INCREASED NEONATAL URINARY AMMONIA: A MARKER FOR IN UTERO CALORIC DEPRIVATION?

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ABSTRACT

The decline in the urinary urea to ammonia ratio represents a simple measure of nutritional status in the adult. We examined the relationship of this ratio to nutrient-related fetal growth retardation. Levels of ammonia and urea nitrogen were measured in the first voided urine and cord blood from 15 term infants exhibiting a wide range of growth. Analysis by multiple regression with neonatal ponderal index as the primary dependent variable revealed a significant correlation between lowered ponderal index and decreased urinary urea and ammonia. The correlation was primarily a function of increasing ammonia levels, with no relationship between fetal leanness and urinary urea. Comparable cord artery and vein ammonia suggest that placental ammoniagenesis was not a major determinant of observed elevations in urinary ammonia. Confirmation of the striking correlation between increased urinary ammonia and lowered neonatal ponderal index may afford a simple test for the identification of nutrient-related growth retardation.

Methods for the detection of the fetus who will be born small for gestational age (SGA) are insensitive to the presence and nonspecific for the cause of intrauterine growth retardation (IUGR). One approach for identifying the nutritionally growth-retarded fetus is measurement of starvation associated metabolites in the amniotic fluid. Associations of amniotic fluid 3-methyl-histidine to creatinine,[1] alkaline ribonuclease, α amino nitrogen,[2] and hydroxyproline[3] with fetal growth have been encouraging. However, their widespread use has been restricted by the need for specialized laboratory analysis.

The decline in the urinary urea to ammonia ratio observed under conditions of prolonged starvation represents a simple measure assessing metabolic state in the child and adult.[4,5] We hypothesized that the nutritionally growth-retarded fetus or neonate might undergo similar changes in these metabolites, with a decline in the urea to ammonia ratio. Our purpose in this preliminary study was to examine the relationship between neonatal leanness and the urea to ammonia ratio in the first voided neonatal urine. Establishing this relationship would be a first step toward sorting out the many, poorly defined causes for IUGR, by defining a method with the potential for identifying cases of nutrient-related IUGR.

PATIENTS AND METHODS

Term infants (37 to 42 weeks) by menstrual, clinical, and sonographic data were selected in a sequential fashion based on the availability of the senior author. To obtain a sample with both normal and aberrant intrauterine growth, two groups were recruited. The first group (IUGR high risk) was chosen on the basis of suspected growth retardation, specifically by the presence of sonographically detected oligohydramnios (maximum pocket of amniotic fluid <1 cm $\times$ 1 cm), a factor previously showing a strong association with the birth of an SGA infant.[6] Documentation of intact amniotic membranes and the absence of sonographically detectable abnormalities of the fetal urinary system was confirmed before patient inclusion. The second group (IUGR low risk) consisted of intrauterine

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pregnancies with no known clinical risk factors for IUGR in whom a recent ultrasound examination documented normal amniotic fluid volume by visual assessment.

At the time of delivery, umbilical cord arterial and venous samples were obtained. The first voided neonatal urine was collected in a pediatric urine collector and immediately placed on ice in a sealed test tube. All samples were analyzed for urea nitrogen and ammonia. Ammonia (total NH_3 and NH_4) was measured on Kodak Ektachem clinical chemistry slides and measured by reflectance spectrophotometry at 600 nm.[7] Urea nitrogen was measured by the enzymatic conductivity rate method on a Beckman Astra 8.[8] Maternal characteristics (height, weight, and weight gain), cord pH, Apgar scores, birthweight and length, and pediatric assessment of gestational age (Ballard)[9] were recorded. Birthweight percentile was based on a sea-level growth curve.

To test the hypothesis that the urinary biochemical markers studied were related to growth, statistical analysis was by unpaired Student's *t* test and stepwise multiple regression. Neonatal ponderal index,[10] (weight [grams]/(length) [cm])^3)×100, was chosen as the primary dependent variable, since weight for length was expected to be the anthropometric measure most directly reflecting fetal leanness and relative caloric deprivation in utero. The primary independent variable was the urea to ammonia ratio. In the follow-up analyses, urinary urea and ammonia were evaluated, along with covariates, including maternal characteristics (prepregnancy body mass index calculated as (weight [kg]/(height) [cm])^2) × 100,[11] and weight gain), and oligohydramnios. A $p < 0.05$ was considered significant.

RESULTS

Eight patients with suspected IUGR and seven with no known risk factors for IUGR were studied. Differences in maternal characteristics of the two patient selection groups did not reach statistical significance (Table 1). Amniotic fluid was clear in all cases, cord pH and Apgar scores were equivalent in the two groups and no infants required resuscitation or ventilatory support. Mean gestational age and birth length of the infants were comparable in the two groups. However, other measure of growth (birthweight, birthweight percentile, and ponderal index) were significantly lower in the IUGR high risk group (Table 2). As documented in Table 3, the urea to ammonia ratio was significantly lower in the IUGR high risk group. Mean urinary urea nitrogen levels were similar, but urinary ammonia was significantly higher in the IUGR high risk group.

Stepwise multiple regression analysis, the results of which are summarized in Table 4, revealed that of the biochemical parameters, in a multivariate setting, only ammonia was significantly related to neonatal ponderal index ($F\ 1,13 = 6.61$, $r = -0.58$, $p < 0.023$). Maternal characteristics explained no significant additional variance in neonatal ponderal index.

The interrelationships of neonatal urinary am-

Table 1. Maternal Characteristics and Neonatal Acid-Base Data

	IUGR High Risk (n = 8)	*IUGR Low Risk (n = 7)*	*p*
Maternal			
Age (yr)	24 ± 4.7	24 ± 5.6	NS*
Gravidity	2.5 ± 1.2	3.4 ± 1.5	NS
Parity	1.0 ± 0.8	1.7 ± 1.0	NS
Body mass index	0.23 ± 0.06	0.26 ± 1.0	NS
Weight gain (kg)	13.0 ± 3.8	17.7 ± 5.0	NS
Neonatal			
Apgar 1 minute	8.1 ± 0.6	8.6 ± 0.5	NS
Apgar 5 minutes	8.9 ± 0.4	9.0 ± 0	NS
Arterial cord pH	7.34 ± 0.04	7.31 ± 0.04	NS

*NS: not significant

Table 2. Neonatal Growth Parameters*

	IUGR High Risk (n = 8)	*IUGR Low Risk (n = 7)*	*p*
Ballard (wk)	39.4 ± 2.1	39.3 ± 1.3	NS†
Birthweight (gm)	2806.1 ± 747	3501.7 ± 452	<0.03
Birth length (cm)	47.9 ± 3.8	49.0 ± 1.9	NS
Birthweight percentile	23 ± 30	72 ± 38	<0.03
Ponderal index	2.51 ± 0.27	2.97 ± 0.20	<0.001

*Mean ± standard deviation.
†NS: not significant.

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Table 3. Neonatal Urinary Biochemical Parameters*

	IUGR High Risk (n = 8)	*IUGR Low Risk (n = 7)*	*p*
Urea to ammonia ratio	3.7 ± 2.5	9.2 ± 2.9	<0.001
Ammonia (mg/dl)	44.9 ± 37.7	8.1 ± 5.1	<0.02
Urea nitrogen (mg/dl)	122 ± 97.5	71.29 ± 45.9	NS†

*Mean ± standard deviation.
†NS: not significant.

Table 4. Results of Multiple Regression with Neonatal Ponderal Index as Dependent Variable

Variable	R^2	*F to Enter at Step 0*	*p*	*F to Remove at Final Step*	*p*
Ammonia	0.34	6.61	<0.05	6.61	<0.05
Urea to ammonia ratio	0.28	5.10	<0.05	0.84	NS
Maternal weight gain	0.18	3.06	NS	1.32	NS
Urea	0.12	1.83	NS	0.47	NS
Maternal height	0.03	0.37	NS	1.23	NS
Maternal body mass index	0.004	0.05	NS	0.37	NS

*NS: not significant.

monia, ponderal index, and oligohydramnios were explored in follow-up regression analyses and summarized in Figure 1. Oligohydramnios exhibited a strong positive correlation with neonatal urinary ammonia ($r = 0.58$) and a strong negative correlation with neonatal ponderal index ($r = -0.72$).

Mean cord arterial and venous urea (6.4 ± 2.3 mg/dl, 5.9 ± 1.9 mg/dl, respectively) and ammonia (104 ± 63 μmol/liter, 77.6 ± 69 μmol/liter, respectively) were not significantly different.

DISCUSSION

The key finding of this study is the striking correlation between increased urinary ammonia levels and lowered neonatal ponderal index. The fact that the association was only moderately strong undoubtedly reflects the influence of factors other than nutrient supply on fetal growth. Since maternal characteristics explained no significant variance in ponderal index, differences in neonatal leanness did not appear to reflect genetically determined constitutional variation in this series. This suggests that measurement of neonatal urinary ammonia may help differentiate constitutionally small infants from those subjected to caloric deprivation in utero.

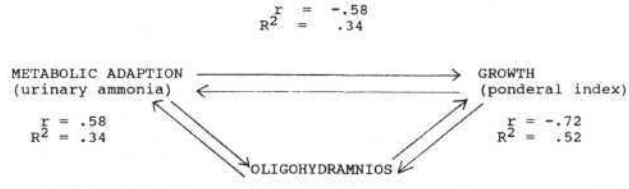

Figure 1. Interrelationship between urinary ammonia, neonatal ponderal index and oligohydramnios.

Although urinary pH was not measured, variance in urinary acidity was unlikely to be an important determinant of observed urinary ammonia levels. Renal capacity for acidification is limited in the first 24 hours of life.[12] Additionally, comparable acid-base status between groups in this study indicated no obvious need for neonatal urinary acidification. In the presence of neonatal acidosis, however, urinary pH should be evaluated.

In the adult, a declining urea to ammonia ratio reflects metabolic adaptation. Under conditions of starvation, increased urinary ammonia, the byproduct of renal gluconeogenesis, maintains acid-base equilibrium by titrating keto acids. A decrease in urinary urea, normally the major osmotic solute, conserves water and reflects sparing of protein stores. Our results suggest that the situation in the calorically growth-retarded neonate is not precisely comparable. The correlation between the urea to ammonia ratio and neonatal ponderal index in this study was primarily a function of increasing ammonia levels, with no relationship between fetal leanness and urinary urea levels. Studies of chronically starved sheep have demonstrated an increase in the rate of urea excretion despite no significant alteration in placental transfer of amino acids. Thus, elevated nitrogen excretion might be a marker for a state of relative catabolism. In addition, large maternal protein stores available for fetal homeostasis would produce relative, rather than absolute, fetal caloric deprivation. Metablic adaptations, such as the decline in urinary urea, might therefore not be fully utilized.[13]

Increased renal, hepatic, and placental ammoniagenesis, as well as impaired placental clearance of ammonia, may all have contributed to the observed variation in neonatal urinary ammonia levels. One possible interpretation of the comparable cord ar-

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tery and vein ammonia levels is that placental ammoniagenesis was not a major determinant of the observed increases in urinary ammonia. It should be noted, however, that this study did not measure uterine or umbilical blood flows. Previous studies in the fetal lamb have shown a positive venoarterial ammonia difference across the placenta, suggesting that placental ammoniagenesis may be a large source of fetal ammonia.[14] This question as well as the relative contributions of renal and hepatic ammoniagenesis will require further study.

The strong correlation of oligohydramnios with both decreased ponderal index and increased urinary ammonia supports the contention that oligohydramnios may be a relatively specific marker for nutrient-related growth retardation. Evidence of metabolic compromise in the urine of neonates with oligohydramnios further explains the utility of this sonographic marker in identifying the at-risk fetus.[15]

Based on this very small series, we are certainly not recommending the use of ammonia or the urea to ammonia ratio in clinical practice. Nonetheless, it is tempting to speculate that confirmation of our findings by additional studies might lead to a simple test for the presence of nutrient-related growth retardation at birth, as well as a practical method of monitoring response to nutritional therapy in both the antenatal and neonatal periods. Studies are currently in progress examining the relationship of amniotic fluid urea and ammonia to fetal growth and the potential utility of these determinations for the antenatal detection of impaired growth in utero.

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