ARTICLE

Effects of Two Different Doses of Amino Acid Supplementation on Growth and Blood Amino Acid Levels in Premature Neonates Admitted to the Neonatal Intensive Care Unit: A Randomized, Controlled Trial

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ABSTRACT

OBJECTIVES. The goal was to measure the effects of 2 distinct strategies for parenteral nutrition on neonatal growth and blood amino acid profiles.

METHODS. In a multicenter trial (n = 11 sites), we randomly allocated premature (23–29 weeks and 6 days of gestation) neonates to 1 of 2 approaches to intravenous amino acid administration. In one group, amino acid supplementation was started at 1.0 g/kg per day and advanced by 0.5 g/kg per day to a maximum of 2.5 g/kg per day (2.5 g/kg per day group). The other group received amino acids starting at 1.5 g/kg per day and advancing by 1.0 g/kg per day to a maximum of 3.5 g/kg per day (3.5 g/kg per day group). Filter paper blood spots were obtained from each infant on the day of random assignment and on days 7 and 28 of age, to monitor blood amino acid levels.

RESULTS. We enrolled 122 neonates (64 in the 3.5 g/kg per day group and 58 in the 2.5 g/kg per day group). There were no differences in demographic or baseline characteristics between the 2 treatment groups. There was no significant difference in growth by day 28 after birth (median weight gain: 12.9 and 11.4 g/kg per day for the 3.5 and 2.5 g/kg per day groups, respectively), and the incidences of secondary morbidities were similar in the 2 groups. On day 7, blood levels of several amino acids and the serum urea nitrogen level were higher in the 3.5 g/kg per day group, compared with the 2.5 g/kg per day group; none of the amino acid levels were lower.

CONCLUSIONS. Higher doses of amino acid supplementation did not improve neonatal growth and were associated with increased blood amino acid and urea nitrogen levels.

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Key Words
neonates, parenteral nutrition, amino acids, acylcarnitines, nutrition

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STUDIES HAVE INDICATED that premature neonates often develop severe nutritional deficits during the first weeks after birth,[1–5] and inhibited growth during the early postnatal period has been associated with poor long-term outcomes.[2,5,6] Variations in dietary intake may account for 45% of the variations in growth.[7,8] As a result, efforts have focused on determining whether nutritional deficiency and growth restriction in premature infants can be prevented through better nutritional intake.[6,9,10]

On the basis of animal studies that showed high levels of in utero amino acid flux during the later phases of gestation, several authors have suggested that higher doses of amino acid supplementation may minimize extrauterine growth restriction and enhance the outcomes of very low birth weight infants.[9–11] Although standard doses of amino acids may be inadequate to promote normal growth, higher doses may lead to elevated blood amino acid levels and increased toxicity. Given the limited metabolic capacity of very low birth weight infants, excess administration of amino acids (protein) may result in saturation of both catabolic and anabolic pathways of protein metabolism, leading to larger amino acid pools for longer periods of time. Through the implementation of a multicenter randomized trial and tandem mass spectrometric analysis of key amino acids from dried filter paper blood spots, the effects of 2 distinct strategies for amino acid supplementation on amino acid profiles and growth in premature infants were evaluated.

METHODS

Study Subjects

Eligible infants had estimated gestational ages between 23 weeks 0 days and 29 weeks and 6 days, were inborn, and had parental consent for participation in the study. Patients were approached for random assignment during the first 48 hours after birth and were excluded if they were >48 hours of age or had a major congenital anomaly. Investigational review boards of each hospital (n = 11) approved the protocol.

Random Assignment and Blinding

We used an electronic system to assign a randomized code, which was delivered to the health care provider responsible for preparing parenteral nutrition. That individual used the random assignment code to determine the treatment assignment and mixed the parenteral solution according to study and pharmacy protocols. The parenteral nutrition solution was labeled "study AA," and the concentrations of amino acids were not indicated on the bag. A pharmacy log tracked the amounts of amino acids in the solution for each patient. This approach blinded the clinical care staff members to the amount of amino acid supplementation that each neonate received. Random assignment was stratified according to site.

Treatment

For the group with a maximal dose of 2.5 g/kg per day (2.5 g/kg per day group), amino acid supplementation was started at 1.0 g/kg per day and advanced 0.5 g/kg per day to a maximum of 2.5 g/kg per day on day 4 of treatment. For the group with a maximal dose of 3.5 g/kg per day (3.5 g/kg per day group), amino acid supplementation started at 1.5 g/kg per day of amino acids and advanced 1 g/kg per day to a maximum of 3.5 g/kg per day on day 3 of treatment.

When enteral feedings were started, parenteral supplementation of amino acids continued, with the goal of delivering the maximal dose of amino acids allowed by the protocol. As feedings were advanced, intravenous fluids were decreased accordingly, to keep total fluids at ≤150 mL/kg per day. Parenteral nutrition was mixed to provide the maximal amount of protein allowed by the protocol. As feedings were advanced, the total amount of protein per kilogram per day increased as a result of the protein in the enteral feedings. When the parenteral nutrition was being administered at a rate of <70 mL/kg per day, the amount of amino acids that could be added to the parenteral nutrition decreased (limited by the solubility of the elements in the parenteral nutrition). Therefore, when feedings reached 80 to 100 mL/kg per day, we decreased amino acid supplementation to 1 g/kg per day for the 2.5 g/kg per day group and to 2.0 g/kg per day for the 3.5 g/kg per day group. For both groups, amino acid supplementation was stopped when feedings reached 100 to 130 mL/kg per day. When enteral nutrition was at the level of 100 to 130 mL/kg per day, the patient was considered to have completed treatment. Subsequent parenteral nutrition was administered at the discretion of the health care team.

Nutritional Support Guidelines

We recommended starting parenteral lipid administration at the same time that the study amino acid supplementation was started, beginning at a rate of 0.5 g/kg per day and advancing 0.5 g/kg per day to a maximum of 3.5 g/kg per day.[12–14] We discouraged the use of insulin and recommended limiting the glucose infusion rate to 8 to 12 mg/kg per minute as tolerated. Although the quantity and quality of enteral nutritional support were not structured in this trial, we offered the following feeding guidelines, in an attempt to maintain consistency: small-volume (<10 mL/kg per day) feedings should begin within the first week of life; low-dose dopamine treatment (defined as <5 μg/kg per minute) was not considered a contraindication for trophic feedings; feedings could be initiated even if umbilical catheters were in place; feedings could be advanced at a rate of as much as 30 mL/kg per day and were not to be advanced at a rate of <10 mL/kg per day; and 150 mL/kg per day, with consistent weight gain of 20 to 30 g/kg per day, was considered adequate nutrition.

Study End Points

Our primary outcome measure was growth, assessed as changes in weight, length, and head circumference over the first 28 days after birth. Weight gain was calculated as weight gain (in grams per kilograms per day) = (weight at 28 days − birth weight) divided by birth weight divided by 28 days. We also assessed length and head growth changes in centimeters per week, as (measurement at 28 days − measurement at birth) divided by 4 weeks.

Secondary outcome measures included blood amino acid profiles and incidence of major morbidities during the first 28 days after birth. Blood amino acid profiles were obtained on the day of random assignment (usually day 1) and on day 7 (parenteral phase of nutrition) and day 28 (enteral phase of nutrition) of life.

Data Collection and Monitoring

All information was collected by using an Internet-based case report form that could not be finalized until all required fields were completed. Site visits were conducted to monitor the accuracy of data collection and adherence to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use good clinical practice guidelines.

Study logs of amino acid contents in the parenteral nutrition were maintained for each subject for the first 28 days of life, by pharmacy personnel. Amino acid contents were recorded each day that the subject received either study or nonstudy parenteral nutrition. Pharmacy logs and source documents were monitored to validate each of the growth measurements and the amount of amino acid supplementation that was recorded on the case report form of each subject.

Measurement of Amino Acid and Acylcarnitine Levels

Amino acid and acylcarnitine profiles were analyzed from dried blood spots by using tandem mass spectrometry, as described by Chace and Kalas.[15] All quantitative results were provided to study investigators and were reported as micromoles per liter.

To provide "normal" reference values (reference values in Fig 1), we calculated the median and the 10th and 90th percentiles for each metabolite by using 1000 normal term newborn values (from samples obtained in the first 7 days after birth). These values were derived from samples sent to our laboratory for screening for inborn errors of metabolism. The values represent our internal normal newborn control values (reference values). To try to identify the amino acid and acylcarnitine values that were commonly (>50% of the patients) abnormal, relative to our internal reference newborn levels, we calculated the proportion of patients with values greater than the 90th percentile (high levels) and the proportion of patients with values less than the 10th percentile (low values) for the overall study population and each treatment group.

Statistical Analyses and Sample Size Calculation

Our hypothesis was that the higher-dose group would have a growth velocity (13.0 g/kg per day) 3 g/kg per day more than that of the lower-dose group (10 g/kg per day). On the basis of previous work with premature neonates,[6] we calculated that a sample size of 108 patients (54 patients in each group) would allow us to detect a difference of 3.0 g/kg per day in weight gain between the 2 treatment groups (α = .025, power = 80%, assuming SD = 5.0 g/kg per day). The α level was set at .025 on the basis of a 2-sided, 2-sample t test. We anticipated a 10% dropout rate resulting from morbidity and death; therefore, we enrolled 122 subjects.

We performed an intent-to-treat analysis. Categorical variables were evaluated by using a 2-tailed χ^2 test and Fisher's exact test. Continuous variables were compared by using a 2-tailed t test for parametrically distributed data and Kruskal-Wallis analysis of variance for nonparametrically distributed data. Rank data were assessed by using a 2-tailed, Mann-Whitney, nonparametric test. Analysis of variance for repeated measures was used to evaluate the changes in all laboratory values over time, from the day of random assignment to days 7 and 28 of age. Both time and treatment groups were evaluated. To evaluate the influence of covariates (gestational age, birth weight, gender, and postnatal exposure to steroids) on our study results, we performed a linear regression analysis. Our goal was to determine the treatment-influenced weight gain when these covariates were included in the regression model.

RESULTS

Enrollment

Between September 1, 2005, and June 1, 2006, site logs showed that 230 neonates were screened and 122 neonates (53%) were enrolled from 11 sites, 64 in the 3.5 g/kg per day group and 58 in the 2.5 g/kg per day group. The 2 reasons for nonenrollment were parent refusal (n = 57) and missed opportunities for obtaining consent (n = 51). The median enrollment per site was 11 patients (range: 3–16 patients).

Report on Patients Who Did Not Complete the Study

Of the 122 patients enrolled, 11 patients did not complete the study. Three patients died before 28 days. Five patients were withdrawn from the study (3 in the 3.5 g/kg per day group and 2 in the 2.5 g/kg per day group). One patient was considered by the attending neonatologist to be too sick to continue in the study, and 2 patients were transferred out of the study facility before 28 days. Follow-up laboratory assessments were not available for those patients. The primary reason for a patient being withdrawn from the study was a serum urea nitrogen level of >50 mg/dL (n = 4). Two of the 11 patients who did not complete the study did not receive study parenteral nutrition. Removal of the 11 patients who failed to complete the

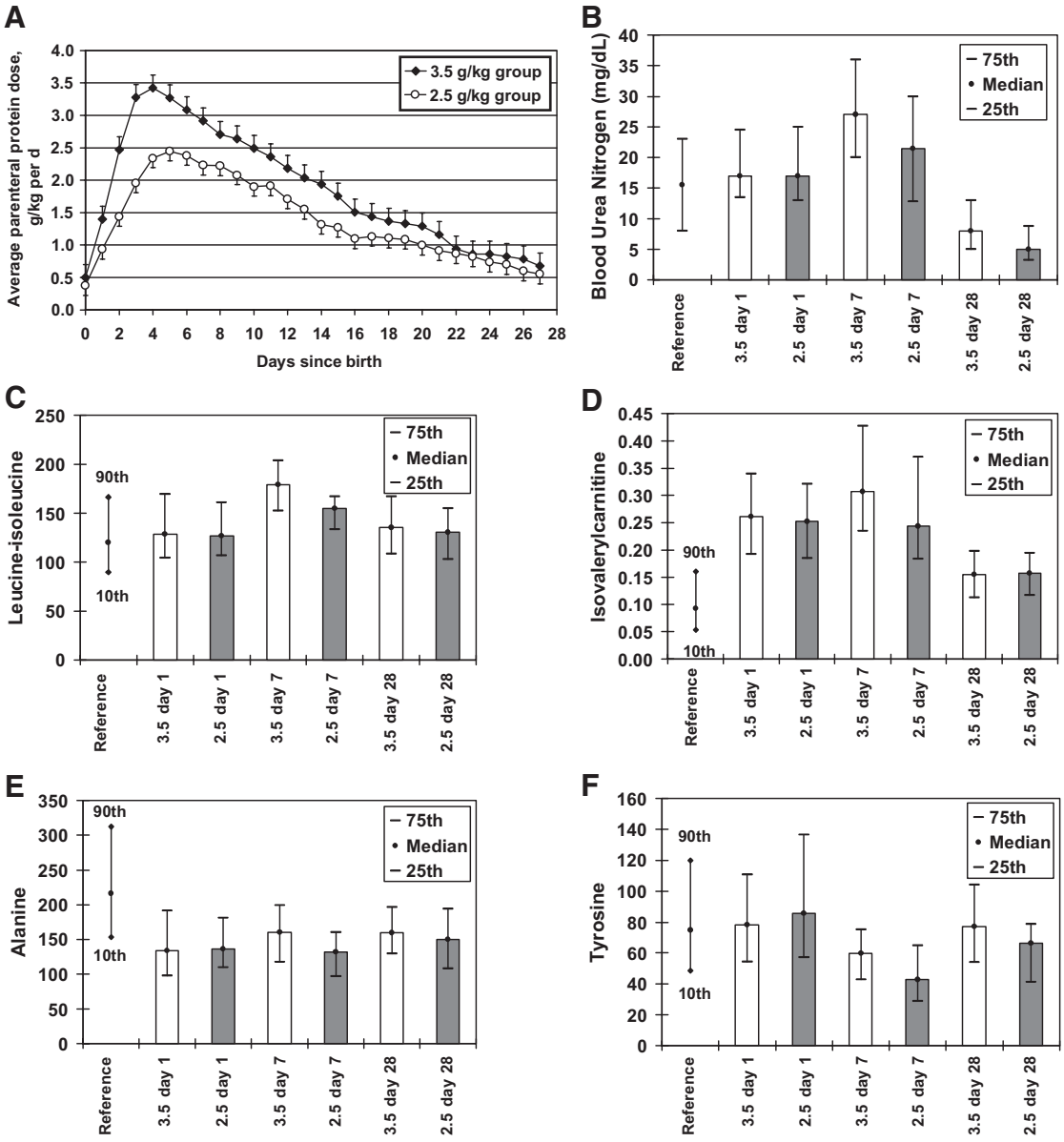

FIGURE 1

A, Mean ± SE of the dose of amino acids over time for neonates receiving a maximal dose of 3.5 g/kg per day and neonates receiving a maximal dose of 2.5 g/kg per day. B, Median and 25th and 75th percentile values for changes in serum urea nitrogen levels over time. C–F, representative changes in levels of key amino acids and acylcarnitines over time (C, leucine/isoleucine; D, isovaleryl carnitine; E, alanine; F, tyrosine). All values are reported in micromoles per liter. Statistical details are presented in Table 3. Graphs represent median and 25th and 75th percentile values for measured values and median and 10th and 90th percentile values for reference values. 3.5 indicates 3.5 g/kg per day group; 2.5, 2.5 g/kg per day group.

study protocol did not change our results, and we report only the intent-to-treat analysis.

Protocol Experience

Fifty percent of the patients were enrolled during the first 24 hours after birth, and there was good separation of the 2 treatment groups regarding the amounts of amino acids delivered after random assignment (Fig 1A). By design, from day 1 to day 28, neonates in the 3.5 g/kg per day group received a larger dose of amino acids than did those in the 2.5 g/kg per day group (Fig 1). There was no difference in the number of days of study amino acid support or the total number of days of any parenteral nutrition (Table 1).

Demographic Data and Primary and Secondary Morbidity Outcomes

We used an intent-to-treat approach, and all tables reflect all enrolled infants, categorized according to treatment group. There were no significant differences in the demographic or baseline characteristics of the 2 treatment groups (Table 1). On day 7, there were no differences in weight, length, head circumference, or degree of nutritional support (Table 1). Similarly, there was no

TABLE 1 **Demographic Characteristics**

	Maximal Dose of 3.5 g/kg per d	Maximal Dose of 2.5 g/kg per d	*P*
N	64	58	
EGA, median (IQR), wk	27 (26–28)	27 (25–28)	.8
Birth weight, median (IQR), g	961 (780–1187)	918 (788–1231)	.99
Birth length, median (IQR), cm	35.5 (33.5–37.5)	35 (32–38)	.6
Birth head circumference, median (IQR), cm	25 (23.1–26.5)	24.5 (23–27)	.9
1-min Apgar score, median (IQR)	5 (4–7)	5 (3–7)	.4
5-min Apgar score, median (IQR)	8 (7–8)	7 (6–8)	.1
Prenatal steroid use reported, *n* (%)	53 (82.8)	49 (84.5)	.8
Cesarean section, *n* (%)	39 (60.9)	39 (67.2)	.6
Male gender, *n* (%)	38 (59.4)	32 (55.2)	.7
Product of multiple gestation, *n* (%)	17 (26.6)	17 (29.3)	.7
Race, *n* (%)			.7
Asian	1 (1.6)	4 (6.9)	
Black	20 (31.3)	17 (29.3)	
Hispanic	8 (12.5)	5 (8.6)	
Other	4 (6.3)	4 (6.9)	
White	31 (48.4)	28 (48.3)	
Surfactant use reported, *n* (%)	50 (78.1)	51 (87.9)	.2
Age at random assignment, median (IQR), d	1 (0.5–1.4)	1 (0.5–1.4)	.8
Type of amino acid, *n* (%)			
Aminosyn	5 (7.8)	3 (5.2)	.7
TrophAmine	59 (92.2)	55 (94.8)	.7
Carnitine added to parenteral nutrition, *n* (%)	24 (37.5)	26 (44.8)	.37
Received protein before study enrollment, *n* (%)	42 (65.6)	38 (65.5)	.9
Total parenteral amino acid dose during first 7 d, median (IQR), g/kg	21.5 (19.5–21.5)	14.5 (13.5–15.4)	.01
Total parenteral amino acid dose during first 28 d, median (IQR), g/kg	51 (28–74)	34.5 (22.5–58)	.01
Time on treatment protocol, median (IQR), d	15 (10–22)	14.5 (10–25)	.5
Total time on parenteral amino acid support, median (IQR), d	18 (11.8–25)	17.5 (11.8–26)	.6
Day 7 growth parameters			
Weight, median (IQR), g	910 (760–1092)	880 (768–1105)	.99
Length, median (IQR), cm	36 (33.5–37.8)	35 (32.1–37.7)	.7
Head circumference, median (IQR), cm	24.5 (22.9–26)	24.5 (23.1–26.5)	.5
Day 7 nutritional support			
Glucose infusion, median (IQR), g/kg per d	11.3 (8.6–13.7)	11.2 (8.5–13.1)	.8
Lipids, median (IQR), g/kg per d	2.3 (1.6–2.9)	2.4 (1.7–2.9)	.7
Receiving some feedings, *n* (%)	44 (68.8)	40 (69)	.99
Enteral feedings, median (IQR), mL/kg per d	12.8 (0–57)	12.9 (0–34)	.2
Intravenous fluids, median (IQR), mL/kg per d	127 (98.6–153.6)	140 (123–160.3)	.4
Total fluids, median (IQR), mL/kg per d	151 (134–171)	158 (147–174)	.99
Day 7 laboratory data			
Serum bicarbonate level, median (IQR), mEq/L	22 (19–24)	22 (20–25)	.24
Serum blood urea nitrogen level, median (IQR), mg/dL	27 (20–36)	21.5 (12.8–30)	.01
Serum creatinine level, median (IQR), mg/dL	0.9 (0.7–1)	1 (0.73–1.18)	.04
Serum total bilirubin level, median (IQR), mg/dL	5.6 (4.5–7.3)	5.5 (4.1–6.6)	.26

EGA indicates estimated gestational age; IQR, interquartile range.

significant difference in growth (or any growth parameter) on day 28 (Table 2). The incidences of morbidities were similar for the 2 treatment groups (Table 2). Multivariate regression analysis showed that gestational age, birth weight, and postnatal exposure to steroids each had an independent influence on weight gain. The neonates with the poorest weight gain were small neonates who were gestationally immature and who were exposed postnatally to dexamethasone. In multivariate analyses, treatment group did not influence growth or the occurrence of the patient's weight being below the 10th percentile for weight at 28 days of age.

Changes in Amino Acid Levels

Differences in Levels According to Treatment Group

There were no differences in amino acid levels on the day of random assignment (Table 3). On day 7 (parenteral phase of nutrition), blood levels of several amino acids (alanine, arginine, glutamate, leucine/isoleucine, methio-

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