RESEARCH PAPER

Effect of Two Different Doses of Parenteral Amino Acid Supplementation on Postnatal Growth of Very Low Birth Weight Neonates – *A Randomized Controlled Trial*

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Objectives: To evaluate the effects of two different doses of parenteral aminoacid supplementation on postnatal growth in Very Low Birth Weight (VLBW) infants receiving partial parenteral nutrition (PPN).

Design: Double blinded randomized controlled trial.

Settings: Level 3 NICU between February 2008 to February 2010.

Participants: 150 inborn babies with birthweight between 900-1250 g, irrespective of gestational age, were randomized to either of the two interventions of amino acid supplementation.

Intervention: Two different initial doses of parenteral amino acids (AA) in the PPN solutions- Low AA group: 1 g/kg/d *versus* High AA group: 3 g/kg/d from day 1 of life with increment by 1 g/kg every day till a maximum of 4 g/kg/d, until babies tolerated 75% enteral feeds.

Main outcome: Average postnatal weight gain (in g/kg/d)) by 28 days of life.

Results: Both groups had similar baseline characteristics. The gain in weight, length and head circumference at 28 days were significantly lower in the High AA group. The average weight gain at 28 days was 8.67g/kg/d in the High AA group and 13.15g/kg/d in the Low AA group (mean difference 123.12, 95% CI 46.67 to 199.37, $P<0.001$). The incidences of neonatal morbidities associated with prematurity were similar in both groups.

Conclusion: Higher initial parenteral aminoacid supplementation, in settings where partial parenteral nutrition is administered, results in poor growth in VLBW infants due to inadequate non-protein calorie intake.

Keywords: *Amino acid, Postnatal growth, Very low birth weight.*

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Postnatal growth of very low birth weight (VLBW) neonates has always remained a challenge in NICU. They are often less than the 10th percentile of reference intrauterine curves at the time of hospital discharge [1]. The AAP Committee on Nutrition recommendation of providing nutrient intakes that permit the rate of postnatal growth to approximate that of a normal fetus of the same postmenstrual age is rarely met for very preterm babies [2].

Early aggressive nutritional management as a solution to postnatal growth failure has been studied. The evidence supports recommendations to administer early parenteral nutrition and enteral nutrition [1]. There is evidence from randomized controlled trials that, early aminoacid supplementation (starting within few hours of birth) as compared to amino acid supplementation after 3-5 days of life, in very preterm babies, results in better post natal growth [3,4]. Previous studies [5-9] have shown that higher doses of amino acids from day 1 result in better plasma amino acid profile and nitrogen accretion and are well tolerated, but its role in improving postnatal growth rate and long term neurodevelopment is still inconclusive.

In resource limited settings, use of lipids and micronutrients is restricted for various reasons, and partial parenteral nutrition (comprising of glucose, amino acids and electrolytes, but no lipids) is administered to the preterm babies. We undertook this trial to assess if a better post natal growth could be demonstrated in VLBW infants by starting parenteral amino acids at a higher dose (3 g/kg/d) from day 1 of life in comparison to gradual increments in dose of parenteral amino acids in the partial parenteral nutrition regimen.

METHODS

This single centre randomized controlled trial enrolled inborn babies with birth weight 900-1250 g from a level 3 NICU in Mumbai between February 2008 and February 2010. Babies missed out in the first 24 hrs of life, having obvious congenital anomalies affecting growth and requiring surgical intervention were excluded from the

study. A written informed consent was obtained from the parents before enrolment. The study was approved by the Institutional ethics committee.

The subjects were randomized into two groups within first 24 hours of life to receive 2 different doses of parenteral amino acid preparation. The Partial Parenteral Nutrition (PPN) administered to the infant in both groups was composed of dextrose, amino acids, sodium and potassium. Lipids, multivitamin and trace elements were not routinely provided. The PPN was administered through peripheral intravenous line. The Low AA group received 1 g/kg/d of parenteral amino acids on day 1 and dose increased by 1 g/kg every day till maximum of 4 g/kg/d. The High AA group received 3 g/kg/d of parenteral amino acids on day 1 and dose increased to 4 g/kg/d on next day.

Sample size calculations: In a pilot observation in our unit, amongst babies with weight between 900 to 1250 grams, average weight gain of a baby at the end of day 28 of life, who receives parenteral amino acid (PPN) similar to those in Low AA group was found to be 10 grams/kg/day. We hypothesized that, by starting amino acid supplementation at 3g/kg/d from day 1 of life, as in High AA group, weight gain would improve at least by 50% (that is 15 g/kg/d). Assuming SD of 10%, a sample size of 63 babies would be required in each group for a 2-tailed alpha error of 0.05 and beta error of 0.20 (power 80%). We enrolled 75 babies in each group to account for drop out due to death or loss of follow up within 28 days of life.

Randomization: A random number sequence was generated in a variable block size of two or four each using a "Random Allocation Software" computer program. The random allocation of sequence was generated by a statistician who was not a part of the study. The random codes were kept in serially numbered, opaque, sealed and identical envelopes to eliminate selection bias. The participants were enrolled by the on call senior residents working in the NICU. Eligible infants were assigned to interventions by them by opening sequentially numbered sealed opaque envelopes.

Blinding: The composition of the study solutions for seven different weight groups of 50 grams each between 900 and 1250 g and for the first 8 days of life (time to reach maximum fluid volume in each group) was provided by the investigator in two tabular sheets (for 2 study groups) to the staff nurse responsible for preparing PPN. This nurse was not involved in patient care. The senior resident who assigned the babies to the intervention was not involved in the study. Thus, blinding was achieved at two levels (clinicians involved in care of the infant and judicial assessors of outcomes).

Primary outcome was postnatal growth at 28 days of age as defined by weight gain by 28 days in g/kg/day. Weight gain by 28 days was calculated as (weight in grams at 28 days - birth wt in grams)/birth wt in kg/28. Secondary outcomes were 1) weight (g), length (cm), and head circumference (cm) at 28 days 2) gain in length and head circumference by 28 days (cm/wk) 3) number of days on PPN 4) the number of days required to regain birth weight 5) duration of hospital stay and 6) the incidence of following morbidities: patent ductus arteriosus (PDA), sepsis (early and late onset), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), chronic lung disease (CLD/BPD), retinopathy of prematurity (ROP), hypoglycemia and anemia in both groups.

Babies were weighed on an electronic weighing scale (accuracy of 5 g) immediately after birth and subsequently daily till discharge. Length and head circumference were measured by investigator at birth and subsequently, weekly till discharge. Weight, length and head circumference were measured on follow-up at 28 days of age. Incidence of PDA, sepsis, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), chronic lung disease (CLD), retinopathy of prematurity (ROP), hypoglycemia were assessed in both groups. PDA was diagnosed by the presence of continuous murmur in the left 2nd intercostal space and one of the following-tachycardia, bounding peripheral pulses, pulse pressure of >25 mm of Hg, hyperkinetic precordium, and hepatomegaly. Sepsis was diagnosed with either a positive body fluid culture or nephalometric CRP>10mg/l (early and late onset sepsis were defined based on the onset of sepsis before or after 72 hrs of life). Ultrasound grading of IVH was performed by grading system described by Papile [10]. NEC was diagnosed by the presence of clinical triad of abdominal distension with thrombocytopenia and hyponatrimia or suggestive radiological findings (pneumatosis intestinalis, pneumoperitoneum). CLD/BPD was defined by the need of supplemental oxygen at 28 days of life [11]. Stages of ROP were diagnosed as per the international classification (ICROP) [12]. Anemia was defined by hemoglobin value of <13g/dL (assessed within 4 weeks of life) and hypoglycemia by a blood sugar of <40mg/dL.

For babies weighing <1 kg and ≥1 kg, total fluid intake on day 1 was 100 mL/kg/d and 80 mL/kg/d, respectively. Additional 20 mL/kg/d were provided in both groups from day 1 for extra insensible water losses due to radiant warmer. Total fluids were increased by 20 mL/kg every day in both groups till a maximum of 190 mL/kg/d and 170 mL/kg/d by day 8 in babies weighing <1kg and ≥1kg respectively. Fluids were administered in

both the groups such that the glucose infusion rate was not less than 5 mg/kg/min and non protein calorie intake not less than 35 Kcal/kg/d. Both groups received parenteral 10% dextrose, parenteral aminoacid preparation and electrolytes (after day 3 of life). In this study, parenteral lipids were administered only to the babies weighing <1 kg beginning at the rate of 0.5 g/kg/d and advancing 0.5 g/kg/d to a maximum of 3.5 g/kg/d. Lipids were not routinely administered in the study group, as per unit policy.

All babies in the study were started on trophic feeds (10 mL/kg/d) on day 1 and feeds were not advanced for the first 4 days (till the dose advancement of parenteral amino acid supplements was complete). Subsequently feeds were advanced at the rate of 10-15 mL/kg/d if babies tolerated feeds and were hemodynamically stable. As enteral feeds were advanced, intravenous administration of PPN study solutions were decreased accordingly keeping the total fluid requirement of the baby constant as per standard practice. Feeds were predominantly expressed breast milk (EBM) / Pasteurized donor milk from Human Milk Bank. Formula feeding is not allowed in our unit. After achieving full enteral feeds, fortification was indicated for babies with moderate and severe CLD, PDA with congestive cardiac failure, where poor weight gain was due to restricted feed volumes of <150 mL/kg/d, which constituted <10% babies in this study. Human milk fortification was discontinued at discharge.

All babies in the study received parenteral amino acids till enteral feeds constituted 75% of the total fluid requirement of the baby

Statistical analysis: All data were analysed using SPSS version 16 statistical package. Continuous variables with normal distribution (gestational age, weight at birth and 28 days, length at birth and 28 days, weight and length gain) were analyzed by two sample *t* test. Head circumference at birth and 28 days were not normally distributed and hence analysed by Mann Whitney U test. Kolmogorov Smirnov test was used to check normality of continuous data. Categorical data was assessed using Fischer Exact test. A *P* value of <0.05 was considered as statistically significant. Analysis was performed by following intention to treat principle.

RESULTS

Between February 2008, and February 2010, 150 neonates were enrolled (***Fig.* 1**). Out of the 150 patients enrolled, 123 completed the study. There were no significant differences in the mean birthweight, gestational age, APGAR scores, number of SGA babies

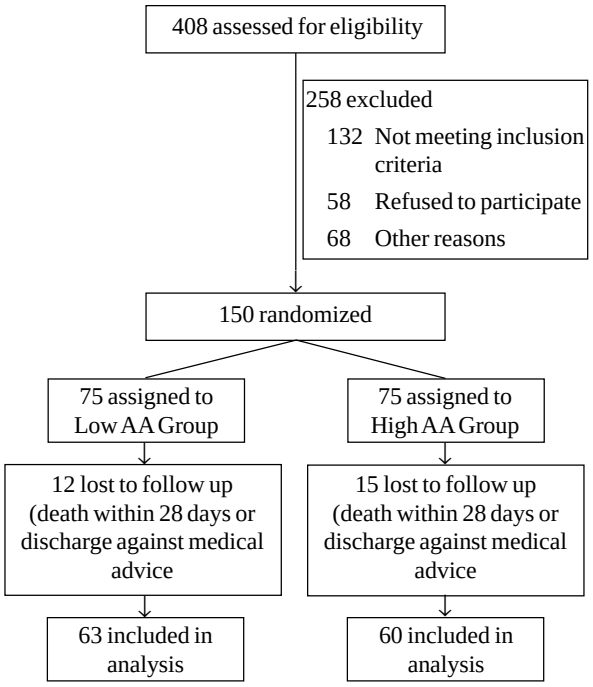

FIG. 1 *Flow diagram of patients in study.*

TABLE I BASELINE CHARACTERISTICS OF INFANTS

Baseline Characteristics	*Low AA Group (n=75)*	*High AA group (n=75)*
Gestational age (wks) *	32.12 (2.3)	31.65 ± 1.97
Birthweight (g)*	1092.4 (105.5)	1103.5 ± 110.1
Length (cm)*	37.63 (2.65)	37.94 ± 2.17
Head circumference † (cm)	26 (26-28)	27 (27-28)
1 min APGAR <5	15 (20)	12 (16)
5 min APGAR<5	6 (8)	5 (6.6)
Male gender	35 (46.6)	26 (34.6)
SGA	30 (40)	24 (32)
Multiple pregnancy	7 (9.3)	5 (6.6)
Antenatal steroid use	10 (13.3)	14 (18.6)
Mechanical ventilation	4 (5.3)	8 (10.6)
CPAP	4 (5.3)	12 (16)
Surfactant	3 (4)	6 (8)
Inotropic support	8 (10.6)	11 (14.6)
Parenteral lipids	7 (9.3)	9 (12)
Fluid restriction	18 (24)	10 (13.3)
Feed fortification	8 (10.6)	6 (8)
Postnatal steroid use	4 (5.3)	5 (6.6)

** Data represented as Mean (± SD), and †median (IQR); Rest in number (%); CPAP: Continous positive air way pressure.*

between two treatment groups (***Table* I**).The baseline characteristics in the neonates that could not be followed up at 28 days of life, were similar in both the groups. Only 16 out of 150 babies received parenteral lipid preparations. There was no difference in the number of days of parenteral amino acid support.

The primary outcome of weight gain by 28 days was 13.15 g/kg/d and 8.67 g/kg/d in Low AA group and High AA group, respectively ($P<0.001$) (***Table* II**).The weight and length at 28 days of life were higher in the Low AA group (***Fig.* 2**). Median gain in head circumference was higher in the Low AA group. After adjusting for important baseline clinical variables that may act as confounders (multiple pregnancy, antenatal steroids, 5 minute APGAR score, birth weight, gender, SGA status, early onset sepsis, late onset sepsis, respiratory support, inotropic support and hypo-glycemia), using multivariate linear regression analysis, High AA group had significantly lower rate of weight gain (g/kg/day) compared to the Low AA group. No other variables were significantly associated with the rate of weight gain.

We did not find significant differences in total duration of PPN, duration of hospital stay, frequency of PDA, sepsis, IVH, NEC, hypoglycemia, anemia, CLD and ROP between the two groups (***Table* III**).

DISCUSSION

We found that 1g/kg/d of parenteral amino acids on day 1 with gradual increments of 1g/kg every day till a maximum of 4g/kg/d resulted in better growth (weight, length and head circumference) than early aggressive parenteral amino acid supplementation (3g/kg on day 1 of life) in very low birth weight infants. There were no significant differences in total duration of PPN, duration of hospital stay, enteral intake and morbidities associated with prematurity, between the two groups.

Strengths of our study were: robust randomized controlled trial design, sufficient sample size with adequate power to detect a difference. The blinding was achieved at two levels: clinicians involved in care of the infant and assessors of outcomes. Limitations of our study include: partial parenteral nutrition with inadequate calories, short term assessment of postnatal growth, and absence of biochemical evidence of protein accretion to support clinical evidence.

Randomized controlled trials [5-9] on aggressive protein supplementation have reported no difference in the weight gain in the high versus low AA group. However, majority of those [5-8] were designed to study amino acid profile and nitrogen retention, and were not powered to study the outcome of postnatal growth.

The mean gestational age in most other studies on aggressive protein supplementation was <30wks and the mean birthweight was between 800-950g [3-7, 9]. Most of the studies included ventilated babies only. Clark, *et al*. [9] reported surfactant use in >75% of the babies, whereas only 18% of our babies needed any form of respiratory support. In other words, babies in our study groups were larger, more mature and required less respiratory support than in most of the trials on aggressive

TABLE II POSTNATAL GROWTH AND NUTRITIONAL INTAKE AMONG THE STUDY SUBJECTS

Outcome	*Low AA Group (n=63)*	*High AA Group (n=60)*	*Mean difference (95% CI)*	*P value*
Weight gain at 28 days (g/kg/d)*	13.15 (5.25)	8.67 ± 4.28	4.48 (2.76-6.19)	<0.001
Weight in g at 28 days*	1494.7 (224.4)	1371.58 ± 202.64	123.12(46.67-199.57)	0.01
Length in cm at 28 days*	40.21 (2.34)	39.19 ± 1.8	1.02(0.27-1.77)	0.008
Length gain (cm/wk)*	0.63 (0.36)	0.36 ± 0.348	0.27(0.14-0.39)	<0.001
Head circumference in cm at 28 days†	29 (27.5-30.5)	28 (27-29)		0.42
Head circumference gain (cm/wk)†	0.625 (0.37-0.875)	0.25 (0.03-0.59)		<0.001
Total days of PPN†	12 (7-15)	10 (7-15)		0.31
Cumulative Enteral intake in first 28 days (kcal/kg/d)*	69.9 (3.99)	71.1 ± 3.34	-1.2(-2.52-0.11)	0.07
Cumulative non protein calorie from PPN (kcal)*	349.1 (55.34)	318.5 ± 60.43	30.6(9.93-51.27)	0.004
Time to regain BW (days)†	12 (10-14)	16 (11-20)		<0.001
Duration of hospital stay (days)†	21 (14-26)	19 (13-26)		0.25

**Data represented as mean (SD); †Data represented as median (IQR).*

TABLE III NEONATAL MORBIDITY IN THE TWO GROUPS NO. (%)

	Low AA Group (n=63)	*High AA Group (n=60)*
Patent ductus arteriosus	16 (25.4)	7 (11.6)
Early onset sepsis	28 (44.4)	19 (31.6)
Late onset sepsis	9 (14.3)	7 (11.6)
Intraventricular hemorrhage	5 (7.9)	6 (10)
Necrotising enterocolitis	6 (9.5)	8 (13.3)
Chronic lung disease	6 (9.5)	6 (10)
Retinopathy of prematurity	2 (3.2)	3 (5)
Anemia	8 (12.7)	6 (10)
Hypoglycemia	10 (15.8)	16 (26.6)

P value >0.05 for all comparisons.

protein supplementation, yet there was poor weight gain in the High AA group.

High AA group had more babies on respiratory support than the low AA group; and 50% of the babies in the study had sepsis. However, the regression analysis showed that the weight difference between the two study groups was influenced only by the dose of amino acids. The cumulative enteral calorie intake, time to reach full feeds and the number of babies on fortified feeds were also similar between the groups suggesting that the nutrition intake in the later part of the study period (after the 1st week) was similar. Therefore, the poor weight gain in high AA group was attributed to the lower non protein calorie supplementation through PPN in the first week of life (more marked in the first three days) of life, resulting in a low calorie nitrogen ratio (CNR) - which occurred because of aggressive protein supplementation in the settings of partial parenteral nutrition

A CNR (ratio of non protein calories: calories from protein) of 150-250 through parenteral nutrition, is desired for post natal growth. [13]. A minimum non protein calorie intake of at least 35-40 kcal/kg/d is required for the utilization of 1g/kg of protein supplemented per day. For higher doses of protein, the non protein calorie intake should also increase proportionately to optimise the CNR and improve postnatal growth. [14]. Most of the studies involving higher doses of amino acids also administered parenteral lipids in both groups from day 1 [5-9]. In current study, 90% of the babies received partial parenteral nutrition (*i.e.* without lipid infusion). This reduction in non-protein calorie intake, along with higher doses of amino acids, caused more than two fold reduction in the CNR in the high AA group in the first three days of life (The non-protein energy intake in the Low AA group and High AA group was 44 kcal/kg and 36 Kcal/kg, respectively on day 1 of life resulting in the calorie nitrogen ratio of 150 in Low AA group and 62.5 in High AA group). After day 4 of life, the advancement of parenteral protein was complete; the enteral intake was increased and the volume of dextrose in the PPN increased to meet the daily fluid requirements. This improved the non-protein calorie intake (56-70 Kcal/kg/d) -though still inadequate in both the groups. After the 1st week of life, increments in the enteral nutrition optimized the non-protein calorie intake. Essentially after day 4, the protein and non-protein calorie intake provided through the study solutions were the same in both groups.

The best option to optimize the CNR in the high AA group would have been the provision of lipids in both groups from day 1 of life. It was not feasible to administer lipids to all babies in this study. The next option would have been provision of higher concentrations of dextrose in the study solutions. As we used 10% dextrose and amino acids in variable proportions to prepare the study solutions, the net dextrose concentration in the High AA solution varied from 7-8%. We did not attempt to achieve the final concentration of dextrose at 10% in PPN fluid in either group for following reason. In past, in our unit, addition of higher concentrations of dextrose to obtain a 10% dextrose study solution resulted in a higher incidence of hyperglycemia in the ELBW babies in the first week of life, especially with infection.

The delay in regaining birth weight in the High AA group could be attributed to the inadequate calorie intake in the High AA group in the first week of life. The mean duration of PPN in our study was lower than that reported in the NICHD Neonatal Research Network study [15]. Interestingly, the babies in the Low AA group did not get discharged earlier than the High AA group, despite a better weight gain, because they had to be nursed till the attainment of full-suck feeds. The duration of hospital stay for the babies in this study was much lower, because we could follow them up in our Kangaroo Mother Care Clinic after discharge, on a daily basis.

This study also highlights the fact that increments in weight, length and head circumference in the study groups were less than those described in the studies done in the Western world. This explains the need for optimizing calorie intake soon after birth, as well as for long term data on growth pattern and neurodevelopmental outcome in these infants.The consistent finding in most studies, that weight gain was unaffected by differing protein intake, could be attributed to the similar calorie intake between the groups, triggering a debate on the

WHAT IS ALREADY KNOWN?

- Early aggressive parenteral protein supplementation in TPN in preterm neonates improves rate of protein accretion, but not post natal growth.

WHAT THIS STUDY ADDS?

- In resource limited settings, where partial parenteral nutrition is provided, an initial dose of 1g/kg/d of parenteral protein followed by gradual advancement to 4g/kg/d results in better postnatal growth than aggressive protein supplementation.

importance of adequate calorie supplementation over protein for preterm babies

This study underscores the need of research in the role of PPN in babies <1000g, that require parenteral nutrition for longer duration. It is also mandatory to know if PPN affected long term growth in the very preterm babies, bearing in mind the implications of rapid catch up growth in the preterm babies with poor weight gain in the first month of life. Also, the long term neurodevelopment in preterm babies that are deprived of the essential fatty acids as a result of PPN in the first week of life, needs to be studied

We conclude that, starting higher doses of amino acids without parenteral lipids and higher dextrose concentrations did not result in a better weight gain than slow dose advancement. Therefore, in resource limited settings, where parenteral lipids are not started within first 3 days of post-natal life, starting amino acids at 1g/kg/d on day 1 and gradual advancement would be a better option.

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