestational age infant/nitric oxide/nitrate/nitrite/small for gestational age infant

Introduction

During normal pregnancy several haemodynamic changes occur, including profound increases in blood flow through uterine blood vessels, altered responses to vasopressor agents, and reduced peripheral resistance and blood pressure (Gant *et al.*, 1987). In contrast to the mother, the fetal circulation is characterized by high cardiac output and low systemic resistance (Yang *et al.*, 1996). The mechanisms underlying these adaptations of normal pregnancy and the low resistance of the fetal circulation are currently unclear.

Nitric oxide formation is up-regulated in pregnancy, as determined by the urinary excretion of cyclic guanosine monophosphate (cGMP) and nitrite–nitrate (Conrad and Vernier, 1989; Conrad *et al.*, 1993), but the role of nitric oxide in maternal adjustments to pregnancy or fetal growth and development is unknown (Diket *et al.*, 1994). Nitric oxide is a potent vasodilator produced coincident with the metabolism of L-arginine to L-citrulline by nitric oxide synthase in endothelial cells (Palmer *et al.*, 1988; Nathan, 1992). Nitric oxide is an endogenous nitrovasodilator that activates cytosolic guanylate cyclase in vascular smooth muscle leading to active vasodilation. Experimental and clinical studies (Yallampalli and Garfield, 1993; Diket *et al.*, 1994; Yang *et al.*, 1996) strongly suggest that increased nitric oxide synthesis may be partially responsible for regulating the vascular adaptation of pregnancy and that chronic reductions of nitric oxide production in rats result in significant intrauterine growth retardation (Yallampalli and Gardfield, 1993; Diket *et al.*, 1994; Salas *et al.*, 1995). However, little information currently exists regarding nitric oxide synthesis in appropriate for gestational age (AGA) and small for gestational age (SGA) infants during pregnancy. The aim of this study was to compare maternal and fetal sera nitric oxide metabolites between pregnancies with AGA and those with SGA, in order to determine whether pregnancies with SGA have lower rates of nitric oxide synthesis, as measured by maternal and fetal sera nitrite and nitrate.

Table I. Clinical characteristics of subjects. Data are presented as mean ± SD

	AGA (n = 30)	SGA (n = 10)
Maternal age (years)	28.6 ± 4.1	28.9 ± 2.4
Parity	0.8 ± 0.9	1.0 ± 0.8
Birth age (weeks)	39.4 ± 1.1	39.0 ± 1.7
Birth weight (gm)	3108 ± 283*	2399 ± 445*
Apgar score at 1 min	8.8 ± 0.8	7.9 ± 1.7
Umbilical artery blood gas analysis		
pH	7.27 ± 0.06	7.27 ± 0.04
PO_2 (mm Hg)	17.2 ± 6.9	16.1 ± 7.3

AGA = appropriate for gestational age baby; SGA = small for gestational age baby.
*P <0.05.

Materials and methods

Patients

Maternal and fetal circulating nitrate and nitrite concentrations were compared between 30 pregnancies with AGA and 10 pregnancies with SGA at birth. All the women were non-smokers, with no indication of maternal complication, or of drug administration. The patients were allocated to each group after birth weight measurement. Those subjects with diabetes, multiple pregnancies, fetal hydrops, previous pregnancy with pre-eclampsia or mole pregnancies were excluded from the study. Clinical characteristics of the subjects are given in Table I. Gestational age was estimated from the first day of the last menstrual period and confirmed by first-trimester and early

second-trimester ultrasound examinations (crown–rump length, biparietal diameter and femur length measurements). Estimated fetal weights by ultrasound during pregnancy and birth weights in AGA were within normal ranges (between the 10th and 90th percentile) of the standard growth curve for the Japanese population (Sato *et al.*, 1982); those in the SGA group were below the normal ranges (i.e. below the 10th percentile). Middle cerebral artery pulsatility index and umbilical artery pulsatility index in AGA infants were within normal ranges (Manabe *et al.*, 1995) respectively. In SGA infants, the middle cerebral artery pulsatility index was below the 5th percentile. In three out of 10 SGA infants, the umbilical artery pulsatility index was above the 95th percentile, and those in the remaining seven SGA infants were within the normal range. All AGA babies were delivered vaginally. Three babies were delivered by Caesarean section due to fetal distress and seven by vaginal delivery in pregnancies with SGA. In all 10 pregnancies with SGA there was no known cause for the SGA. No neonate had congenital malformations or genetic disorders. The study was approved by the local ethical committee of Shimane Medical University and standardized informed consent was obtained from each patient.

Sampling technique

In the case of vaginal delivery, 10 ml samples of the maternal blood were obtained before the onset of delivery. In the case of Caesarean delivered pregnancies, an elective Caesarean section was conducted, and 10 ml samples of the maternal blood were obtained before operation, to avoid the effect of timing of sampling in relation to delivery on maternal blood nitrate and nitrite concentrations (Okutomi *et al.*, 1997). All women were fasted for at least 24 h within 1 week before delivery, when the maternal blood was obtained. The mean number of days between sampling and delivery was 2.7 (range 1–6 days). After delivery of the placenta, a 10 ml sample of the cord blood was obtained. This was performed by needle puncture of the maternal cubital vein and umbilical vein. Plasma was separated by centrifugation at 1000 *g* for 10 min and stored in batches for measurement of circulating nitric oxide metabolites. Simultaneously, a 1 ml sample of umbilical artery blood was obtained, and blood gas analysis was then performed on a Ciba Corning 278 (Ciba Corning Diagnostic Co. Ltd., Tokyo, Japan) pH blood gas analyser.

Plasma nitrite and nitrate concentrations were measured using methods adapted from Misko *et al.* (1993). Briefly, plasma was filtered through a Centricon 10 (Amicon, Beverly, MA, USA) for 1 h at 4°C at 3000 *g* to remove contaminating haemoglobin. A 50 µl sample of plasma was incubated with 40 µM NADPH and 14 mIU of nitrate reductase (from *Aspergillus niger*; Sigma, St. Louis, MO, USA) in a final volume of 50 µl of 20 mM Tris, pH 7.6. The reaction was terminated after 5 min at 20°C by addition of 10 µl of 2,3-diaminonaphthalene (0.05 mg/ml in 0.62 M HCl). After a 10 min incubation at 20°C, 5 µl of 2.8 N NaOH was added and the intensity of the fluorescence was measured using a Hitachi 850 Fluorescence spectrophotometer (Hitachi Co Ltd, Tokyo, Japan). Nitrite standards (>98% pure, Sigma) were routinely made fresh, dissolved in double-deionized water, and kept on ice prior to use. Nitrite was detectable at a concentration of 10 pmol/ml.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis for comparison of birth age, birth weight, umbilical artery blood pH and PO_2, and maternal and fetal circulating nitrate and nitrite concentrations between AGA and SGA infants was done using an unpaired *t*-test. Maternal age, parity, and Apgar score between the two groups were compared using a Wilcoxon–Mann–Whitney test. The Spearman rank test was used to assess correlation between maternal and fetal nitrate and nitrite concentrations from the same patients. $P < 0.05$ was considered to be significant.

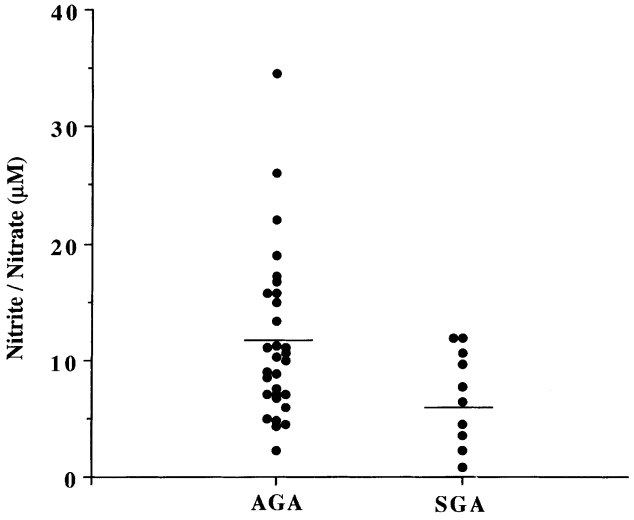

Figure 1. Measurement of individual nitrite and nitrate levels in maternal serum of appropriate for gestational age infants (AGA) and small for gestational age infants (SGA). Horizontal lines represent mean values for each group, which were statistically significantly different ($P = 0.015$; unpaired *t*-test).

Results

There were no significant differences for maternal age, parity, birth age, Apgar score, and umbilical artery blood pH and PO_2 between AGA and SGA infants (Table I). Birth weight (2399 ± 445 g) of the SGA infants was significantly lower than that (3108 ± 283 g) of the AGA infants ($P < 0.05$; Table I). Maternal circulating nitrate and nitrite concentrations (6.91 ± 1.27 µM) in pregnancies with SGA were significantly lower than those (11.69 ± 1.33 µM) in pregnancies with AGA ($P = 0.015$; Figure 1). Fetal circulating nitrate and nitrite concentrations (7.54 ± 1.09 µM) in pregnancies with SGA were also significantly lower than those (11.24 ± 1.08 µM) in pregnancies with AGA ($P = 0.024$; Figure 2). There was a linear correlation between maternal and fetal nitrate and nitrate concentrations from the same patient ($R^2 = 40.0$; $P < 0.0001$).

Discussion

Pronounced vasodilation of the maternal vasculature occurs during normal pregnancy in women and in other mammals, including the rat (Robson *et al.*, 1989; Gilson *et al.*, 1992; Conrad, 1984, 1992; Buhimschi *et al.*, 1996; Purcell *et al.*, 1997). Also, pressor responsiveness and vascular reactivity to infused vasoconstrictors are attenuated (Gant *et al.*, 1973; Nisell *et al.*, 1985; Conrad and Colpoys, 1986; Conrad *et al.*, 1991; Liao *et al.*, 1996). These cardiovascular changes ultimately assure the adequate delivery of oxygen and nutrients to the fetus (Conrad *et al.*, 1993). Nitrate concentrations in pregnant sheep and their fetuses are increased (Yang *et al.*, 1996).

The increased nitrate concentrations in the maternal and fetal circulation may reflect increased nitric oxide synthesis

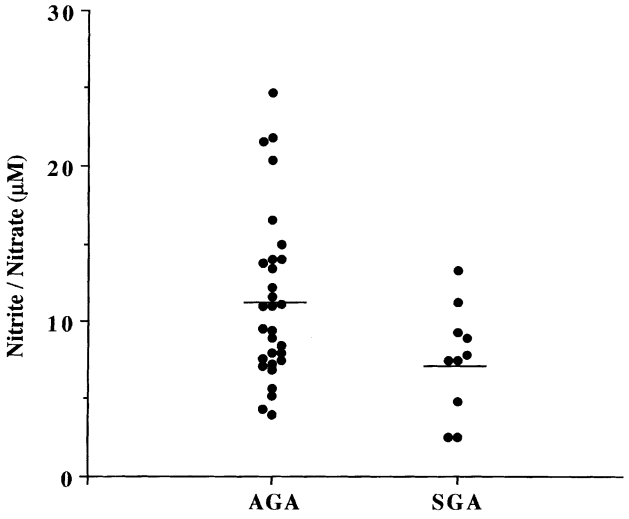

Figure 2. Measurement of individual nitrite and nitrate levels in cord serum of appropriate for gestational age infants (AGA) and small for gestational age infants (SGA). Horizontal lines represent mean values for each group, which were statistically significantly different ($P = 0.024$; unpaired *t*-test).

which may, in part, mediate the cardiovascular adaptations to normal pregnancy and the low systemic and umbilical vascular resistance in the fetus. However, hypoxia reduces nitric oxide production (Kim *et al.*, 1993). Fetoplacental hypoxia, commonly associated with intrauterine growth retardation, may well result in reduced placental endothelial and trophoblast nitric oxide synthesis. Several reports have suggested that, in pregnancies complicated by pre-eclampsia and fetal growth restriction, a deficiency exists in nitric oxide synthase activity (Brennecke *et al.*, 1994; Morris *et al.*, 1995). Moreover, the infusion of nitric oxide synthase inhibitors in pregnant rats caused intrauterine growth retardation (Yallampalli and Garfield, 1993; Diket *et al.*, 1994; Molnar *et al.*, 1994; Salas *et al.*, 1995).

In this study, maternal and fetal nitric oxide metabolites in SGA infants were significantly lower than those in AGA infants. These results suggest that maternal and fetal nitric oxide synthesis are decreased in pregnancies with intrauterine growth restricted infants. On the other hand, Lyall *et al.* (1996) reported that significantly higher nitrate concentrations were found in umbilical venous plasma in pregnancies complicated by intrauterine growth restriction in comparison with normal pregnancies, and indicated that increased nitric oxide production may be a compensatory response to improve blood flow in the placenta. These authors (Myatt *et al.*, 1997) also reported increased endothelial nitric oxide synthase expression and hence increased nitric oxide production in the fetal–placental vasculature in pregnancies with intrauterine growth restriction. The reason for differences of nitric oxide metabolite concentrations in cord blood and nitric oxide synthase activity in placental tissues in pregnancies with intrauterine growth restriction is currently unknown.

One possible explanation for these differences may be the difference in definition of intrauterine growth restriction. In our study, estimated fetal weights at examination and birth weights in AGA were below normal ranges (below the 10th percentile), and middle cerebral artery pulsatility index was below the 5th percentile. In three out of 10 SGA infants, the umbilical artery pulsatility index was above the 95th percentile, while in the remaining seven SGA infants it was within the normal range. In the study by Lyall *et al.* (1996), however, the estimated fetal weight was below the 5th percentile, and Doppler ultrasound of the umbilical artery demonstrated absent end-diastolic flow velocity. Doppler studies demonstrated that fetal hypoxaemia caused a relative redistribution of fetal cardiac output with an increased blood flow to the brain at the expense of the viscera in human growth-restricted fetuses (Wladimiroff *et al.*, 1986). Hata *et al.* (1994) showed that there was a redistribution in human fetuses with only an increase in blood flow to the brain in cases of mild-to-moderate hypoxia. Arduini *et al.* (1992) reported that a nadir of vasodilatation in cerebral arteries was reached 2 weeks before the onset of antepartum fetal heart rate late decelerations, whereas significant changes in the umbilical artery occurred close to the onset of abnormal fetal heart rate patterns. Therefore, growth restriction of infants in our study may be less severe than those in Lyall's study (1996). The precise physiological significance of nitric oxide synthesis for intrauterine fetal growth remains to be determined.

With respect to the relationship between nitric oxide synthesis and Doppler ultrasound umbilical artery flow velocity waveforms, there is a statistically significant reduction in nitric oxide synthase activity in placentas from pregnancies with abnormal umbilical artery flow velocity waveforms (Giles *et al.*, 1997a). Inhibition of nitric oxide synthase activity in the lamb fetoplacental circulation with N^{ω}-nitro-L-arginine is associated with an increase in the abnormal umbilical artery blood flow velocity waveforms (Giles *et al.*, 1997b). In our study intrauterine growth restricted pregnancies showed abnormal middle cerebral artery blood flow velocities and relatively abnormal umbilical artery blood flow velocities. These results suggest that decreased nitric oxide production might result in abnormal umbilical artery flow velocity waveforms.

In summary, the current study demonstrates that maternal and fetal plasma nitric oxide metabolite concentrations are decreased in pregnancies with intrauterine growth restricted infants, and may reflect decreased nitric oxide synthesis.

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