Relationship between asymmetric dimethylarginine in umbilical cord plasma and birth weight follows a U-shaped curve

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Abstract. Asymmetric dimethylarginine (ADMA) is a nonselective nitric oxide (NO) synthase inhibitor associated with cardiovascular and metabolic disorders. NO regulates placental blood flow, which plays an important role in fetal growth. Many epidemiological studies have disclosed that restricted fetal growth is associated with an increased risk of insulin resistance in adult life. We studied the relationship between ADMA in cord blood and birth size. Nine small for gestational age (SGA) and 32 appropriate for gestational age (AGA) infants were studied. Their cord plasma ADMA, insulin, insulin-like growth factor-1 (IGF-1), and adipocytokine levels were determined using enzyme-linked immunosorbent assays. The relationship between birth weight and ADMA levels followed a U-shaped curve rather than inverse linear associations expected over a full range of birth weight distribution. ADMA positively correlated with birth weight in the AGA group (p<0.001, R=0.590), and inversely correlated with birth weight in the SGA group (p<0.05, R=-0.741). ADMA inversely correlated with adiponectin (p<0.05, R=-0.289) and quantitative insulin sensitivity check index (QUICKI) (p<0.05, R=-0.294) in all subjects, and did not correlate with nitrogen oxides (NOx). Insulin, IGF-1, leptin, adiponectin and QUICKI were lower in the SGA than the AGA group. Plasma ADMA levels in cord blood may be a marker of fetal growth and insulin resistance.

Key words: Appropriate for gestational age, Asymmetric dimethylarginine, Nitric oxide, Small for gestational age, Quantitative insulin sensitivity check index

PLACENTAL TRANSPORT of nutrients is dependent on vascular development, which determines blood flow to the placenta. The ability of the uterine artery to dilate during pregnancy may be specifically related to upregulation of multiple pathways involving the production of nitric oxide (NO) [1]. Asymmetric dimethylarginine (ADMA), an L-arginine analog and a natural inhibitor of NO synthase, is a key regulator of NO production by the vascular endothelium [2]. Increased maternal plasma ADMA levels are associated with a decrease in NO levels and hence decreased placental blood flow [3]. ADMA is produced as a by-product of protein modification processes in human cells and can be found in human plasma [4]. Its normal range of healthy children is 0.36 to 1.61 μmol/L [5]. In normal pregnancies, maternal plasma ADMA levels are reduced in the early stages of pregnancy, but increase with advancing gestational age [6]; on the other hand, in compromised pregnancies, such as in patients with preeclampsia, maternal ADMA levels increase from early gestation itself [7]. Maternal ADMA levels increase in preeclampsia and/or intrauterine fetal growth restriction [8]. Braekke et al. reported that not only maternal concentrations of ADMA, but also L-arginine, are significantly higher in women with preeclampsia than in controls [9]. Elevation of ADMA has also been reported in adult cardiovascular diseases [10-13]. Oxidative stress is also responsible for the increased synthesis and/or inhibition of catabolism of ADMA [14] that are observed in patients with hypercholesterolemia, hyperglycemia, diabetes and hypertension [10, 15-17].

It is well known that low birth weight is associated with adult disorders characterized by insulin resistance, such as type 2 diabetes, hypertension, dyslipidemia and coronary heart disease [18, 19]. It has been proposed that this association results from fetal programming in response to the intrauterine environment [20].
Moreover, few studies on the association between neonatal birth weight and ADMA exist [21]. We hypothesized that decreased NO and increased ADMA might underlie the initial pathophysiologic events leading to insulin resistance. To test this hypothesis, the relationship between birth weight and ADMA or insulin resistance parameters was evaluated.

**Methods**

**Subjects**

This study was conducted from February 2004 to January 2013. The study group consisted of 41 singleton subjects with gestational ages ranging from 36 to 41 weeks, and birth weights ranging from 1,798 to 3,822 g. Some of these subjects had been previously recruited for an observational study on intracellular magnesium [22]. Gestational age was assessed at the time of registration by dating the last menstrual period. This study was retrospective and the neonates were enrolled in the study when they were at the time of birth. None of the subjects were on any medication or showed any evidence of endocrine malfunction or recent use of drugs. All mothers of the neonates were Japanese with no remarkable past medical histories including diabetes mellitus, and they manifested no abnormal findings during pregnancy, such as preeclampsia. Cord blood from infants with diabetic mothers, such as those with preexisting diabetes and gestational diabetes mellitus, was excluded. Infants were excluded if they had neural tube defects, chromosomal abnormalities, or other severe congenital diseases. For these reasons, SGA subjects in this study were all asymmetrical SGA, not including symmetrical SGA. This investigation was conducted according to the principles expressed in the Declaration of Helsinki. The study protocol was approved by Kansai Medical University Review Board for Human Studies. Written informed parental consent was obtained before recruitment.

**Definition of small for gestational age (SGA)**

SGA was defined as birth weight below –1.5 standard deviations (SD) of the Japanese standard birth-weight curve [23]. Ponderal index (birth weight [in kilograms] divided by birth length [in meters] cubed) was used as a measure of relative birth weight. Appropriate for gestational age (AGA) was defined as birth weight, birth length and Ponderal index ≥10th percentile and <90th percentile of the respective means for the gestational age.

**Collection**

Cord blood samples were collected by the labor ward staff at the time of birth of the neonates, and kept at 4 °C. Plasma was separated immediately, stored at -80 °C, and thawed only once before analysis. Cord plasma glucose was measured using a standard assay.

**ELISA assay**

Plasma ADMA levels were detected using the ADMA enzyme-linked immunosorbent assay (ELISA) kit (Immundiagnostik AG, Bensheim, Germany). The cross-reactivity was ≤0.5% for symmetric dimethyl-arginine (SDMA), an isomer of ADMA, and <0.02% for L-arginine. The lower limit of detection was 0.05 μmol/L. The inter- and intra-assay levels were 0.03 (SD) % and 0.04 (SD) %, respectively. Cord plasma leptin levels were determined with the use of a commercially available ELISA kit (Immuno-Biological Laboratories Co., LTD, Gunma, Japan) with a detection limit of 195 pg/mL [intra-assay and inter-assay coefficient of variations (CVs) of 6.9% and 7.7%, respectively]. Plasma insulin-like growth factor-1 (IGF-1) assay was performed using a commercially available ELISA kit (R&D System Inc. Minneapolis, MN) with a detection limit of 7 pg/L (intra-assay and inter-assay CVs of 4.3-5.3% and 8.1-9.8%, respectively). Plasma insulin concentrations were determined with the use of a commercially available ELISA kit (BIOSOURCE EUROPE S. A., Nivelles, Belgium) with a detection limit of 1.08 pmol/L (intra-assay and inter-assay CVs of 5.3% and 9.8%, respectively). Plasma adiponectin assay was performed using a commercially available ELISA kit (R&D System Inc. Minneapolis, MN) with a detection limit of 0.246 ng/mL (intra-assay and inter-assay CVs of 3.4% and 6.8%, respectively). Plasma resistin assay was performed using a commercially available ELISA kit (R&D System Inc. Minneapolis, MN) with a detection limit of 0.026 ng/mL (intra-assay and inter-assay CVs of 5.3% and 8.2%, respectively).

**Plasma nitrate/nitrite levels**

Plasma NOX [nitrite (NO₂⁻) + nitrate (NO₃⁻)] levels were measured using a colorimetric assay kit (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). Before measurements, samples were centrifuged through Microcon YM-10 Centrifugal Filters (Micropore, Bedford, MA) to remove proteins from the serum. The range of measurement value was 16.4 to 75.7 μmol/L and CV was 0.31.
Statistical analysis

Statistical analysis was performed with JMP 6 software (SAS Institute Inc., Cary, NC). Data are expressed as means ± SD. Comparisons between the groups were performed using Student’s t-test. Continuous data were compared using either the two-tailed t-test for independent samples or the Mann–Whitney U-test, as appropriate. The correlation between cord blood ADMA levels and birth size, IGF-1, and insulin were examined by linear regression and Pearson product-moment correlation coefficient analyses. Associations of ADMA with several variables were analyzed by univariate regression or non-linear logistic analysis. A value of \(p<0.05\) was considered significant.

Results

Profile of each group

The subjects consisted of 9 SGA infants and 32 AGA infants. Table 1 summarizes the clinical characteristics, anthropometric indices and plasma biomarkers of patients in the SGA and AGA groups. Mean gestational age in the SGA group was lower than that in the AGA group. The two groups did not differ significantly in terms of maternal age and parity. Mean plasma glucose, ADMA, NO\(_X\), and resistin concentrations did not differ significantly between SGA and AGA groups, whereas mean plasma adiponectin, leptin and IGF-1 were significantly lower in the SGA group. There were no gender differences in adipocytokine and leptin values. However, mean circulating levels of ADMA were significantly lower in female than in male subjects in the patients as a whole (male: 1.37±0.35 μmol/L, female: 1.08±0.30 μmol/L, \(p<0.05\)). ADMA was inversely related to adiponectin (\(p=0.048\), \(R=-0.289\)) and the quantitative insulin sensitivity check index (QUICKI) (\(p<0.05\), \(R=-0.294\)) in all subjects, while it did not correlate with NO\(_2^-\)+NO\(_3^-\) (NO\(_X\)) (Table 2).

Correlation between ADMA and birth weight

The relationship between ADMA levels and birth weight followed a U-shaped curve rather than an inverse linear association expected over a full range of birth weight distribution (Fig. 1, ADMA (μmol/L) = 0.509 + 0.000198 × birth weight (g) + 0.0000007 × [birth weight – 2876.7]\(^2\). F value=8.063, \(p=0.0011\)). ADMA was positively related to birth weight in the AGA group (\(p<0.001\), \(R=0.590\)), while it was inversely related to that in the SGA group (\(p<0.05\), \(R=-0.741\)).
Discussion

Increased circulating ADMA levels occur in adult patients with diabetes mellitus and vascular diseases [10-13, 15]. We tested whether the origin of ADMA dysregulation starts in fetal life. The fact that the prenatal environment can modify adult diseases is now firmly established and is supported by both epidemiologic data [18, 19] and experimental studies [24]. However, the processes responsible for the link between reduced fetal growth and insulin resistance or glucose intolerance in adult life are not fully understood. Chievaroli et al. reported that plasma ADMA was significantly higher in SGA children (6 to 7 years old) than AGA peers [21]. We hypothesized that increased ADMA and decreased NO might underlie the initial pathophysiologic events leading to insulin resistance.

The ability of the uterine artery to dilate during pregnancy may be specifically related to upregulation of multiple pathways for the production of NO [1]. Maternal plasma NO regulates feto-placental vascular activity and placental flow [3]. The activity of constitutive NO synthase is dependent on ADMA. Several studies have reported elevated maternal plasma ADMA levels in preeclampsia; some studies also suggested that ADMA is elevated in early stages of pregnancy in women who later develop preeclampsia [25].

In this study, however, circulating ADMA levels had no correlation with NO levels. Dimethylarginine dimethylaminohydrolase (DDAH) metabolizes free ADMA to citrulline that can be recycled to arginine [26, 27]. DDAH activity or arginine concentrations may also regulate NO levels as well as ADMA. The different kinetics of the reaction of NO with these oxygen-derived radicals and their varying concentration in different cells and organs may account for the slight inconsistency of the results on in vivo degradation of NO [27].

In addition, circulating levels of ADMA did not uniformly correlate with birth weight in all subjects. In fact, ADMA correlated positively with birth weight in the AGA group, while it was inversely related to birth weight in the SGA group. We initially evaluated ADMA levels using continuous data and observed a potential non-linear relationship between ADMA and birth weight, thus suggesting that conventional linear regression would not be appropriate. The relationship between birth weight and ADMA levels followed a U-shaped curve rather than inverse linear associations expected over a full range of birth weight distribution. In some populations, there is an inverse association between birth size and disease. However, epidemiologic studies confirm that the relationships between human birth weight and adult obesity, hypertension, and insulin resistance follow U-shaped curves [28-30]. An analysis of all of these reports suggests that the relationship between birth weight and these metabolic abnormalities in adult life can be described by a U-shaped curve. SGA is a sign of increased stress exposure during the fetal period, caused by under-nutrition in utero, leading to up-regulation of ADMA. Decreased NO production, induced by increased ADMA activity is associated with risk factors of cardiovascular disease such as impaired glucose regulation, hypertension, and hypothalamic-pituitary-adrenal axis programming is an important putative mechanism in linking these disorders with SGA [31]. The subjects with the higher birth weight part of the U-shaped curve affected maternal hyperglycemia during pregnancy leads to increased fetal insulin levels, resulting in up-regulation of ADMA.

Since measurement of glucose tolerance and insulin sensitivity is practically difficult and ethically not acceptable in newborns, we instead quantified QUICKI, calculated by cord plasma insulin and glucose levels, to assess insulin resistance in the fetuses (a lower QUICKI indicates greater insulin resistance) [32]. Mean QUICKI levels in the SGA group were significantly lower than in the AGA group, suggesting that SGA subjects have a high tendency toward insulin resistance [33]. Furthermore, ADMA levels inversely correlated with QUICKI (p<0.05) and adiponectin (p<0.05). In the present study, we confirmed that cord blood plasma adiponectin levels were lower in the SGA than AGA group. These findings suggest the importance of adiponectin in the regulation of body fat mass in newborn infants. A growing body of evidence suggests that adiponectin may also play a role in the development of insulin resistance [34]. Kajantie et al. reported that adiponectin concentrations in fetal circulation showed a 20-fold rise between 24 week gestation [35]. In our study, the difference in gestational age between SGA and AGA may affect the U-shaped curve of ADMA, as ADMA was inversely related to adiponectin.

Our study has several limitations that need to be addressed. First, although it is the largest study of its type, the sample size is still small. The power limitations
and type II error should therefore be taken into account when interpreting the results. Second, neither L-arginine nor DDAH levels were measured. Moonen et al. reported that L-arginine:ADMA ratio was a better indicator of NO availability [36]. Finally, we performed a cross-sectional study with analysis of ADMA values at one point and this is not as valuable as a longitudinal study with several values. Further studies will be needed to assess the possible role of ADMA in the initial pathophysiologic events leading to insulin resistance.

In conclusion, ADMA inversely correlated with adiponectin and QUICKI in all subjects, while followed a U-shaped curve with birth weight. Plasma ADMA levels in cord blood might be a marker of fetal growth and insulin resistance.

Author Contributions

J. Takaya and K. Kaneko designed the research, and Y. Tanabe and Y. Kuroyanagi conducted the research. All authors read and approved the final manuscript.

Author Disclosures

None of the authors have any potential conflicts of interest associated with this research.

References


