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Effect of enteral glutamine supplementation for low-birth-weight infants on weight gain patterns and levels of fecal secretory immunoglobulin A

Mahendra Sampurna[1], Dina Angelika[1], Martono Tri Utomo[1], Nur Aisiyah Wijaya[1], Budiono Budiono[2], Firas Farisi Alkaff[3], Roedi Irawan[1], Risa Etika[1]

[1]Department of Pediatrics, Airlangga University School of Medicine, Surabaya, Indonesia
[2]Department of Public Health, Airlangga University School of Medicine, Surabaya, Indonesia
[3]Airlangga University School of Medicine, Surabaya, Indonesia

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Abstract

Aim: Glutamine is needed for optimal cell growth and for the immune system, especially in the enterocytes of gut mucosal immune responses. Low birth weight makes infants susceptible to glutamine depletion because nutrition is limited in the first week of life. To determine the effect of enteral glutamine supplementation on weight gain patterns and fecal secretory immunoglobulin A.

Material and Methods: This study is a double-blind, randomized controlled trial. Infants were randomly assigned to the glutamine group and placebo group. The glutamine group was supplemented with glutamine 400 mg/kg/day for 14 days, and placebo group received glucose 400 mg/kg/day for 14 days. The infants were observed for 30 days. Return-to-birth-weight, weight gain velocity, and fecal secretory immunoglobulin A levels were monitored during the study.

Results: Thirty-seven low-birth-weight infants were randomly assigned to the glutamine and placebo groups. The glutamine group had a shorter return-to-birth-weight time than the placebo group (8.1±0.9 vs. 11.0±1.6 days) and faster weight gain velocity (20.0±1.8 vs. 15.5±2.2 g/kg/day) ($p<0.001$). Secretory immunoglobulin A levels after glutamine supplementation were higher than in the placebo group (0.456±0.057 vs. 0.376±0.035 mg/g) ($p<0.001$). Levels of secretory immunoglobulin A after treatment in each group were increased. However, there was a significant difference before and after supplementation between the glutamine and placebo groups (0.247±0.024 vs. 0.140±0.016 mg/g) ($p<0.001$).

Conclusion: Enteral glutamine supplementation in low-birth-weight infants accelerates return to birth weight, increases the weight gain velocity, and the levels of fecal secretory immunoglobulin A.

Keywords: Enteral glutamine supplementation, Fecal sIgA, low birth weight, weight gain pattern

Introduction

Low birth weight (LBW) is one of the most important health problems in the world nowadays. The proportion of LBW is about 14% of total live births, and accounts for 60-80% of deaths in neonates (1). The annual report from the Neonatology Division Department of Pediatrics Faculty of Medicine Universitas Airlangga, Dr. Soetomo RSUD in 2011 stated that the prevalence of LBW was 47% (2). Low-birth-weight infants are at a greater risk of growth and neurologic developmental restriction compared with infants who are able to spur growth within the first year of life, because LBW infants lack optimal nutrition (3).

Breast milk contains optimal nutrition for term infants, but not in LBW infants with regard to the protein, calcium, vitamin D, and sodium content. Various

Corresponding Author : Mahendra Sampurna E-mail: mahendra.tri@fk.unair.ac.id
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methods have been studied to overcome the growth restriction in LBW such as giving a human milk fortifier or low-birth-weight formula (4). Unfortunately, the use of human milk fortifier is still rare in Indonesia, and the use of low-birth- weight formula has a higher incidence necrotizing enterocolitis compared with breast milk fortification (5, 6).

Optimal cell growth requires several types of amino acids, the most important of which is glutamine (7). Low-birth-weight babies are susceptible to glutamine deficiency because glutamine supply from the placenta suddenly stops after birth (8). Several studies have shown that glutamine is beneficial for LBW, especially in growth parameters, the gastrointestinal system, immunity, allergy, morbidity, and mortality (9-11). Moreover, glutamine supplementation in animal studies was also proven to be useful in gut-associated lymphoid tissue (GALT). It prevents the decrease of immunoglobulin A (IgA)-producing plasma cells, increases levels of secretory fecal IgA (sIgA), and also prevents adherence and bacterial translocation from the gut (12).

The optimal dose of glutamine supplementation as an enteral nutrition in LBW is still controversial (13). A recent systematic review stated that parenteral and enteral glutamine supplementation in preterm infants had no significant effect on mortality, sepsis, and enterocolitis. Further studies about enteral glutamine supplementation have been suggested, especially related to the gastrointestinal system (14).

The aim of the study was to determine the effect of glutamine supplementation in LBW infants on recovery time for a return to birth weight (RTBW), weight gain velocity (WGV), and the effects on the fecal Ig A secretory intestinal (sIgA) levels.

Material and Method

This study was an experimental study with a double-blind, randomized trial design, conducted in the Neonatal Intensive Care Unit (NICU) at Dr Soetomo General Hospital Surabaya from December 2012 to February 2013. The sample of this study comprised LBW infants born in the NICU and Neonatus Intermediate in Dr. Soetomo General Hospital who fulfilled met the inclusion criteria. The inclusion criteria of this study were LBW infants with birth weight between 1500-2500 grams. The exclusion criteria were LBW infants with asphyxia neonatorum, severe congenital abnormalities, neonatal sepsis, or LBW due to twin pregnancy. Drop out criteria of this study were infants with feeding intolerance, infants whose parents withdrew their consent for the study, infants outside Dr. Soetomo Hospital where follow-up was not possible, or infants who died during the study period.

Sample Size

The sample size was determined using the Federrer formula (15) as follows:

$$(K-1)\,(r-1)\geq 15,$$

where K was the number of groups and r was number of samples needed in each group. The minimum sample for each group was 16 infants because there were 2 groups in this study.

Intervention

The subjects were divided into 2 groups, the glutamine group and the placebo group. The treatment group received breast milk and low-birth-weight formula- supplemented capsules containing 400 mg/kg/day glutamine for 14 days from day 3 to day 17, and the placebo group received breast milk and low-birth-weight formula-supplemented capsules containing 400 mg/kg/day of glucose for 14 days from day 3 to day 17.

Evaluation

Stool sampling was performed on day 0, then repeated on day 17 (after treatment). The evaluation of the sIgA levels was performed after all the samples were collected. Fecal sIgA levels were calculated based on the conversion from Optical Density, which was generated from reading using a Biorad enzyme-linked immunosorbent assay (ELISA) reader and assessed with units of mg/g feces. For the calculation of body weight, weight measurements were conducted every 3 days for the first 30 days of life.

Ethical clearance

This study was granted ethical approval by the Ethics Committee of Dr. Soetomo General Hospital before conducting the study (Ethics Committee Approval Number: 81/Panke.KKE/VIII/2010) which complied to principles of Helsinki. Parents gave their informed

consent prior to their inclusion in the study. Before signing the informed consent form, information on informed consent was given. Details that might disclose the identity of the subjects under study were omitted.

Table 1. Characteristics of the study sample

Characteristic		Glutamine (n=18)	Placebo (n=19)	p
Sex				
Male (n)		7	10	
Female (n)		11	9	0.309[a]
Birth weight (mean)		2055 g	2025 g	0.664[c]
Lowest weight (mean)		1822 g	1791 g	0.600[c]
Preterm/LBW/SGA (n)		5	6	
Preterm/LBW/AGA (n)		12	10	
Aterm/LBW/SGA (n)		1	3	0.512[b]
APGAR Score	3-7 (n)	4	6	
	7-9 (n)	14	13	0.714[a]
Antibiotic prophylaxis (n)		7	11	0.330[a]
Mode of Delivery				
Vaginal delivery (n)		7	9	
C-section (n)		11	10	0.743[a]
Meconium stained amniotic fluid		4	7	0.476[a]
PROM		4	4	0.999[a]
Hypoglycemia		3	1	0.340[b]
Sepsis		0	2	0.486[b]

LBW: low birth weight; SGA: small for gestational age; AGA: appropriate for gestational age; PROM: premature rupture of membrane
^Chi-Square test was used
*Fisher's exact test was used
#Mann-Whitney Test was used
p<0.05 was considered significant

Table 2. Low-Birth-Weight Growth Pattern

	Glutamine (n=18)	Placebo (n=19)	p
RTBW (day)	8.1±0.9	11.0±1.6	<0.001
WGV (g/kg/day)	20.0±1.8	15.5±2.2	<0.001

WGV: weight gain velocity; RTBW: return to birth weight
Pearson correlation test was used
p<0.05 was considered significant

Statistical Analysis

Data from this study were analyzed using the SPSS 16.0 statistics program. The Chi-square test, Fisher's exact test, and the Mann-Whitney U test were used to analyze the characteristics of the subject. The Shapiro-Wilk test was used to analyze the normality of the samples. Pearson's correlation test was used to analyze the growth pattern of RTBW and WGV between both groups, and the paired-samples t-test was used to analyze fecal sIgA level differences before and after the treatment. The independent samples t-test was used to analyze the difference in fecal sIgA levels between both groups before treatment, after treatment, and the Δ from before and after treatment. Bivariate Pearson's correlation was used to analyze the different amounts of formula given between the groups as a confounding factor for WGV, RTBW, and fecal sIgA levels. Confidence intervals (95%) (α=0.05) were used in this study. The data are presented using tables and figures.

Result

Thirty-seven infants, 18 infants in the glutamine group and 19 infants in the placebo group, were included in this study. The characteristics of the study sample were homogeneous in terms of sex, birthweight, antibiotic exposure, mode of delivery, APGAR score, and infection risk (p>0.05) (Table 1).

Effect of glutamine supplementation on LBW growth pattern

The glutamine group RTBW time was shorter than in the placebo group (8.1±0.9 days vs. 11.0±1.6 days) and faster in WGV (20.0±1.8 g/kg/day vs. 15.5±2.2 g/kg/day). Supplementation with 400 mg/kg/day glutamine for 14 days showed a statistically significant better growth pattern than placebo in LBW infants (p<0.001) (Table 2).

After the first day of birth, there was similar weight loss between the glutamine and placebo group. The lowest body weight (nadir) of the glutamine group occurred at 4.3±0.7 days and the placebo group at 4.8±1.0 days (p=0.082). On the 30th day of birth, the mean body weight of the glutamine group was higher than that of the placebo group (2871±294 g vs. 2602±279 g; p=0.007) (Figure 1).

Effect of glutamine supplementation on fecal sIgA levels

There was a significant increase in fecal sIgA levels before and after treatment in both the glutamine and placebo groups (0.209±0.045 mg/gram vs. 0.456±0.057 mg/gram, 0.236±0.040 mg/gram vs. 0.376±0.035 mg/gram; p<0.001, respectively). The mean increase of sIgA levels in the glutamine group was greater than in the placebo group (p<0.001) (Table 3).

There was no significant difference in fecal sIgA levels between the glutamine and placebo groups before treatment (0.209±0.045 mg/gram vs. 0.239±0.040 mg/gram, p=0.066). However, fecal sIgA levels after treatment showed a significant difference between the glutamine and placebo groups (0.456±0.057 mg/gram vs. 0.376±0.035 mg/gram; p<0.001). There was also a significant difference in Δ fecal sIgA levels between the glutamine and placebo groups (p<0.001) (Table 4).

Confounding factor

The average amounts of formula consumed by the glutamine placebo groups were 2980±529 mL/kg and 2595±419 mL/kg, respectively (p=0.019). Bivariate analysis showed that the mean difference of the formula did not change the mean value of RTBW, WGV, and fecal sIgA levels before and after treatment (p=0.612, 0.914, and 0.731, respectively) (Table 5).

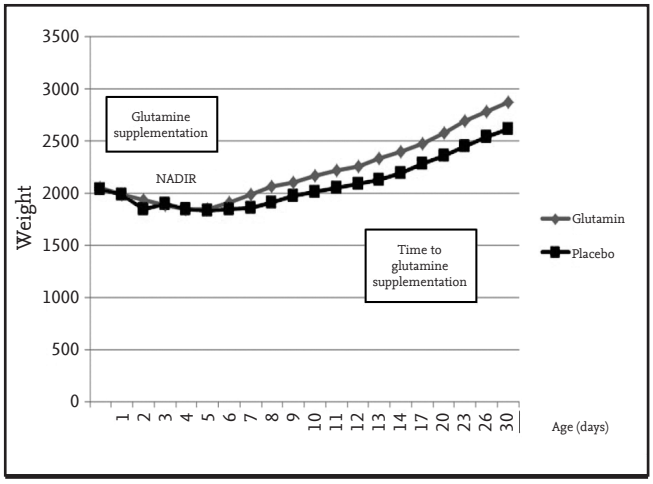

Figure 1. Growth Pattern in the Glutamine and Placebo Groups

Table 3. Fecal secretory immunoglobulin A level before and after treatment in both groups

	fecal sIgA level (mg/g fecal)		
Groups	Before treatment	After treatment	p
Glutamine	0.209±0.045	0.456±0.057	<0.001
Placebo	0.236±0.040	0.376±0.035	<0.001

Paired t-test was used
p<0.05 was considered significant

Discussion

Effect of glutamine supplementation on LBW growth patterns

In our study, the glutamine group had a shorter RTBW and faster WGV compared with the placebo group. A previous study in China found that glutamine supplementation in LBW infants made a shorter RTBW time in the glutamine group than in the placebo group (11). Another study in Russia that used glutamine enteral supplementation with a dose of 300mg/kg/day found no significant results in growth parameters at the end of the first and second month. However, a significant difference was present at the end of the third month, and the difference was even greater at the end of the fourth month (9).

Table 4. Fecal secretory immunoglobulin A level before and after treatment in the glutamine and placebo groups

Fecal sIgA Level (mg/g fecal)	Glutamine (n=18)	Placebo (n=19)	p
Before treatment	0.209±0.045	0.236±0.040	0.066
After treatment	0.456±0.057	0.376±0.035	<0.001
Δ SIgA	0.247±0.024	0.140±0.016	<0.001

Independent sample t-test was used
p<0.05 was considered significant

Table 5. Confounding Factor Analysis

Dependent Variable	Independent Variable	r	p
RTBW	Average Formula Groups	0.262	0.612
WGV	Average Formula Groups	0.012	0.914
sIgA	Average Formula Groups	0.121	0.731

RTBW: return to birth weight; WGV: weight gain velocity
Bivariate Pearson's correlation test was used
p<0.05 was considered significant

A preliminary study using glutamine supplementation at 200-500 mg/kg/day showed that LBW growth pattern improvement started to appear at a dosage of 300 mg/kg/day and more significant improvements appeared at a dosage of 400-500 mg/kg/day. Glutamine kinetics in LBW suggest that enteral glutamine supplementation had more anabolic effects on increasing protein synthesis rather than preventing protein breakdown. Glutamine supplementation at a dosage of 200 mg/kg/day had fewer adverse effects than 500 mg/kg/day in maintaining the balance of positive nitrogen ratio (nitrogen/leusine). Glutamine also had a site action on the gastrointestinal tract by improving oral intake tolerance (16). However, there was a significantly higher elevation in blood urea in the glutamine group at a dosage of 500 mg/kg/day in our preliminary study compared with placebo, although when it was converted to laboratory parameters it was still within the acceptable range.

Term or preterm infants lose 10-15% of their weight in their first week of life. RTBW time ranges from 10 to 14 days and is expected to be achieved within 14-21 days, with WGV between 15 to 20 g/kg/day until it reaches 2000-2500 grams (3, 17). In growth and nutritional adequacy assessment, the targets are to increase in weight (10-20 grams/kg/day), increase in length (1 cm /week), and increase in head circumference (0.5-1 cm/week).

The increase in weight shows that LBW infants need additional calories and a large amount of protein. Protein deficit-induced growth restriction in LBW infants, which occurs in the first week of life because most of the protein intake is used to maintain protein deficits so that anabolic phase, marked with optimal weight gain, would not occur in this period. High levels of protein breakdown are relatively reduced by the parenteral amino acid administration and early breastfeeding in the early phase of life (18). The addition of protein would help to increase body weight, both through the addition of calories and through the addition of lean body mass. It requires a P/E ratio (protein/energy) of at least 3.1-3.25 grams/100 kcal to add to lean body mass, higher than the breast milk P/E ratio, which only has 1.4 grams/100 kcal (4).

Effect of enteral glutamine supplementation on fecal sIgA levels

In this study, the glutamine group showed a higher increase in fecal sIgA levels after treatment compared with the placebo group. Several factors that can affect the synthesis of sIgA include infant age, breastfeeding milk or formula milk, the presence of intestinal bacterial colonization, intraluminal infection, and antibiotic administration (19, 20). The important site for intestinal bacterial colonization is the mucus layer (21). A previous study showed that glutamine supplementation increased the thickness and optical density of intestinal mucus, and therefore prevented bacterial colonization (22). Modulation of intestinal bacteria colonization is considered as the most important stimulus for the development of Th1 and cytokine response, which will eventually stimulate the production of sIgA (21, 23).

Immunoglobulin A secretory is the main product of the mucosal immune system and is the most dominant immunoglobulin on the mucosal surface. Measuring sIgA levels from intestinal fluids and feces can reflect the competence of the intestinal mucus immune response because intestinal sIgA is realitvely stable in the intestinal lumen (20, 24).

Immunoglobulin A secretory plays role in the gastrointestinal defense mechanism to counteract dietary and microbial antigens through immune exclusion. It also plays role in inhibiting adhesion and invasion of potentially harmful antigens into mucosal tissue and neutralizing toxins from pathogenic microbes (19, 24). An increase in intestinal or fecal sIgA levels is triggered by glutamine-induced supplementation, without being preceded by a gastrointestinal infection process, which is highly beneficial for the survival of the infant's intestinal mucous. Stimulation of the intestinal immune response triggers the mucosal immune response elsewhere according to the common mucosal system principle; therefore, early glutamine supplementation will be beneficial for preventing infection in another mucosa.

Limitation of the study

In this study, the average amount of formula consumed by the glutamine group was significantly higher than the placebo group. Not all factors that affect IgA levels could be controlled. The mixing percentage of breastfeeding and the low birth weight formula could not be equated between the two groups. This study did not measure the baseline levels of IgA in

every breast milk. Therefore, it was unknown whether the increase of IgA in the stool was because of glutamine supplementation or because of high levels of IgA in the breast milk.

Conclusion

Enteral glutamine nutrition supplementation (400 mg/kg/day) in LBW infants shortens the RTBW time, increases the WGV, and increases fecal sIgA levels.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Dr Soetomo General Hospital (81/Panke.KKE/VIII/2010).

Informed Consent: Written informed consent were obtained from patients' parents.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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