Maternal and fetal amino acid concentrations and fetal outcomes during pre-eclampsia

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The pathophysiology of pre-eclampsia is contested, but one hypothesis indicates that it is a heterogeneous condition in which only a subset of affected women bear small-for-gestational age (SGA) babies. In intrauterine growth-restricted (IUGR) infants, placental transport of amino acids is diminished and the resulting decrease in cord-blood amino acid concentrations is thought to contribute to their stunted growth. In contrast, the metabolic syndrome (dyslipidaemia, hyperinsulinaemia, hyperglycaemia, hypertension and obesity) which is associated with high amino acid concentrations is more prevalent in women with pre-eclampsia. The focus of this study was to compare maternal and fetal serum amino acid concentrations during normal pregnancy and pre-eclampsia and to evaluate the associations between the amino acid concentrations

and fetal growth. The results indicate that maternal and cord-blood amino acid concentrations were significantly higher in women with pre-eclampsia compared with normal pregnant women and the concentrations were inversely associated with measures of infant growth. Maternal and cord-blood amino acid concentrations were also significantly higher in pre-eclamptic mothers with SGA infants compared with pre-eclamptic mothers whose babies were not SGA. These data indicate that, in contrast to IUGR, pre-eclampsia is associated with enhanced placental amino acid transport or reduced fetal amino acid utilization. Furthermore, the data are consistent with the hypothesis that pre-eclampsia is a heterogeneous disease associated with the metabolic syndrome.

Introduction

The pathophysiology of pre-eclampsia is not well understood and empirical strategies to prevent this major cause of maternal and infant mortality have been unproductive. A central debate about the genesis of pre-eclampsia is whether the disease invariably involves placental hypoperfusion or has a more heterogeneous pathophysiology (Freidman *et al.*, 1991; Lain and Roberts, 2002). Infants born to pre-eclamptic mothers at term are generally not small for their gestational age (Chesley, 1999). However, when pre-eclamptic mothers deliver pre-term, their babies tend to be smaller than other pre-term babies (Xiong *et al.*, 2002).

Women with early disease, but not necessarily women with pre-eclampsia at term, may have a pre-existing hypermetabolic syndrome, termed the metabolic syndrome (Ness and Roberts, 1996). This metabolic syndrome consists of hypertension in concert with adiposity, dyslipidaemia, hyperglycaemia and hyperinsulinaemia.

Although fetuses of women with the metabolic syndrome appear to be exposed to an excess of circulating nutrients, they may not benefit. Indeed, in pre-eclampsia, nutrient abundance may mark the presence of a predisposition to poor fetal growth.

Amino acid concentrations, which are increased in the metabolic syndrome, serve as one of the main nutrient sources for fetal growth, accounting for 20–40% of fetal energy requirements (Dicke and Henderson, 1988; Herrera, 2000; Kalhan, 2000). Amino acids are actively transported by the placenta from the maternal to the fetal circulation (Young and Prenton, 1969; Ronzoni *et al.*, 1999; Battaglia and Regnault, 2001; Jansson, 2001). Indeed, the placental and fetal uptake of amino acids is in excess of fetal protein synthesis needs (Battaglia and Regnault, 2001; Jansson, 2001).

This study compared maternal and cord-blood amino acid concentrations during normal pregnancy and pre-eclampsia and evaluated the associations between amino acid concentrations and fetal growth. Because of the evidence that pre-eclampsia is associated with the metabolic syndrome, it was hypothesized that maternal and

fetal amino acid concentrations would be increased during pre-eclampsia and, paradoxically, would be highest among pre-eclamptic women bearing small-for-gestational age (SGA) infants. This would contrast with intrauterine growth-restricted (IUGR) pregnancies in which deficient placental amino acid transport is related to reduced fetal growth (Cetin *et al.*, 1992; Jansson *et al.*, 1998; Paolini *et al.*, 2001).

Materials and Methods

Subjects

Fifty-nine nulliparous subjects were recruited (1994–1997) and gave their informed consent at admission to labour and delivery at Magee-Women's Hospital as part of an ongoing investigation of pre-eclampsia approved by the hospital's institutional review board. Twenty-nine of the women (cases) had pre-eclampsia using the criteria of hypertension, proteinuria, hyperuricaemia and the reversal of hypertension and proteinuria after pregnancy. Hypertension was defined as an absolute blood pressure > 140/90 mmHg after 20 weeks of gestation. Proteinuria was defined as > 300 mg per 24 h urine collection, > 2+ on a voided, > 1+ on a catheterized random urine sample, or a protein:creatinine ratio > 0.3. Hyperuricaemia was defined as > 1 SD above usual values at the gestational age at which the sample was obtained (at term > 5.5 mg dl⁻¹). Severe pre-eclampsia was defined as absolute blood pressure values > 160 mmHg systolic blood pressure or > 110 mmHg diastolic blood pressure, proteinuria > 4+ on a voided urine sample, hyperuricaemia or HELLP syndrome. HELLP syndrome included the definition of pre-eclampsia as well as thrombocytopaenia (< 100 000 platelets ml⁻¹), serum lactate dehydrogenase ≥ 164 U l⁻¹ and serum aspartate aminotransferase = 48 U l⁻¹. Thirty women (controls) had uncomplicated pregnancies. These women had no known medical problems, were normotensive and were without proteinuria or hyperuricaemia throughout the pregnancy. The mothers in the two groups were frequency-matched for age, pre-pregnancy body mass index and race. Fetal gestational age was confirmed using sonography at approximately the twentieth week of gestation. Gestational age at the time of sampling of the maternal blood was noted. For the neonates, values for gestational age, birth weight, birth length, head circumference, sex and whether they were SGA or large-for-gestational age (LGA) were recorded. SGA infants were defined as those who were less than the tenth centile for birth weight corrected for gestational age, sex and race. In a similar way, LGA infants were defined as those who were greater than the ninetieth centile for birth weight corrected for gestational age, sex and race. The incidence of self-reported smoking status for both groups was similar (8 of 29 pre-eclamptic women and 8 of 30 controls), and none of

the subjects in this study reported taking aspirin or antioxidants.

Blood samples

Maternal and cord-blood serum samples were allowed to stand at room temperature (about 18°C) for 1 h, centrifuged at 2000 *g* for 20 min, aliquoted under sterile conditions and then stored at –80°C until they were assayed. The fasting status of the patients who provided samples was not available, and was assumed to be non-fasting.

Amino acid analysis

Samples were centrifuged through an Amicon centrifuge filter at 800 *g* and then combined with norleucine and lithium diluent, pH 2.2 (Pickering Labs, Mountain View, CA). The amino acids were separated by cation exchange chromatography (Pickering Labs) using a four-solvent delivery system (Perkin-Elmer, Wellesley, MA). The amino acids were then derivatized with ninhydrin in a post-column reaction (Pickering amino acid analysis module, AT3000) and detected at 500 nm (UV-visible detector). The Pickering amino acid standard was used to calibrate the system. Total run time was 180 min. Data are not reported for asparagine, glutamate, serine and threonine in the cord-blood samples as chromatographic interferences prevented the measurement of these amino acids in some samples.

Statistical analysis

Means and standard errors are reported. A *t* test was used to compare means. ANOVA, followed by Tukey's test, was used to compare three means. Fisher's Exact test was used to compare the percentages of SGA and LGA among cases and controls. Partial correlations between amino acid concentrations and fetal outcomes were made after adjusting for gestational age. Significance was accepted at *P* < 0.05.

Results

The characteristics of the subjects are shown (Table 1). The mothers in the two groups were frequency-matched for age, body mass index and race; these values were similar. Hypertension is a requirement for the diagnosis of pre-eclampsia, hence, the cases had markedly higher systolic and diastolic blood pressures than controls. The infants in the pre-eclamptic group were on average smaller (weight, length, head circumference) at birth than the infants of control mothers and this is primarily, although not entirely, explained by their earlier gestational age.

Table 1. Clinical characteristics of the mothers and infants involved in the study

Parameter		Normal pregnancy (n = 30)	Pre-eclampsia (n)
Mothers	Age (years)	24.8 ± 0.9	27.0 ± 1.2 (27)
	Body mass index (kg m ⁻²)	26.2 ± 1.4	26.2 ± 1.0 (27)
	Systolic blood pressure (mmHg)	118.9 ± 1.8	155.6 ± 2.8 (29)*
	Diastolic blood pressure (mmHg)	72.0 ± 1.1	92.3 ± 1.5 (29)*
	Serum haemoglobin (g dl ⁻¹)	12.9 ± 0.2	13.3 ± 0.1 (24)
	Hematocrit (%)	37.4 ± 0.5	38.2 ± 0.4 (24)
	Urine protein (g per 24 h urine collection)	0.24 ± 0.05	1.82 ± 0.21 (28)*
	Severe pre-eclampsia (n)	na	15
	Race (%)		
	Caucasian	76.7	75.9
	Black	23.3	20.7
	Other	0.0	3.4
	Sampling age (weeks)	38.8 ± 0.6	35.5 ± 0.5 (29)*
Neonates	Gestational age (weeks)	39.5 ± 0.2	35.5 ± 0.5 (29)*
	Birth weight (g)	3576 ± 75	2456 ± 152 (26)*
	Birth length (cm)	51.9 ± 0.3	46.9 ± 0.7 (24)*
	Head circumference (cm)	34.6 ± 0.2	31.8 ± 0.5 (24)*
	SGA (n)	1	8 [‡]
	LGA (n)	6	2
	Sex (n) [‡]		
	Male	21	19
	Female	9	9

Small-for-gestational age (SGA) and large-for-gestational age (LGA) values were compared using Fisher's Exact test. All other comparisons used the *t* test; **P* < 0.001; †*P* < 0.01 compared with normal pregnancy values. ‡Sex of one child was not recorded.

Maternal amino acids during pre-eclampsia

The mean concentration of most maternal amino acids was higher in women with pre-eclampsia compared with controls (Table 2). Results for all individual amino acids required for protein synthesis, except for tryptophan, and their summed total are shown. Ten of the 19 individual amino acids and the total amino acid concentration were significantly higher among pre-eclamptic women. Two of four amino acids that are products of intermediary metabolism (citrulline and 3-methylhistidine) were also higher during pre-eclampsia.

Proteinuria is a clinical feature of pre-eclampsia and could lead to a decrease in maternal amino acid concentrations. However, the results indicated an increase in plasma amino acid concentrations during pre-eclampsia. Furthermore, no significant correlations were observed between the maternal amino acid concentrations and urinary protein concentrations.

The mean total amino acid concentration for the women with pre-eclampsia who had SGA infants ($392.8 \pm 49.9 \mu\text{mol } 100 \text{ ml}^{-1}$) was significantly increased compared with the pre-eclamptic women who did not have SGA infants ($286.6 \pm 9.5 \mu\text{mol } 100 \text{ ml}^{-1}$, *P* < 0.005) and compared with controls with non-SGA infants ($256.3 \pm 7.0 \mu\text{mol } 100 \text{ ml}^{-1}$, *P* < 0.001). The maternal total amino acid concentration in women with

pre-eclampsia and non-SGA infants was also significantly increased compared with controls with non-SGA infants (*P* < 0.02).

Cord-blood amino acid concentrations during pre-eclampsia

Among the 15 normal pregnancy and 16 pre-eclamptic subjects who had matched cord-blood samples available for this analysis, several amino acids were significantly higher among the infants of pre-eclamptic mothers compared with the infants of controls (Table 3). These included alanine, lysine, methionine, proline, taurine and total amino acids.

Infants were again divided into either SGA or non-SGA groups and the mean cord-blood total amino acid concentrations were compared. Similar to maternal total amino acid concentrations, mean cord-blood total amino acid concentration in SGA infants from pre-eclamptic women ($320.8 \pm 32.5 \mu\text{mol } 100 \text{ ml}^{-1}$) was significantly higher compared with the pre-eclamptic women with non-SGA infants ($251.9 \pm 16.7 \mu\text{mol } 100 \text{ ml}^{-1}$, *P* = 0.05), as well as with the controls with non-SGA infants ($231.1 \pm 11.8 \mu\text{mol } 100 \text{ ml}^{-1}$, *P* < 0.005). However, cord-blood total amino acid concentrations in non-SGA infants of women with pre-eclampsia were not

Table 2. Serum amino acid concentrations in normal pregnant women and women with pre-eclampsia

Amino acid	Normal pregnancy (n = 30)	Pre-eclampsia (n = 29)
Essential		
Arginine	7.9 ± 0.4	7.9 ± 0.5
Histidine	8.8 ± 0.3	11.5 ± 0.8 [‡]
Isoleucine	5.2 ± 0.3	6.4 ± 0.3 [‡]
Leucine	10.4 ± 0.5	11.9 ± 0.7
Lysine	13.9 ± 0.7	16.1 ± 0.9
Methionine	2.3 ± 0.1	3.0 ± 0.2 [‡]
Phenylalanine	6.9 ± 0.4	8.2 ± 0.6
Valine	15.9 ± 0.7	19.6 ± 0.9 [‡]
Non-essential		
Alanine	37.2 ± 1.3	51.1 ± 5.0*
Asparagine	4.9 ± 0.2	5.5 ± 0.4
Aspartic acid	5.1 ± 0.4	5.1 ± 0.4
Cystine	2.8 ± 0.1	3.7 ± 0.3 [‡]
Glutamate	11.4 ± 1.3	14.9 ± 2.0
Glutamine	42.8 ± 1.1	52.4 ± 2.8 [‡]
Glycine	20.0 ± 1.0	23.2 ± 2.0
Proline	12.6 ± 0.6	16.0 ± 1.0 [‡]
Serine	11.5 ± 0.5	12.4 ± 0.8
Threonine	16.1 ± 0.7	19.8 ± 1.5*
Tyrosine	4.5 ± 0.3	5.9 ± 0.3 [‡]
Total	260.2 ± 7.8	315.9 ± 17.3 [‡]
Citrulline	1.6 ± 0.1	2.2 ± 0.1 [‡]
3-Methylhistidine	0.20 ± 0.06	0.42 ± 0.07*
Ornithine	4.5 ± 0.5	5.9 ± 0.7
Taurine	12.2 ± 0.5	11.3 ± 0.8

Values are means ($\mu\text{mol } 100 \text{ ml}^{-1}$) $\pm$ SEM; * $P < 0.05$; [‡] $P < 0.01$; [‡] $P < 0.005$ (*t* test), compared with normal pregnancy values.

significantly different from non-SGA infants of normal pregnant women.

Maternal amino acid concentrations and fetal growth

Overall, there were consistent inverse relationships between maternal amino acid concentrations and birth weight and birth length of infants after adjustment for gestational age, within both the control subjects and the women with pre-eclampsia. Specifically, during normal pregnancy, the amino acids significantly ($P < 0.05$) negatively correlated with birth weight were the three branched-chain amino acids isoleucine ($r = -0.470$), leucine ($r = -0.498$, $P < 0.01$) and valine ($r = -0.458$), as well as lysine ($r = -0.456$) and total amino acids ($r = -0.389$). Valine ($r = -0.470$), methionine ($r = -0.415$) and tyrosine ($r = -0.457$) were significantly and inversely correlated with birth weight during pre-eclampsia. For birth length, during normal pregnancy, isoleucine ($r = -0.388$) and valine ($r = -0.394$) were significantly and inversely associated with this outcome. Valine ($r = -0.443$), asparagine

Table 3. Cord-blood amino acid concentrations in infants of normal pregnant women and women with pre-eclampsia

Amino acid	Normal pregnancy (n = 15)	Pre-eclampsia (n = 16)
Essential		
Arginine	5.1 ± 0.5	4.0 ± 0.6
Histidine	8.7 ± 0.9	11.1 ± 0.9
Isoleucine	6.4 ± 0.5	6.6 ± 0.3
Leucine	11.2 ± 0.9	10.9 ± 0.5
Lysine	27.2 ± 2.2	32.8 ± 1.4*
Methionine	2.9 ± 0.2	3.8 ± 0.3*
Phenylalanine	7.5 ± 0.4	8.1 ± 0.6
Valine	20.0 ± 1.1	20.4 ± 0.6
Non-essential		
Alanine	48.9 ± 3.9	72.3 ± 9.9*
Aspartic acid	1.8 ± 0.3	4.2 ± 1.1
Cystine	0.4 ± 0.2	2.1 ± 0.9
Glutamine	45.4 ± 2.1	48.8 ± 2.0
Glycine	25.1 ± 1.2	31.5 ± 4.6
Proline	13.5 ± 0.8	17.1 ± 1.5*
Tyrosine	6.5 ± 0.5	7.6 ± 0.5
Total	230.6 ± 11.3	281.4 ± 17.6*
Citrulline	1.01 ± 0.06	1.19 ± 0.1
3-Methylhistidine	0.22 ± 0.2	0.53 ± 0.07
Ornithine	11.0 ± 1.5	10.8 ± 1.2
Taurine	16.5 ± 1.9	28.1 ± 3.5 [‡]

Values are means ($\mu\text{mol } 100 \text{ ml}^{-1}$) $\pm$ SEM; * $P < 0.05$; [‡] $P < 0.01$ compared with normal pregnancy values.

($r = -0.604$, $P < 0.005$), glutamine ($r = -0.422$) and tyrosine ($r = -0.419$) were inversely correlated with birth length during pre-eclampsia.

There were no significant correlations between any maternal amino acid and head circumference of the infants among the women with a normal pregnancy outcome after adjusting for gestational age (Table 4). However, among the women with pre-eclampsia, several amino acids showed significant inverse correlations with head circumference: asparagine ($r = -0.636$, $P < 0.005$), methionine ($r = -0.564$, $P < 0.01$), tyrosine ($r = -0.553$, $P < 0.01$), valine ($r = -0.494$, $P < 0.05$) and total amino acids ($r = -0.465$, $P < 0.05$). For three of these amino acids (asparagine, tyrosine and valine), correlations with head circumference were also significantly different when comparing the normal and pre-eclamptic groups. The relationship between head circumference of the infant and total amino acids (Fig. 1) is representative of the significant associations found for individual amino acids. At low amino acid concentrations the head circumferences were similar for both groups, but as maternal amino acid concentrations increased, the head circumference values of infants for the controls remained constant, whereas they decreased for the pre-eclamptic women.

Table 4. Correlation (*r*) of maternal amino acid concentration with head circumference, adjusted for gestational age, of infants of normal pregnant women and women with pre-eclampsia

Amino acid	Normal pregnancy (<i>n</i> = 30)	Pre-eclampsia (<i>n</i> = 24)
Essential		
Arginine	−0.107	−0.042
Histidine	0.098	−0.363
Isoleucine	−0.202	−0.167
Leucine	−0.129	−0.366
Lysine	−0.127	−0.347 [§]
Methionine	−0.060	−0.564 [†]
Phenylalanine	0.148	−0.257 [§]
Valine	−0.116	−0.494 ^{*,§}
Non-essential		
Alanine	0.090	−0.338
Asparagine	0.021	−0.636 ^{‡,§}
Aspartic acid	0.093	−0.110
Cystine	−0.352	−0.078
Glutamate	0.007	−0.022
Glutamine	−0.104	−0.377
Glycine	0.151	−0.348
Proline	0.174	−0.238 [§]
Serine	0.077	−0.209
Threonine	−0.134	−0.489 [*]
Tyrosine	0.079	−0.553 ^{‡,¶}
Total	−0.006	−0.465 [*]
Citrulline	0.036	−0.099
Ornithine	0.075	0.027
Taurine	−0.019	0.033

**P* < 0.05; [†]*P* < 0.01; [‡]*P* < 0.005 (*r* values), partial correlations;
[§]*P* < 0.05; [¶]*P* < 0.005 (comparison of the relationship between head circumference and amino acid concentrations among pre-eclamptic and normal pregnancies; slopes and intercepts compared simultaneously).

Discussion

The main findings of this study are: (1) maternal and cord-blood amino acid concentrations were significantly increased during pre-eclampsia; (2) the smaller the infant, the higher the maternal amino acid concentrations, a relationship that, for head circumference, was restricted to pre-eclamptic pregnancies; and (3) pre-eclamptic women with SGA infants had higher maternal and cord-blood amino acid concentrations than women without SGA (pre-eclamptics or controls), whereas the cord-blood amino acid concentrations in pre-eclamptic and control women without SGA did not differ.

The largest differences in mean maternal amino acid concentrations between the pre-eclamptic women and controls were for alanine and glutamine (13.9 $\mu\text{mol } 100 \text{ ml}^{-1}$ and 9.6 $\mu\text{mol } 100 \text{ ml}^{-1}$, respectively), and these are the most important amino acids for gluconeogenesis (Stumvoll *et al.*, 1999). These amino acids could fuel the hyperglycaemia characteristic of the metabolic syndrome.

Fig. 1. Head circumference of infants adjusted for gestational age versus total serum amino acid concentration of normal pregnant women (■) and pre-eclamptic women (□).

Two previous studies, consistent with this report, found increased maternal amino acid concentrations during pre-eclampsia (Cockburn *et al.*, 1971; Li *et al.*, 1996). A third study reported significantly lower maternal amino acid concentrations during pre-eclampsia (D'Aniello *et al.*, 2001). However, it is noteworthy that the amino acid values reported in this third study were two orders of magnitude higher than those generally observed.

The present findings provide new insight into the inter-relationships among amino acid concentrations, pre-eclampsia and fetal growth, because they indicate that the most severely growth-restricted babies were exposed, via cord blood, to the highest amino acid concentrations. In particular, reduced head circumference, the most preserved fetal growth parameter, was uniquely related to high amino acid concentrations in pre-eclamptic women. Moreover, pre-eclamptic women with SGA babies had higher amino acid concentrations than controls but pre-eclamptic women whose babies were not SGA did not have higher amino acid concentrations. These observations are consistent with the hypothesis that pre-eclampsia does not involve one uniform pathophysiological process. The subset of women with pre-eclampsia having poor fetal outcomes may have the metabolic syndrome (Lain and Roberts, 2002), a syndrome characterized by increased amino acid concentrations (Pijl *et al.*, 1994).

These findings contrast with observations in IUGR pregnancies wherein maternal amino acid concentrations are generally not different from controls and cord-blood amino acid concentrations are lower due to reduced placental amino acid transport (Harrington *et al.*, 1999; Bajoria *et al.*, 2001; Paolini *et al.*, 2001; Jansson

et al., 2002). Many transport systems for amino acids, including A, ASC and L, have been identified within the human placenta (Jansson, 2001). There is considerable redundancy between the systems and competition for a carrier within a system. SGA babies from pre-eclamptic pregnancies may have increased placental amino acid transport or they may have aberrant utilization. The cord-blood measurements in the present study represented mixed arterial and venous cord blood; thus the cord-blood concentrations provide limited information about what the fetus utilized. Further limitations of this study include the retrospective design, the small sample size and the unclear fasting status of the subjects who provided the samples. Multiple comparisons were made but this restriction is mitigated by the number of differences observed that were significant at $P < 0.005$. The gestational age at sampling was also different for the two groups of pregnant women. However, amino acid concentrations did not change during the sampling range studied (data not shown). In a similar way, adjusting the amino acid concentrations for sampling age does not alter the conclusions reached.

In conclusion, the results of this study emphasize the differences in maternal and fetal amino acid concentrations observed between pre-eclamptic and normal pregnancies and the divergent associations between amino acid concentrations and fetal outcome in the two groups. In particular, the concentrations of several amino acids were inversely correlated with head circumference of infants in pre-eclamptic pregnancies but not in normal pregnancies. In addition, total amino acid concentrations were significantly higher in maternal and cord blood of pre-eclamptic women with SGA infants compared with either pre-eclamptic or control women without SGA infants. This result is intriguing, as it contrasts with the observation that cord-blood amino acid concentrations are reduced in IUGR infants. These data provide support for the notion that pre-eclampsia is a heterogeneous disorder associated with a hypermetabolic state wherein, paradoxically, the more abundant the availability of amino acids to the placenta, the less the fetus grows.

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