Long-term enteral glutamine supplementation in very low birth weight infants: effects on growth parameters

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Enteral and parenteral glutamine supplementation in preterm infants has been shown to have some beneficial effects on neonatal morbidity and mortality, although the results are controversial. In this study, we aimed to determine if long-term glutamine-supplemented enteral nutrition affects growth parameters in very-low-birth-weight (VLBW) preterm infants. Preterm infants with a birth weight of ≤1500 g were assigned to receive enteral glutamine supplementation (300 mg/kg/day) or placebo between 8-120 days (4 months) of life. At the end of each month, growth parameters [weight, length, head circumference, left upper mid-arm circumference (MAC) and left mid-thigh circumference (MTC)] were determined and enteral glutamine dose was adjusted according to the current weight. In VLBW infants (n=69), the glutamine-supplemented group (n=36) had significantly higher mean weight, length, head circumference, MAC and MTC than the control group (n=33) at the end of the fourth month. These findings suggest that long-term enteral glutamine supplementation may lead to significant improvements in growth in all body measures in VLBW infants, possibly in a time-dependent pattern.

Key words: preterm infant, growth, nutrition, amino acid supplementation.

Glutamine, the most abundant amino acid in the human body, plasma and breast milk, is an important substrate for protein synthesis and growth. It is also a major energy source for rapidly dividing cells such as enterocytes and immune cells[1,2]. It can be synthesized in the body, but glutamine stores may be depleted, and biosynthetic pathways frequently cannot meet the increased demands in the presence of severe catabolism. Thus, glutamine is considered a "conditionally essential amino acid" and its supplementation has been shown to decrease infectious morbidity and mortality in seriously ill adult patients[3].

After preterm birth, the sudden cessation of glutamine supply from the mother and placenta to the preterm infant, who is under high catabolic stress and undergoing rapid growth, is potentially detrimental. Glutamine is not included in total parenteral nutrition solutions and very preterm infants frequently are not enterally fed adequately for weeks. Preterm infants seem to depend on an adequate supply of glutamine and its metabolites for growth and normal physiologic development[4,5]. Although there is no clear evidence from randomized trials to support the routine use of parenteral or enteral glutamine supplementation in preterm infants[6], some investigations have shown that parenteral or enteral glutamine supplementation might have some beneficial effects[7-12]. These benefits can be summarized as: reduced risk of infectious morbidity, although there are controversial results, decreased time required for mechanical ventilation, decreased incidence of feeding intolerance and time to achieving full enteral nutrition, fewer neurologic sequelae and decreased hospital costs[7-12]. However, although growth was not a primary outcome point in these studies, it was similar both in the study and control groups during the 28-30 or highly variable (32.1±23.5) days of the study periods[8,10,12].

Postnatal growth of very-low-birth-weight (VLBW) (≤1500 g) infants is a multifactorial process which mainly depends on the intrauterine

nutritional status, severity of clinical illnesses and the adequacy of parenteral and enteral nutrition (protein, lipid and caloric intake). Protein deficits, particularly those produced in the first weeks of life, contribute substantially to poor growth. Growth of VLBW infants is generally accelerated after discharge from the hospital as the initial catabolic period eventually subsides[13]. Thus, in addition to earlier, more aggressive parenteral and enteral nutrition practices during hospitalization, recent efforts have focused on postdischarge interventions. Special formulas providing more protein, energy and other nutrients (postdisharge formulas) have been shown to lead to a better growth when compared with standard term formulas or human milk in preterm infants[14,15]. Therefore, as glutamine is the most abundant amino acid in the human body and an important structural component of proteins, we aimed to study the effects of long-term enteral glutamine supplementation especially on growth parameters in preterm infants.

Material and Methods

Subjects

This study was performed at the Neonatal Intensive Care Unit and Neonatal Follow-Up Clinic of Hacettepe University, İhsan Doğramacı Children's Hospital, Ankara, Turkey, between 1 October 2001 and 30 September 2003. VLBW infants who were appropriate for gestational age were included in the study. The Investigational Ethical Committee of the Medical Faculty of Hacettepe University approved the study protocol and informed consent forms were obtained from the parents of each infant. Exclusion criteria included infants with congenital malformations, chromosomal abnormalities and inherited metabolic diseases; small- and large-for-gestational-age infants; infants who developed necrotizing enterocolitis and did not receive any enteral feedings for more than one week in the hospitalization period; infants who had mechanical ventilation longer than four weeks; and infants who developed posthemorrhagic hydrocephalus after grade III-IV intraventricular hemorrhage.

Clinical and Anthropometric Data

Prenatal history recorded for each infant consisted of maternal chronic or gestational diseases that complicated the pregnancy and antenatal corticosteroid therapy; demographic and clinical characteristics of the infants recorded included gestational age, gender, mode of birth, fifth minute Apgar score, anthropometric findings [birth weight, length, head circumference, left upper mid-arm circumference (MAC) and left mid-thigh circumference (MTC)], duration of mechanical ventilation, duration of total parenteral nutrition, time to achieve full enteral nutrition, clinical diagnoses and total duration of hospitalization. MAC was measured on the left side at exactly the mid-distance between the acromion and the tip of the olecranon, while MTC was measured on the left side at exactly the mid-distance between left spina iliaca anterior superior and upper margin of patella. The scale was Seca 728, Germany and measuring rod was Seca 207, Germany. Standard paper tape measures were used for measuring head circumference, MAC and MTC. All the anthropometric parameters were measured by one investigator.

Study Design

Hospitalization period

Eligible infants were included in the study or in the control group according to the order of admission to the Neonatal Intensive Care Unit. In the study group, infants received enteral glutamine supplementation from the 8th day of life until the end of 120 days (postnatal 4 months) at a dose of 300 mg/kg/day in two divided doses (q12 hours). Glutamine, which was supplied as 500 mg capsule (L-glutamine 500 mg, GNC-General Nutrition Center, Pittsburgh, USA), was divided into sterile closed vials, each containing 250 mg glutamine, by the hospital pharmacy. Powder glutamine (250 mg) was mixed with sterile water (10 ml) and a study solution was formed just before it was given to the infants by the Unit Nursery. The solution was clear, odorless and tasteless. Glutamine study solution was given at the same time but separate from the feedings by an orogastric tube or orally. Weight, length, head circumference, left upper MAC and left MTC were measured at the end of each postnatal month during the hospitalization and glutamine dose was adjusted according to the current weight. Infants in the control group did not receive any glutamine supplementation but they received placebo (sterile water). During the hospitalization period, the parents of the

infants in the study group were trained by the nursing staff regarding the preparation of the glutamine study solution at home.

Parenteral nutrition was initiated at the end of 24 hours in each infant. Amino acids (TrophAmine 6%, Eczacıbaşı/Baxter, Istanbul, Turkey) were started at a dose of 0.5 g/kg/day and reached 3 g/kg/day on the third day of life, while lipid emulsion (Lipofundin MCT/LCT 20%, B. Braun Melsungen AG, Germany) was started at a dose of 0.5 g/kg/day on the third day of life, reaching 2.5 g/kg/day on the seventh day life. Parenteral glucose was started at 6-8 mg/kg/min on the first day of life and increased as tolerated to 12 mg/kg/min. All patients received 80-100 kcal/kg/day at the end of the first week. Minimal enteral nutrition with breast milk or 1:1 diluted preterm formula was initiated at the end of 24 hours at a dose of 10 ml/kg/day and the enteral feedings were increased as tolerated due to the nutrition protocol of the nursery in the second week of life. Full enteral feedings were defined as 150 ml/kg/day. Both during the hospitalization and postdischarge periods, all infants received only one of these enteral feeding types: (1) exclusively breast milk (contains approximately 285 μm/L glutamine)[16], (2) breast milk + human milk fortifier (Eoprotin® Milupa GmbH, Friedrichsdorf, Germany, 100 ml breast milk + 4.2 g Eoprotin contains 2.1 g protein and 170 mg L-glutamine), (3) breast milk + preterm formula (Prematil-LCP®, Milupa, GmbH, Friedrichsdorf, Germany, 100 ml formula contains 2.4 g protein and 479 mg L-glutamine), and (4) only preterm formula (Prematil-LCP®, Milupa) due to the decision of the parents.

Postdischarge period

After discharge, all infants were followed up in the Neonatal Follow-Up Clinic of Hacettepe University Ihsan Doğramacı Children's Hospital. At the end of each postnatal month, every infant was called in for a follow-up visit and weight, length, head circumference, left upper MAC and left MTC were measured. Parents were queried regarding glutamine administration. Enteral glutamine dose was adjusted according to the current weight. Parents were advised not to give additional feedings (cow's milk or weaning semi-solid foods) to the infants until the end of the postnatal fourth month. Glutamine supplementation was discontinued at the end of the postnatal fourth month.

At the end of the postnatal fourth month, blood glucose, blood urea nitrogen, creatinine, total protein, albumin and venous blood pH and bicarbonate levels were measured in each infant. Blood ammonia, glutamine and glutamate levels were planned to be measured only in infants with suspected signs of toxicity in the study group. The signs of toxicity were defined as lethargy, poor feeding, vomiting, and metabolic acidosis of undefined etiology[17]. In each infant with suspected toxicity, infectious screening tests including complete blood count, differential leukocyte count, C-reactive protein (CRP) level, blood and urine cultures, and chest X-ray were also performed.

Statistical Analysis

Statistical data were analyzed using SPSS 11.0 software on a personal computer. Continuous variables were compared using two-tailed *t* test for parametrically distributed data or Mann-Whitney for non-parametrically distributed data. Categorical variables were analyzed by χ^2 test or Fisher's exact test. A p value of <0.05 was accepted as statistically significant.

Results

A total of 69 infants were included in the study: 33 (47.8%) in the control group and 36 (52.2%) in the study group. Of all infants, 17 (24.6%) were extremely-low-birth-weight (ELBW) (≤1000 g), while 52 (75.4%) were VLBW infants.

The demographic, nutritional and clinical characteristics of the study and the control groups were similar (Tables I and II). There were no significant differences in growth parameters at birth and in the first two months of life between the study and the control groups. However, the study group had significantly higher mean weight, length, head circumference, MAC and MTC at the end of the third and fourth months when compared to the control group (Table III). The pattern of mean body weight changes at each postnatal month in each group is shown in Figure 1.

None of the infants in the study group showed signs of glutamine toxicity. Blood glucose, blood urea nitrogen, creatinine, total protein, albumin and venous blood pH levels were

Table I. Demographic and Nutritional Characteristics of the VLBW Infants

	Control Group (n=33)	Glutamine Group (n=36)	p
Gender (F/M), n	15/18	12/24	0.303
Gestational age (wk), mean±SD	29.4±2.2	29.7±2.3	0.509
Mode of birth (V/CS), n	5/28	5/31	0.882
Apgar score at 5 min, mean±SD	7.0±1.3	7.1±1.4	0.675
Prenatal maternal/gestational disease, n (%)	20 (60.6)	24 (66.6)	0.601
Antenatal steroids, n (%)	24 (72.7)	29 (80.6)	0.441
Duration of total parenteral nutrition (day), mean±SD	11.8±8.0	9.8±6.5	0.275
Time of full enteral nutrition (day), mean±SD	14.5±8.7	12.3±7.2	0.262
Enteral nutrition (pre- and post-discharge period)			
Type of feeding, n (%)			
1. Exclusively breast milk	–	–	
2. Breast milk + fortifier	13 (39.4)	13 (36.1)	0.779
3. Breast milk + preterm formula	17 (51.5)	19 (52.8)	0.916
4. Preterm formula	3 (9.1)	4 (11.1)	1.000

VLBW: Very-low-birth weight. V: Vaginal. C/S: Cesarean section.

Table II. Clinical Characteristics of the VLBW Infants

	Control Group (n=33)	Glutamine Group (n=36)	p
Duration of ventilator support (day), median (min-max)	5 (0-28)	3.5 (0-28)	0.833
Total period of hospitalization (day), mean±SD	30.3±21.1	28.9±23.9	0.803
Treatment with steroids after 14 d, n (%)	4 (12.1)	2 (5.5)	0.416
Morbidities, n (%)			
– Respiratory distress syndrome	12 (36.4)	16 (44.4)	0.495
– Patent ductus arteriosus	11 (33.3)	13 (36.1)	0.809
– Sepsis (blood-culture proven)	7 (21.2)	4 (11.1)	0.330
– Pneumonia	6 (18.2)	5 (13.8)	0.627
– Intraventricular hemorrhage	2 (6)	3 (8.3)	1.000
– Periventricular leukomalacia	2 (6)	1 (2.8)	0.603
– Chronic lung disease	6 (18.2)	4 (11.1)	0.502
– Retinopathy of prematurity	4 (12.1)	6 (16.7)	0.737

VLBW: Very-low-birth weight.

Table III. Growth Parameters of the VLBW Infants at the End of Each Month from Birth to Postnatal Four Months (mean±SD)

	Control Group (n=33)	Glutamine Group (n=36)	p
Body weight (g)			
At birth	1222±234	1260±254	0.518
1st month	1501±309	1593±326	0.232
2nd month	2023±445	2219±491	0.086
3rd month	2883±548	3169±468	0.024
4th month	3787±605	4280±438	0.000
Length (cm)			
At birth	37.6±2.7	38.2±2.5	0.340
1st month	39.6±2.8	40.8±2.8	0.114
2nd month	43.4±2.9	44.3±2.8	0.202
3rd month	46.9±2.8	48.9±3.1	0.008
4th month	51.1±2.6	53.0±2.4	0.002
Head circumference (cm)			
At birth	27.3±1.8	27.5±2.1	0.657
1st month	29.6±1.9	29.8±2.1	0.616
2nd month	32.4±1.9	33.1±2.2	0.183
3rd month	35.0±1.8	35.9±1.9	0.044
4th month	37.5±1.7	38.5±1.7	0.013
Mid-arm circumference (cm)			
At birth	6.1±0.7	6.2±0.8	0.396
1st month	7.1±0.8	7.4±0.9	0.124
2nd month	8.4±0.8	8.8±0.8	0.030
3rd month	10.6±0.9	11.3±0.8	0.001
4th month	12.9±0.9	14.1±1.1	0.000
Mid-thigh circumference (cm)			
At birth	7.6±0.8	7.9±0.9	0.199
1st month	8.9±0.9	9.3±1.2	0.097
2nd month	11.3±1.1	11.8±1.3	0.078
3rd month	14.2±1.2	15.1±0.9	0.001
4th month	16.7±1.3	18.4±1.2	0.000

VLBW: Very-low-birth weight.

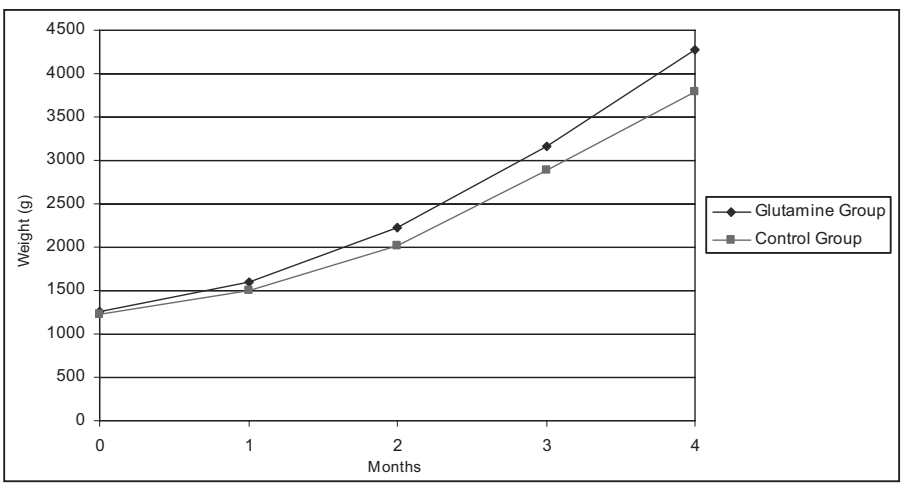

Fig. 1. The pattern of mean body weight changes at each postnatal month in each group of very-low-birth-weight infants.

within normal ranges in all infants at the end of the fourth month and were similar between the study and control groups (Table IV).

Table IV. Biochemical Parameters of the VLBW Infants at the End of the Fourth Month (mean±SD)

	Control Group (n=33)	Glutamine Group (n=36)	p
Biochemical Parameters			
Blood fasting glucose (mg/dl)	86.8±12.6	88.6±13.7	0.593
Blood urea nitrogen (mg/dl)	13.8±2.9	14.9±3.2	0.179
Creatinine (mg/dl)	0.7±0.2	0.8±0.2	0.745
Total protein (g/dl)	6.1±0.9	6.5±0.9	0.069
Albumin (g/dl)	4.5±0.4	4.7±0.5	0.581
Blood pH	7.38±0.04	7.36±0.04	0.183

VLBW: Very-low-birth weight.

Discussion

In the present study, long-term enteral glutamine supplementation in VLBW infants was shown to promote growth in all body measures, possibly in a duration-dependent pattern.

The effects of glutamine supplementation on protein metabolism in VLBW infants have been investigated in few studies. Short-term (4-24 hours) intravenous and enteral (1-5 days) glutamine supplementations have been associated with a lower rate of protein breakdown, but no effect in whole body protein balance was measured by leucine kinetics in VLBW infants[18-20]. Similarly, in healthy and critically ill adult patients, short durations of intravenous and enteral supplementations of glutamine have neither stimulated protein synthesis nor attenuated breakdown in skeletal muscles determined by kinetic studies[21-24]. In addition, although the durations of enteral glutamine supplementations in VLBW infants were relatively longer (28-30 days and 32.1±23.5 days), no significant differences in growth parameters between the study and control groups were observed in three studies[8,10,12]. However, in our study, long-term and continuous enteral glutamine supplementation led to significant enhancement of growth at the end of the third and fourth months in VLBW infants. We think that continuous and long-term enteral glutamine supplementation, which covered part of the postdischarge period, were the most critical factors which led to enhanced growth in VLBW infants. Preterm infants, either because of immaturity or intercurrent illnesses, cannot get enough caloric and protein intake, and have high rates of catabolism and protein breakdown in the early weeks of life. Respiratory distress syndrome, sepsis, necrotizing enterocolitis, anemia, intraventricular hemorrhage, chronic lung disease and prolonged ventilatory support are the major morbidities which contribute to this catabolic phase[15]. In addition, postnatal steroid therapy for chronic lung disease of prematurity increases protein breakdown and inhibits protein synthesis in almost all tissues[25]. It is apparent that protein deficits contribute substantially to poor growth, particularly those produced in the first few weeks of life. Most of the protein intake is used for the maintenance of the protein losses so that an anabolic phase charaterized by adequate weight gain is almost impossible during this period. This high rate of protein breakdown could be relatively diminished by early parenteral amino acid administration and early introduction of enteral feedings. However, these interventions fail to provide an adequate protein accretion and growth in the first weeks of life, and postnatal growth failure is nearly universal in VLBW infants[26,27]. Consistent with this situation, we did not observe any significant differences in growth parameters at the end the first and second months between the study and control groups. However, all the mean measures of growth parameters at the end of the third month were significantly higher in the study group than in the control group. In addition, the differences in growth parameters between the study and control groups were observed to be more prominent at the end of the fourth month. So it could be hypothesized that better growth was achieved by continuation of glutamine supplementation beyond the early weeks of life, namely in the postdischarge period in which the catabolic process eventually subsides. However, it is not clear whether

enteral glutamine supplementation during the early weeks of life stimulated protein synthesis in specific tissues, such as the intestine for the preservation of gut barrier or intestinal immune cells, without leading to a net increase in whole body protein synthesis in preterm infants[19].

Although we did not measure the triceps skinfold thicknesses and calculate the arm muscle and fat areas in preterm infants in our study, it is generally assumed that arm size defined by MAC (and also thigh size defined by MTC) reflects the reserve of protein[28]. Glutamine is an anabolic and trophic factor for muscle and has been termed as a "competence factor" in that it serves to stimulate protein synthesis by a mechanism(s) that is not fully understood[29]. Glutamine is synthesized primarily in skeletal muscle, but in hypercatabolic patients, the muscle glutamine pool depletes, muscle glutamine synthesis is suppressed and it is refractory to externally supplied glutamine[30]. This physiological process is consistent with our finding that significant increases in mean MACs and MTCs were observed after a refractory period, in other words, a catabolic period of 2-3 months. During this period, enterally supplemented glutamine was possibly used as an energy source for intestinal and immune cells and for other metabolic reactions, rather than as a substrate for protein synthesis in the muscle. Therefore, we think that muscle was possibly the primary target tissue for protein accretion in long-term glutamine supplementation in VLBW infants.

The safety of enteral glutamine supplementation without clinical and biochemical signs of toxicity in a dose range of 300-600 mg/kg/d has been well documented in preterm infants[17]. This was consistent with our findings in that we did not observe any signs of clinical or biochemical toxicity of glutamine in our study group.

In conclusion, long-term enteral glutamine supplementation has been shown to enhance growth in a time-dependent pattern in VLBW infants. Further randomized, controlled prospective studies are needed for the assessment of the effects of long-term glutamine supplementation on growth and other systems in preterm infants.

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