Asymptomatic Hyperammonemia in Low Birthweight Infants

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Summary

At 0-3 days of age the plasma ammonium concentration in full term appropriate for gestational age (AGA) infants was (mean $\pm$ SEM) $27.5 \pm 0.5 \mu\text{M}$; a value similar to that reported in adults. Ammonium levels in low birthweight AGA and SGA groups were $47.0 \pm 2.0 \mu\text{M}$ and $45.1 \pm 3.3 \mu\text{M}$ respectively; significantly elevated ($P < 0.001$) as compared to the full term group. These increased ammonium levels persisted at 3-5 weeks of age. Associated with the hyperammonemia was a significant ($P < 0.01$) decrease in plasma α -ketoglutarate concentration: $11.8 \pm 1.0 \mu\text{M}$, in the low birthweight AGA as compared to $20.7 \pm 0.6 \mu\text{M}$ in the full term AGA infants. There was an inverse linear correlation between plasma concentrations of ammonium and α -ketoglutarate $r = -0.86$, $P < 0.001$. Urinary orotate excretion was significantly elevated ($P < 0.05$) in low birthweight AGA infants. There was no difference in the plasma concentrations of glutamine, glutamate, or alanine among the various groups. Hyperammonemia was not associated with neurologic dysfunction.

Speculation

There is a greater accumulation of ammonium in low birthweight as compared to full term infants. This may be a consequence of a developmental delay of one or more of the enzymes required for urea synthesis. Alternatively, the hyperammonemia may be related to arginine and/or ornithine diversion out of the cycle and incomplete repletion of ornithine from glutamate via the Δ^1 -pyrroline-5-carboxylate pathway.

Hyperammonemia in childhood is most commonly seen in liver disease, parenteral nutrition, Reye's syndrome, and congenital urea cycle enzyme defects. We have noted hyperammonemia in low birthweight infants and present here a study of changes in plasma levels of ammonium and other substrates related to ammonium disposition during the first 2 months of life in full term, low birthweight SGA, and low birthweight AGA infants.

MATERIALS AND METHODS

Newborn infants admitted to the Infant Special Care Unit and the Newborn Nursery at Johns Hopkins Hospital comprised the study groups. None of the infants had pre-existing liver disease or received parenteral nutrition. The study group included 52 full term AGA infants, 9 low birthweight SGA infants, and 27 low birthweight AGA infants. Low birthweight is defined as a birthweight below 2500 g as recommended by the WHO (29). SGA is defined as a birth weight less than the tenth percentile for gestational age (9), using the Colorado indices (17).

Informed consent was obtained for the study, which was approved by the Joint Committee on Clinical Investigation. Plasma levels of ammonium, glutamine, alanine, and glutamate

were measured once or twice a week on capillary blood during the first 2 months of life. Plasma α -ketoglutarate concentration and urinary orotate excretion were also measured at 0-3 days of age in 30 infants. Plasma ammonium determinations were performed within 30 min of blood drawing using a cation exchange resin, as previously described (3). The normal adult range is 15-33 μM . Glutamine (18), glutamate (4), alanine (30), and α -ketoglutarate (16) were measured by modifications of enzymatic fluorometric techniques, allowing capillary plasma to be used. Urinary orotate was measured by the method of Goldstein *et al.* (11). A neurologic examination as described by Prechtl and Beintema (20) was performed on the low birthweight infants at 0-3 days of age.

RESULTS

The full term group consisted of 25 males (22 were black) and 27 females (25 were black); the low birthweight groups consisted of 14 males (11 were black) and 22 females (20 were black). Birthweights of the groups were (mean $\pm$ SEM): full term 3261 ± 59 g, low birthweight SGA 1848 ± 185 g, and low birthweight AGA 1963 ± 79 g. Gestational age in the groups were (mean $\pm$ SD): full term AGA 39 $\pm$ 1.5 weeks, low birthweight SGA 37 $\pm$ 3 weeks and low birthweight AGA 33 $\pm$ 2.5 weeks. All infants received a proprietary milk formula (Similac) containing 1.5 g protein/100 ml, 18 parts bovine whey protein to 82 parts bovine caseins. Mean protein intake was similar in all groups at 3-5 weeks of age (3.0 g/kg/day) and was higher in the full term group during the first 3 days of life (1.7 compared to 1.2 g/kg/day). At 0-3 days of age, mean daily caloric intake was 70 cal/kg in the low birthweight infants and 60 cal/kg in the full term infants. From 3-5 weeks of age, the mean caloric intake was 140 cal/kg and 120 cal/kg in the low birthweight and full term groups, respectively.

Table 1 lists plasma ammonium levels during the first 2 months of life in the three groups: full term, low birthweight SGA, and low birthweight AGA infants. The full term group comprised 41 delivered spontaneously and 11 by cesarean section. At 0-3 days of age, there was no significant difference in plasma ammonium levels between full term infants delivered vaginally and by cesarean section (mean $\pm$ SEM): 27.2 ± 0.6 and $28.8 \pm 1.0 \mu\text{M}$, respectively. Similar levels were obtained at 3-5 weeks of age.

Plasma ammonium levels in low birthweight AGA and low birthweight SGA groups were $47.0 \pm 2.0 \mu\text{M}$ and $45.1 \pm 3.3 \mu\text{M}$, respectively, at 0-3 days of age. Of the 36 low birthweight infants, four had plasma ammonium concentrations below 33 μM (Fig. 1) and were not followed. The remaining infants were followed until they were 6-8 weeks of age. The significant elevation in ammonium levels persisted in the low birthweight groups at 3-5 weeks of age with levels of $42.9 \pm 1.0 \mu\text{M}$ in the SGA groups and $47.7 \pm 3.0 \mu\text{M}$ in the AGA group. When the infants reached full term by weight (2500 g) or by gestational age (38 weeks) they were still hyperammonemic (mean $\pm$ SEM): $40.0 \pm 2.9 \mu\text{M}$ and $47.2 \pm 3.3 \mu\text{M}$, respectively. At 6-

Table 1. Concentrations of plasma ammonium in full term, low birth weight SGA and low birthweight AGA infants during the first 2 months of life

	0-3 days	3-5 weeks	6-8 weeks
Full term	27.5 ± 0.5 n = 52	25.9 ± 3.1 n = 8	
Low birthweight SGA	45.1 ± 3.3 ¹ n = 9	42.9 ± 1 ² n = 8	29.8 ± 1.2 n = 9
Low birthweight AGA	47.0 ± 2.0 ¹ n = 27	47.7 ± 3.0 ² n = 15	26.9 ± 1.6 n = 9

¹ $P < 0.001$ compared to full term.

² $P < 0.005$ compared to full term.

Fig. 1. The relationship between birthweight and plasma concentration of ammonium at 0-3 days of life ($n = 80$). Normal range of plasma ammonium in adults is indicated by cross-hatching.

8 weeks of postnatal age, plasma ammonium levels were normal in both low birthweight AGA and low birthweight SGA groups: 26.9 ± 1.6 and 29.8 ± 1.2 μM , respectively. No statistically significant relationships were noted when plasma ammonium concentration was correlated with gestational age below 40 weeks, sex, race, protein intake, or plasma glutamine level. The distribution of plasma ammonium levels at 0-3 days as related to birthweight is shown in Figure 1. At birthweights below 2500 g more than 85% of the infants were hyperammonemic; at birthweights above 2500 g, virtually all infants had normal ammonium levels.

Plasma α -ketoglutarate concentration was measured because of its relationship to ammonium metabolism. We found a significant ($P < 0.01$) decrease in plasma α -ketoglutarate concentration, 11.8 ± 1.0 μM , in the low birthweight AGA as compared to 20.7 ± 0.6 μM in the full term infants (2). Using data from the full term and low birthweight AGA infants, we found an inverse linear correlation between plasma concentration of ammonium and α -ketoglutarate ($r = -0.86$, $P < 0.001$) (Fig. 2).

Urinary orotic acid excretion was measured because it has been described as being increased in urea cycle enzyme defects other than carbamylphosphate synthetase deficiency. We found a small but significant ($P < 0.05$) increase in orotic acid excretion in low birthweight AGA group as compared to the full term group (Table 2). There was no difference in the plasma concentrations of glutamine, glutamate, or alanine among the various groups (Table 3). On neurologic examination, the level of alertness, symmetry of movement, deep tendon reflexes, and primitive reflexes were not abnormal in the hyperammonemic infants.

Fig. 2. The relationship between plasma ammonium and α -ketoglutarate levels in low birthweight AGA and fullterm infants ($n = 35$, $r = -0.86$, $y = -0.39x + 31.6$).

Table 2. Plasma α -ketoglutarate concentration and urinary orotate excretion in full term and low birthweight infants at 0-3 days of age

	α -Ketoglutarate, $\mu\text{M} \pm \text{SEM}$	Orotic acid, $\mu\text{g}/\text{mg}$ creatinine $\pm \text{SEM}$
Full term	20.7 ± 0.6 $n = 17$	4.3 ± 0.9 $n = 14$
Low birthweight AGA	11.8 ± 1.0 ¹ $n = 13$	9.3 ± 2.0 ² $n = 18$
Normal (2, 11)	18-25	0-8.7 ³

¹ $P < 0.01$ compared to full term.

² $P < 0.05$ compared to full term.

³ Micrograms per milligram.

DISCUSSION

PLASMA AMMONIUM LEVELS IN NEONATES

Clemmens (7) measured whole blood ammonia in 46 infants between 30 min and 36 days of age, using the alkali distillation method of Seligson (26), and found no difference between low birthweight and full term infants, range 7-116 μM . This test is nonspecific for ammonia as it also measures other alkali labile nitrogen (24): the range of normal is 50-100 μM , whereas the range of normal for the true blood ammonium (as measured by cation-exchange resin or ammonium electrode) is 15-30 μM (21).

Rubaltelli (25), using a cation-exchange method, found that full term SGA infants, less than 5 days of age, had higher plasma ammonium levels (48 ± 10 μM) than did full term AGA infants (35 ± 5 μM). Rih *et al.* (22) found plasma ammonium levels of low birthweight infants less than 6 weeks of age to vary between 60-90 μM when measured with an ammonium electrode. They commented that these levels were not abnormal. Yet when Proelss *et al.* (21), who also used an ammonium

Table 3. Concentrations of plasma glutamine, alanine, and glutamate in study patients during first 5 weeks of life¹

	Concentration, $\mu\text{M} \pm \text{SEM}$					
	Glutamine		Alanine		Glutamate	
	0-3 days	3-5 weeks	0-3 days	3-5 weeks	0-3 days	3-5 weeks
Full term	613 ± 15 n = 18	609 ± 34 n = 8	314 ± 7	338 ± 28	68 ± 2	78 ± 4
Low birthweight AGA	684 ± 30 n = 12	690 ± 38 n = 9	340 ± 22	359 ± 18	74 ± 5	80 ± 5
Low birthweight SGA	669 ± 29 n = 8	713 ± 41 n = 8	308 ± 21	303 ± 23	72 ± 5	77 ± 4

¹ Normal ranges: glutamine, 342-1046; alanine, 177-476; glutamate, 16-64.

electrode, compared plasma ammonium levels measured by the electrode and by an ion-exchange technique, they found a correlation coefficient of 0.994 and an upper limit of normal of 30 μM . Oberholzer *et al.* (19) showed significant differences in resin plasma ammonium levels of full term infants 0–3 days of age as compared to 1–12 weeks of age (mean $\pm$ SD): 86 ± 20 compared to 25 ± 5 μM .

Although the reasons for these discrepancies are not apparent, they may relate in part to the care needed in obtaining the blood sample and the rapidity of performing the test. In our study free-flowing capillary blood was collected in heparinized tubes and immediately placed on ice. The cation-exchange assay was performed less than 0.5 hr after obtaining blood. Using this method, we observed persistent hyperammonemia (37–76 μM) for 2 months postnatally in almost all low birthweight infants tested, whereas it was not found in infants with birthweights over 2500 g. Hyperammonemia was frequently associated with an elevation in urinary orotate excretion and was always associated with a decrease in the plasma concentration of α -ketoglutarate. No intergroups differences were noted in plasma concentrations of glutamine, glutamate, and alanine.

AMMONIUM ACCUMULATION

Although the mechanism of hyperammonemia in low birth-weight infants is unclear, it could result from portal-systemic shunting, urea cycle enzyme deficiency, or ornithine or arginine depletion. Little is known about the possibility of portal-systemic shunting from persistence of the patent ductus venosus in low birthweight infants. Portal-systemic shunting in adults results in hyperammonemia associated with normal or increased plasma levels of α -ketoglutarate (8, 15). In the low birthweight infants, α -ketoglutarate levels were found to be low, suggesting that portal-systemic shunting may not be responsible for the hyperammonemia.

The substrates, enzymes and possible regulatory steps in the synthesis of urea are depicted in Figure 3. Also included in the figure are the pathways for depletion and repletion of the cycle intermediates.

Räihä (23) found that all the urea cycle enzymes had sufficient *in vitro* activity by 28 weeks of gestation to preclude accumulation of substrates or significantly retard urea synthesis. However, it is possible that *in vivo* activity in low birthweight infants may not be sufficient to cope with high protein intakes. One might then have expected an inverse correlation between gestational age and plasma ammonium levels. This was not observed. We also did not find a direct relationship between ammonium and other labile nitrogen components, glutamine, glutamate, and alanine as we have observed in patients with urea cycle defects. Acetylglutamate synthetase activity has not been evaluated; a defect in this enzyme might result in impaired carbamylphosphate synthetase activity.

The finding of an increase in orotic acid excretion suggests accumulation of carbamylphosphate as a consequence of low

Fig. 3. The enzymes and substrates related to ammonium disposition and urea synthesis. GD: glutamate dehydrogenase; CPSI: carbamyl-phosphate synthetase I; Ac glu: acetylglutamate; AGS: acetylglutamate synthetase; cp: carbamylphosphate; OTC: ornithine transcarbamylase, ASA-S: argininosuccinic acid synthetase; ASA-L: ASA-lyase; ODC: ornithine decarboxylase; ORN TR: ornithine translocation; OAT: ornithine δ -amino transferase; pc: Δ^1 -pyrroline-5-carboxylate; GT: glycine transamidinase. Reactions enclosed in the box are mitochondrial. Enzymes are denoted by capital letters, substrates by lower case letters. *: Arginine is an activator of acetylglutamate synthetase (28).

intramitochondrial levels of ornithine. This could result from defective ornithine translocation (10) or a developmental delay in ornithine synthesis from substrates other than arginine; glutamate, proline, or Δ^1 -pyrroline-5-carboxylate.

That preterm infants may have some inadequacy in the *de novo* synthesis of Δ^1 -pyrroline 5-carboxylate or ornithine may be adduced from the findings of Snyderman (27) that preterm infants become hypoargininemic on marginal protein intakes. Furthermore, the requirement of preterm infants for supplemental ornithine or arginine to prevent hyperammonemia while they are receiving total parenteral nutrition (13) also suggests a limited ability of the preterm infant to synthesize sufficient ornithine for the formation of protein and polyamines (only catalytic amounts are needed for synthesis of creatine and urea).

α -KETOGLUTARATE DEPLETION

The inverse correlation between plasma ammonium and α -ketoglutarate (Fig. 2) is consistent with the evidence that ammonium alters the redox state of liver mitochondria in the direction of oxidation, accompanied by a decrease in mitochondrial levels of α -ketoglutarate (5, 28). Studies of the brain response to hyperammonemia also suggest that as the mitochondrial $\text{NAD}^+:\text{NADH}$ ratio increases (12, 31), α -ketoglutarate content may decrease (14). The significant negative correlation we have found between plasma levels of ammonium and α -ketoglutarate is compatible with the hypothesis that the mitochondria are more oxidized in the hyperammonemic state. We have also noted an inverse relationship between plasma concentrations of ammonium and α -ketoglutarate in patients with urea cycle enzyme deficiencies (6). Recently Krebs (32) suggested that *in vitro*, gluconeogenesis may be impaired by high concentration of ammonium. The finding of hyperammonemia in SGA infants who are known to have impaired gluconeogenesis suggests that there may be a relationship between the two, perhaps as a result of decreased formation of glyceraldehyde-3-phosphate secondary to NADH depletion.

The present study shows that there is greater ammonium accumulation in low birthweight as compared to full term AGA infants. The mechanisms responsible are unknown but may involve one or more of the enzymes responsible for urea synthesis. Alternatively, the hyperammonemia may be related to arginine and/or ornithine diversion out of the cycle and incomplete repletion of ornithine from glutamate via the Δ^1 -pyrroline-5-carboxylate pathway. There were no acute neurologic abnormalities associated with the hyperammonemia, but whether hyperammonemia places the low birthweight infant at risk for future neurodevelopmental problems remains to be determined.

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