

# Effect of Glutamine Supplementation on Diarrhea, Interleukin-8 and Secretory Immunoglobulin A in Children With Acute Diarrhea

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## ABSTRACT

**Objective:** Glutamine is an important fuel for rapidly dividing cells such as enterocytes and lymphocytes. Exogenous glutamine supplementation in catabolic states preserves intestinal mucosal structure and function, decreases bacterial translocation, and supports normal immunologic responses. This study was planned to assess the effect of glutamine supplementation on duration and severity of diarrhea and to assess its immunomodulatory effect by measuring serum interleukin-8 (IL-8) and salivary immunoglobulin A (sIgA) in children with acute diarrhea.

**Methods:** In this placebo-controlled, double-blind and randomized trial, 6- to 24-month-old otherwise healthy children admitted to the Diarrheal Diseases Training and Treatment Center with acute diarrhea received either 0.3 g/kg/day of glutamine (n = 63) or placebo (n = 65) for 7 days. Serum IL-8 and sIgA levels were determined on admission and 7 days later. All cases

were followed until the diarrheal episode ended. Anthropometric measurements and history of subsequent infectious diseases were monitored monthly for 3 months after treatment.

**Results:** Mean duration of diarrhea in the glutamine treated group was significantly shorter than that of the placebo group ( $3.40 \pm 1.96$  days,  $4.57 \pm 2.48$  days, respectively;  $P = 0.004$ ). No differences in serum IL-8 and sIgA were found between groups on admission or 1 week later. During 3 month follow-up, mean weight gain and incidence of infectious diseases were similar in both groups.

**Conclusion:** Duration of diarrhea was shorter in children supplemented with glutamine. The beneficial impact of glutamine supplementation seems to be through effects on gastrointestinal mucosa rather than the host immune response. *JPGN* 38:494–501, 2004. **Key Words:** Acute diarrhea— Children— Glutamine supplementation— Interleukin-8— Salivary immunoglobulin A. © 2004 Lippincott Williams & Wilkins

Glutamine is the major energy source for rapidly dividing cells such as enterocytes and lymphocytes (1–3). Animal and human studies have documented roles for glutamine in ameliorating mucosal atrophy occurring during prolonged parenteral nutrition (4), in healing of gastrointestinal mucosa after damage from radio- or chemotherapy (5), in improving gut and systemic immune function (6,7), in attaining positive nitrogen balance (8), and in reducing episodes of bacterial translocation and sepsis (9,10).

Glutamine stimulates NaCl absorption in intestinal mucosa. Glutamine intake is coupled to both electrogenic

and neutral sodium absorption (11). It has been reported that glutamine is superior to glucose alone in promoting net water, sodium, and potassium absorption in normal rabbit ileum (12) and in a rabbit model of secretory diarrhea induced by cholera toxin (13). Nath et al. (14) demonstrated that enteral glutamine could enhance intestinal sodium absorption in rabbits infected with diarrhea producing *Escherichia coli*. Studies have also shown that a glutamine-based oral rehydration solution is more effective than glucose-based ORS in infected pigs with rotavirus or *Cryptosporidium* (15,16). Therefore, glutamine-based ORS is considered the ideal solution to improve mucosal healing from a gastrointestinal infection and to facilitate sodium and water absorption (15–17).

In a randomized, double-blind jejunal perfusion study of adults with acute cholera, glutamine plus glucose significantly reduced net water and sodium secretion and stimulated water absorption to the same degree as glucose and alanine (18). Indeed, preliminary studies in

Received November 6, 2002; accepted December 15, 2003.

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This research was supported by The Scientific and Technical Research Council of Turkey (TUBITAK SBAG-2203).

adult patients with cholera from Indonesia (19) showed a 25% reduction in stool volume during the first 24 hours and a 30% reduction in total stool volume in the group treated with glutamine-based ORS compared with glucose ORS (with 90 mmol/L each of glutamine and glucose). Riberio et al. (20) found glutamine-ORS to be well tolerated but with a therapeutic effect no different from glucose-ORS in treating infants 1-12 months of age with acute non-cholera diarrhea. However, glutamine plus glucose ORS in this study had a higher osmolarity than the control ORS, and study patients were only mildly dehydrated. ORS is more effective when it is hypo-osmolar and given to dehydrated patients (21,22). Unfortunately, the studies of glutamine in patients with diarrhea to date have only evaluated solutions in which glutamine is added to standard, isotonic ORS (19,20).

In addition to its role as a component of ORS, glutamine supplementation may be of benefit to all children with diarrhea, even those without dehydration. As a nutrient promoting intestinal growth and maturation, glutamine may aid in prevention and control of gastroenteritis. It may also promote the appropriate maturation and function of the immune system when given as supplement, rather than in ORS. Although glutamine plays important roles in gut integrity and immunologic responses (2-5,7), there is no single study clarifying the role of glutamine supplementation alone during acute diarrhea.

Given the key role of cytokines in regulating the function of cells of the immune system, there has been some interest in the role of glutamine in modulating cytokine production. Serum interleukin-8 (IL-8) secretion has been found to be inversely related to the severity and outcome of childhood adenovirus infection (23,24). In a study of patients with pancreatitis, de Beaux et al. (25) found that serum IL-8 release was reduced in patients treated with glutamine-supplemented total parenteral nutrition (TPN) but was increased in the conventional TPN group. The administration of glutamine enterally also improves the primary immune defenses of all mucosal surfaces against infections in mice (26). Burke et al. (6) found that glutamine-supplemented TPN maintained salivary immunoglobulin A (sIgA) concentration at normal levels in rats, suggesting that glutamine might have a role in the regulation of the gut-associated lymphoid tissues. Therefore, the immunomodulatory effect of glutamine supplementation in cases of acute diarrhea could be reflected by serum IL-8 levels, as well as the salivary IgA levels.

We planned this study to evaluate the effect of glutamine supplementation, not in ORS but as a medication, on the duration and severity of diarrhea and on the levels of IL-8 and sIgA in children 6 to 24 months of age admitted to Ihsan Doğramacı Children's Hospital Diarrheal Diseases Training and Treatment Center with acute diarrhea.

## PATIENTS AND METHODS

### Design

A randomized, double-blind, placebo-controlled trial was conducted from January 2000 until February 2001 at Hacettepe University Ihsan Doğramacı Children's Hospital Diarrheal Disease Training and Treatment Unit, a 24-hour working clinic where the patients can receive ORS therapy as long as they need on an ambulatory basis. Children ages 6 to 24 months with diarrhea of less than 10 days' duration were included in the trial. Those with chronic illness, severe malnutrition (weight was below 60% of weight standard for age according to National Center for Health Statistics [NCHS]) (27), associated infectious diseases (urinary tract infections, pneumonia), prior antibiotic or antidiarrheal drug use, dysentery, or stool smear containing more than 5 leukocytes per 40x high power field were not admitted to the study.

### Clinical Procedures and Baseline Assessment

One of the authors (SSY) explained the purpose and procedures to the parents. Only mothers who gave written informed consent were allowed to participate. At entry infants were examined and dehydration was assessed and corrected according to World Health Organization (WHO) guidelines by using glucose-ORS (22). Nutritional status (weight for age) was expressed as a percent of the median NCHS standard.

A predesigned, pretested questionnaire was filled out on admission. Data included patient age, weight, length, birth order, and birth weight; age and education level of parents; diarrheal duration, frequency of diarrhea and vomiting, and presence of fever (axillary temperature  $\geq 38^{\circ}\text{C}$ ). Saliva, serum, stool and urine samples were obtained on admission. Stool samples were examined for leukocytes and parasites. Stools were cultured for *Salmonella* and *Shigella* only. Urine analysis and culture were obtained. Samples of venous blood and saliva were obtained only from patients enrolled in the morning. Venous blood (3 mL) was obtained on admission for IL-8 measurement. All breast-fed infants were given 20 mL of water 30 minutes before collection of saliva to avoid contamination of saliva by sIgA in maternal milk. Whole, unstimulated (resting) saliva for sIgA was collected 1 hour after feeding by suction on a plastic syringe without a needle.

### Randomization

Eligible cases were randomized to two groups according to their hospital file number on admission. Patients with odd file numbers received capsules numbered G1 and those with an even number received capsules numbered G4. Capsules of the first dose were opened and dissolved in 50 mL of water and administered by the mother with the help of a nurse. Infants received the supplement, administered by their mothers, in three daily doses for 7 days. The subjects and investigators were blinded to the allocation of treatment group at initial recruitment and during the follow-up period. The randomization code was not opened until the statistical analysis had been completed.

### Follow-up Visits

Mothers were given an observation chart and were instructed to note the frequency of diarrhea, any side effects, and the number of capsules administered every day until the termination of the diarrheal episode. All infants returned to the clinic the day after enrollment and examined. Observation charts were reviewed for accuracy with the mothers. On the second day of intervention, infants with positive urine or stool cultures for pathogenic bacteria and infants non-compliant with the supplement regimen were excluded. The progress of all infants was monitored by telephone interview every other day by one of the authors (SSY) until the infant's recovery from diarrhea.

The transit of enterocytes from crypt to villus tip, including proliferation-maturation-migration-slough of cells, takes 3 to 6 days (28). Therefore, the second venous blood sample and unstimulated saliva sample were obtained 7 days after enrollment.

All cases were followed until the end of the diarrheal episode. Infants were subsequently examined monthly for the next 3 months. At each visit, anthropometrics were obtained, patients were examined for evidence of acute infection, and caregivers were questioned about potential infectious disease occurring in the preceding month.

### Definitions of Primary Outcomes

Clinical recovery was defined as the passage of a soft-formed stool as described by the mother for at least 24 hours. Persistent diarrhea was defined as an episode lasting 14 or more days. Watery diarrhea was defined as severe if the child passed  $\geq 8$  movements/day.

### The Dose and Preparation of Supplements

The optimal dose of glutamine is undetermined. Most investigators suggest that doses of 20 to 40 g/day are required to enhance immunologic function and maintain structure and function of the gastrointestinal tract in adults (2). Previous studies have established the safety of enteral-administered glutamine in normal adults and low-birth-weight infants at 0.1 and 0.3 g/kg/day (10,29). We therefore chose a dose of 0.3 g/kg/day (3–4 g/day). The capsules were prepared and coded as G1 or G4 by the pharmacist (LÖ) at Hacettepe University Faculty of Pharmacy. Treatment capsules containing 250 mg of glutamine and 18 mg of cornstarch and placebo capsules containing 250 mg of cornstarch were manufactured and packed in black cupping glasses. Glutamine capsules were prepared from Power Glutamine® (HPLC 100% Pure L-Glutamine, lot no: 93901 AA 84/9112, Champion Nutrition, CA, U.S.A.). The appearance of the capsules and the cupping glasses in the two groups were identical.

### Compliance

Compliance with the intervention was based on the reports of telephone calls, mother's daily checklist, and the number of capsules in the bottles at the 7th day of the intervention. Non-compliance was defined as the infant receiving either less than half of the study capsules or less than 3 days of supplement. Noncompliant infants were excluded from the study.

### Laboratory Investigations

Samples of saliva and serum were stored at  $-20^{\circ}\text{C}$  until analyzed. When all samples were collected, IL-8 levels were examined by Human IL-8 ELISA/NA-1/KHC0082 kit (lot no: 001901, Cytoscreen, Biosource International, Inc., CA, U.S.A.) and sIgA by Human Secretory IgA RID kit (The Binding Site RN 148.3, lot no: 038189, BIND A RID, Birmingham, United Kingdom) at the Laboratory of Immunology Unit in the Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

### Ethical Consideration

The study was approved by the Ethical Committee for Medical, Surgery and Drug Research at Hacettepe University Faculty of Medicine, Ankara, Turkey (29/7/1999; TBK 99/2–10).

### Sample Size

A preliminary study of 15 patients performed between January and February 2000 found that the mean duration of diarrhea after intervention was 4.61 days (SD = 2.33 days). Using this preliminary study, a prestudy analysis showed that a sample size of 63 infants per group was required (80% power; 5% significance level; two-tailed test) to detect a difference in diarrheal duration of at least 1 day among groups (30). Assuming a dropout rate of 20%, we planned to recruit at least 75 infants for each group.

### Statistical Analysis

All analyses were compared with SPSS for Windows (SPSS Inc., Chicago, IL, U.S.A.). The normality of data distribution was checked using the Kolmogorov-Smirnov test. Serum IL-8 and sIgA were log-transformed for the analysis and presented as the geometric means and 95% confidence intervals because the distributions of the raw data values were skewed. Statistical methods used were *t* test for comparing means and  $\chi^2$  for comparing proportions. The Fisher exact test was used when applicable. Paired *t* test was used for the differences between baseline and the seventh-day examination. Kaplan Meier analysis was performed to determine whether there was a significant difference in the probability of improvement relative to time in the two groups. Log-rank test was done to assess the equality of success across the two groups.

## RESULTS

### Initial Characteristics of the Study Subjects

A total of 159 infants were recruited and randomized into two groups (79 glutamine, 80 placebo). Of these, 16 (9 glutamine, 7 placebo) were excluded because of urinary tract infection detected by culture at the second day of intervention. Stool culture revealed *Shigella* in one case in the placebo group, and stool examination revealed *Entamoeba histolytica* in one of the glutamine group. One infant in the placebo group refused to take the medication at home. In addition, 11 mothers (6 glutamine, 5 placebo) gave capsules at a rate that did not

**TABLE 1.** General characteristics of the study infants at the enrollment (mean ± SD)\*

	Glutamine group n = 63	Placebo group n = 65
Age, months	13.1 ± 5.1	12.1 ± 4.8
Male, n (%)	30 (47.6)	36 (55.4)
Body weight, kg	9.79 ± 1.97	9.25 ± 1.57
Cases with weight/age <90% of NCHS median (percentage)	15 (23.8)	21 (32.3)
Length, cm	76.1 ± 6.5	74.5 ± 5.9
Birth weight, kg	3.34 ± 0.50	3.29 ± 0.45
Duration of breast-feeding, months	9.10 ± 5.56	8.01 ± 5.36
Breast-feeding, n (%)	29 (46.0)	24 (36.9)
Mother's age, years	27.0 ± 6.1	28.2 ± 5.6
Father's age, years	31.2 ± 6.0	32.1 ± 6.3
Mother's education level >8 years, n (%)	30 (47.6)	34 (52.3)
Father's education level >8 y, n (%)	46 (73.0)	47 (72.3)
Housewife, n (%)	50 (79.4)	47 (72.3)
Birth order		
1	33 (52.4)	29 (44.6)
2	15 (23.8)	21 (32.3)
3+	15 (23.8)	15 (23.1)
Duration of diarrhea on admission, day	3.51 ± 2.37	3.72 ± 2.10
Purging rate in the last 24 hours	6.21 ± 2.87	6.76 ± 3.22
Cases with urging rate ≥8/day	17 (27.0)	24 (36.9)
Number of vomiting in the last 24 hours	1.31 ± 1.90	1.84 ± 2.32
Cases with vomiting frequency ≥3/day	15 (23.8)	16 (24.6)
History of dehydration	30 (47.6)	36 (55.4)
Cases with axillary temperature >38°C	12 (19.0)	14 (21.5)

\* *P* > 0.05 for all parameters between groups.  
NCHS = National Center for Health Statistics.

meet the definition for compliance. In the placebo group, one case had persistent diarrhea. Cow's milk intolerance was suspected and confirmed by clinical response to withdrawal of cow's milk and rechallenge. The remaining 128 infants were included in the statistical analyses. The dropout rate was not significantly different between the groups (16 glutamine, 15 placebo). There were no significant differences between dropouts and nondropouts in age, gender, weight, height, duration and frequency of diarrhea, frequency of vomiting, breast-feeding status, presence of malnutrition, birth order, or birth weight.

The admission age, gender, weight, height, duration and frequency of diarrhea, frequency of vomiting, breast-feeding status, presence of malnutrition and dehydration, birth order, birth weight, and mother's and father's age and education were comparable in the two groups (Table 1).

### Duration and Severity of Diarrhea After the Intervention

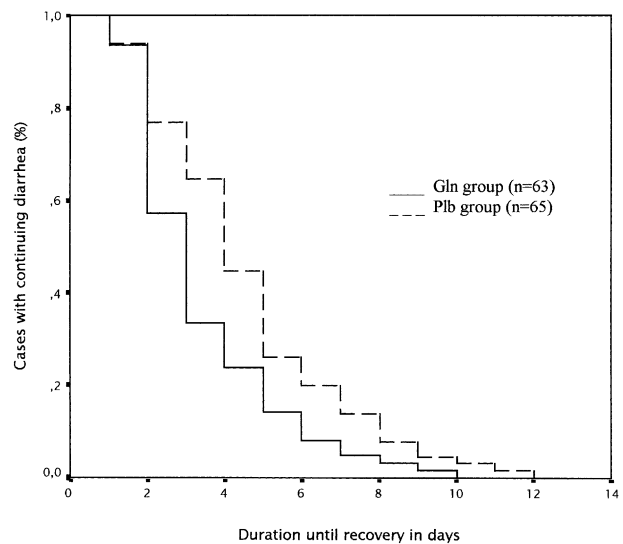
The mean duration of diarrhea after treatment in the glutamine group was significantly shorter than in the

placebo group (3.40 ± 1.96 days, 4.57 ± 2.48 days, respectively; *P* = 0.004). Five infants in the glutamine group and 13 in the placebo group still had diarrhea 7 days after enrollment (7.9%, 20.0%, respectively; *P* = 0.088). Duration of diarrhea in the glutamine group was significantly shorter than in the placebo group with Kaplan Meier analysis (log-rank, *P* = 0.004, Fig. 1).

The mean total duration of diarrhea (the sum of diarrheal duration on admission and diarrheal recovery after the intervention) in the glutamine group was shorter than in the placebo group (6.90 ± 3.24 days, 8.29 ± 3.39 days, respectively; *P* = 0.020). The proportion of persistent diarrhea was 3.2% in the glutamine group and 9.2% in the placebo group (2/63, 6/65, respectively; *P* = 0.27).

Twelve (19.0%) infants in the glutamine group and 19 (29.2%) in the placebo group had a high stool frequency (≥ 8/day) for at least 1 day during follow-up period, but the difference between groups was not statistically significant. Vomiting was observed in 24 (38.1%) infants in the glutamine group and 32 (49.2%) in the placebo group (*P* > 0.05). Six (9.5%) patients in the glutamine group vomited more than 3 times a day in at least one of the intervention days compared with 15 (23.1%) patients in the placebo group (*P* = 0.067).

In the glutamine group, at the seventh day of the intervention, three infants had urinary tract infection and one had upper respiratory tract infection, while in the placebo group three had upper respiratory tract infection symptoms, one had bronchiolitis, and five had urinary tract infection. One infant in the placebo group was hospitalized because of a high stool frequency, recurrent, severe dehydration, and acidosis on the seventh day of the intervention. She was discharged from the hospital after 5 days of treatment.



**FIG. 1.** Kaplan-Meier plot of time-to-diarrheal recovery after initiation of intervention in glutamine (gln) versus placebo (plb) groups (log-rank, *P* = 0.004).

### Diarrheal Recovery Time According to Subject Characteristics and Intervention Group

Glutamine supplementation reduced diarrhea recovery time in both breast-fed and non-breast-fed infants (Table 2). When data were stratified by stool frequency on admission, infants in the glutamine group with a stool frequency < 8/day had shorter duration of diarrhea after the intervention than did those in the placebo group ( $3.30 \pm 1.96$ ,  $4.68 \pm 2.30$  days, respectively;  $P = 0.006$ ). No significant differences were observed for the infants with high stool frequency >8/day. Similarly infants who vomited less than three times daily in the glutamine group had significantly shorter duration of diarrhea compared with similar infants who received placebo ( $3.54 \pm 2.05$ ,  $4.73 \pm 2.62$  days, respectively;  $P = 0.015$ ). The diarrheal duration was significantly shorter the glutamine group with normal nutritional status compared with normally nourished subjects in the placebo group ( $3.42 \pm 1.91$ ,  $4.27 \pm 2.12$  days, respectively;  $P = 0.044$ ). When the duration of diarrhea was compared among dehydrated infants, the duration of diarrheal recovery was shorter in the glutamine group than in the placebo group ( $3.50 \pm 1.98$ ,  $4.72 \pm 2.41$  days, respectively;  $P = 0.030$ ). Diarrheal duration was slightly shorter in the glutamine group with mild to moderate malnutrition and no dehydration than in similar patients in the placebo group. Among patients > 12 months of age, duration of diarrhea was significantly shorter in the glutamine group than in the placebo group ( $3.06 \pm 1.88$ ,  $4.45 \pm 2.53$  days, respectively;  $P = 0.014$ ). No such significant difference in duration was found among patients <12 months.

### Dose Effect

Because daily glutamine intake varied according to compliance level, a dose effect was seen in the glutamine group. When the daily intake of the supplement increased, the duration of diarrhea decreased in the glutamine group only ( $r = -0.253$ ,  $P = 0.046$ ), and there was no similar dose effect in the placebo group ( $r = 0.04$ ,  $P > 0.05$ ).

### Biochemical and Immunologic Analysis

Blood samples were taken from 36 glutamine cases and 39 placebo cases on admission and day 7. Samples of saliva were adequate for analysis in 35 cases from each group. The patients whose blood and saliva samples were studied were similar for baseline characteristics to patients with no blood sample. No differences were found between groups in geometric mean levels of serum IL-8 and sIgA on admission and at day 7 (Table 3). Serum IL-8 levels were significantly less after treatment in both groups ( $P < 0.05$ ), whereas sIgA levels showed no change.

### Weight Gain and Frequency of Infectious Disease

Mean weight gain within the first month in the glutamine group was greater than that in the placebo group ( $489 \pm 365$  g,  $359 \pm 309$  g respectively;  $P = 0.05$ ). Mean weight gain was similar in both groups in the second and third months ( $695 \pm 367$  g in the glutamine group,  $650 \pm 359$  g in the placebo group;  $P > 0.05$  for the second month; and  $1,086 \pm 540$  g in the glutamine group,  $989 \pm$

**TABLE 2.** Effect of glutamine supplementation on diarrheal duration (days) according to subject characteristics in acute diarrhea

	Glutamine group		Placebo group		P
	n	Mean $\pm$ SD	n	Mean $\pm$ SD	
Age					
<12 mo	28	3.82 $\pm$ 2.00	36	4.67 $\pm$ 2.47	0.146
$\geq$ 12 mo	35	3.06 $\pm$ 1.88	29	4.45 $\pm$ 2.53	0.014
Nutritional status					
Cases with weight/age <90% of NCHS median	15	3.33 $\pm$ 2.16	21	5.19 $\pm$ 3.08	0.053
Cases with weight/age $\geq$ 90% of NCHS median	48	3.42 $\pm$ 1.91	44	4.27 $\pm$ 2.12	0.044
Breast-feeding					
No	34	3.44 $\pm$ 2.18	41	4.61 $\pm$ 2.53	0.038
Yes	29	3.34 $\pm$ 1.70	24	4.50 $\pm$ 2.43	0.047
History of dehydration					
No dehydration	33	3.30 $\pm$ 1.96	29	4.38 $\pm$ 2.60	0.069
Mild + moderate	30	3.50 $\pm$ 1.98	36	4.72 $\pm$ 2.41	0.030
Purging rate on admission					
<8/day	46	3.30 $\pm$ 1.96	41	4.68 $\pm$ 2.60	0.006
$\geq$ 8/day	17	3.65 $\pm$ 1.97	24	2.37 $\pm$ 2.30	0.296
Vomiting frequency on admission					
<3/day	48	3.54 $\pm$ 2.05	49	4.73 $\pm$ 2.62	0.015
$\geq$ 3/day	15	2.93 $\pm$ 1.58	16	4.06 $\pm$ 1.95	0.088

NCHS = National Center for Health Statistics.

**TABLE 3.** Effect of glutamine supplementation on levels of serum IL-8 and salivary IgA levels in patients with acute diarrhea

	Glutamine group		Placebo group	
	n	GMT (95% CI)*	n	GMT (95% CI)*
IL-8, pg/mL				
On admission	36	89.4 (72.3–110.6)	39	70.9 (59.7–84.2)
After 7 days	36	54.5 (45.4–66.4)†	39	53.6 (46.9–61.3)‡
sIgA, mg/L				
On admission	35	351 (280–39)	35	321 (261–396)
After 7 days	35	309 (251–379)	35	277 (227–339)

\* Geometric mean, 95% confidence interval.

$P > 0.05$  for comparison of all parameters between glutamine and placebo groups.

Significantly different from baseline values † $P = 0.001$ , ‡ $P = 0.004$ .

395 g in the placebo group;  $P > 0.05$  for the third month). During 3 months of follow-up period, the frequency of infection was not different between the two groups.

## DISCUSSION

In this study, glutamine supplementation had a beneficial effect on diarrheal recovery in children with acute diarrhea. Normally, the human body synthesizes glutamine sufficient for metabolic needs; however, the intestinal mucosa has limited de novo synthetic capacity (1–3). In childhood diarrhea the supply of glutamine might be inadequate to support mucosal recovery and an external supply might be necessary to promote repair and regeneration of the damaged mucosa. Similarly, in catabolic conditions such as infection, malnutrition, surgery, or stress, glutamine is often depleted and becomes a “provisionally essential” amino acid (1–3,6). Indeed, exogenous glutamine supplementation has been shown to improve nitrogen balance after major surgery (3,8), decrease hospital stay after bone marrow transplantation (9), reduce mortality of chronic intensive care unit patients (7,31), stimulate the growth of intestinal mucosa in patients on bowel rest, decrease intestinal permeability and improve absorption (3,32).

In this study, glutamine supplementation seemed to be effective in children >12 months, children with low stool frequency and those with a low vomiting rate. In cases with high stool frequency and vomiting rate glutamine supplementation had no significant impact on diarrhea duration. One possible explanation is that quick transport might decrease enteral glutamine absorption, and the vomiting and purging may reduce surface contact for glutamine and salt absorption, necessitating a higher intake. Another possibility is that the small number of children in these subgroups might have limited the study’s power to detect significant association.

Despite the decrease in diarrheal duration, the frequency of persistent diarrhea was not affected by glutamine

supplementation. One explanation could be that the number of patients was too small to detect changes in this infrequent complication. The study was powered to detect only a one day difference in diarrheal duration. To demonstrate whether glutamine supplementation significantly reduces the incidence of persistent diarrhea, a sample size of more than 100 infants per group would be required (30).

Glutamine supplementation did not have a beneficial effect on subsequent disease prevention. Previous studies have established the safety of enterally administered glutamine in healthy individuals and premature infants at doses of 0.1 to 0.3 g/kg/day (2,10,29). The dose used in our study was .3g/kg/day. However, the optimal dose of glutamine has yet to be determined, and most investigators suggest that doses of 20 to 40 g/day are required in adults to enhance immunologic function and maintain structure and function of the gastrointestinal tract after catabolic insult (2,29).

Ameho et al. (33) showed that glutamine may have inhibited synthesis, release, and/or action of the proinflammatory cytokines IL-8 and TNF- $\alpha$ , resulting in the improvement in disease outcome in experimental trinitrobenzene sulfuric acid-induced colitis in rats. Similarly, in cases of acute pancreatitis, serum IL-8 levels were found to be decreased in a glutamine-supplemented TPN group (25). However, our data indicate that glutamine supplementation for children with acute diarrhea (0.3 g/kg/day of glutamine for 7 days) did not change serum IL-8 levels, despite significant clinical improvement in diarrheal recovery. This finding may be partly attributable to our patient selection. Patients with mild-moderate diarrhea were enrolled, whereas patients with either persistent diarrhea or bloody diarrhea were not enrolled. It is also possible that the glutamine dose in our study might be less than that needed to affect immunologic parameters. Ameho et al. (33) reported that a high-dose (4% glutamine, 40 g/kg/day) glutamine group performed better than did the 2% glutamine group. In our study, only systemic cytokine response was studied. Previous studies have shown that bacterial and viral infections elicit IL-8 secretion in cultured epithelial cells (34,35). Secretion of IL-8 in stool extracts has been correlated with disease severity in a rabbit model of shigellosis (36). Additional studies are necessary to detect the effect of glutamine supplementation on mucosal cytokine production.

Secretory IgA plays an important role in protection against infections caused by enteropathogens in both human and animal models (37). Burke et al. (6) found that glutamine-supplemented TPN maintained biliary secretory IgA concentration at normal levels in rats. Kudsk et al. (38) reported that glutamine-enriched TPN preserved both extraintestinal and intestinal IgA levels and had a normalizing effect on Th2-type IgA-stimulating cytokines in mice. Salivary IgA secretion may be induced by both oral antigens stimulating the proliferation and dif-

ferentiation of lymphoid cells locally in salivary glands and by the migration of antigen-sensitized IgA precursor B cells from gut-associated lymphoid tissues to salivary glands (37). Considering this mechanism and the ease of obtaining salivary samples, salivary secretory IgA was studied in our study. Our data suggest that glutamine supplementation did not affect the level of salivary IgA in patients with acute watery diarrhea. The dose and duration of glutamine supplementation and severity of disease could be plausible explanations for the lack of effect.

Although the mean duration of diarrhea was significantly decreased, glutamine supplementation did not influence immunologic parameters in the current study. We suspect therefore that the reduction in diarrheal duration by glutamine was a result of its local beneficial effect on the mucosa rather than a modulation of the modulation of systemic humoral and cytokine response. However, mucosal immune responses were not the purpose of this experiment and were not examined in this study. Additional studies including the local immune response of glutamine supplementation would clarify this subject.

There remains controversy about the impact of glutamine on the integrity of the mucosal barrier, as well as the maintenance of its metabolic and immune function (3,12,17–20,32,39–41). Studies have shown limited evidence that glutamine supplementation has a benefit for patients with short bowel syndrome, inflammatory bowel disease and radiation induced diarrhea (3,8,28,42–44). Although beneficial effects of glutamine are not consistently observed, some studies suggest that, under certain circumstances, glutamine supplementation may be beneficial to some patients (2,7,9,10,19,25,28,31,45). Additional studies are required to determine which individuals are the best candidates for such therapy.

The reduction in duration and severity of diarrhea by glutamine supplementation may be perceived as desirable by caregivers of children with acute diarrhea. Perhaps the use of glutamine would be helpful in reducing the use of antibiotics and other antidiarrheal drugs often prescribed for children with acute diarrhea. Additional studies, with large numbers of cases, are needed to determine the efficacy and cost-benefit analysis of glutamine-ORS in dehydrated children. Because these findings may have important implications for the reduction of morbidity and mortality caused by diarrhea in children, they need to be confirmed in other developing countries.

## REFERENCES

- Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? *Nutr Rev* 1990;48:297–309.
- Sacks GS. Glutamine supplementation in catabolic patients. *Ann Pharmacother* 1999;33:348–54.
- Souba WW, Klimberg VS, Plumley DA, et al. The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *J Surg Res* 1990;48:383–91.
- Tamada H, Nezu R, Imamura I, et al. The peptide alanyl-glutamine prevents intestinal mucosal atrophy in parenterally fed rats. *JPEN J Parenter Enteral Nutr* 1992;16:110–6.
- O'Dwyer S, Smith R, Hwang T, et al. Maintenance of small bowel mucosa with glutamine enriched parenteral nutrition. *JPEN J Parenter Enteral Nutr* 1989;13:579–85.
- Burke DJ, Alverdy JC, Aoys E, et al. Glutamine-supplemented total parenteral nutrition improves gut immune function. *Arch Surg* 1989;124:1396–9.
- O'Riordian M, Fearon KCH, Ross JA, et al. Glutamine-supplemented total parenteral nutrition enhances T-lymphocyte response in surgical patients undergoing colorectal resection. *Ann Surg* 1994;220:212–21.
- Stehle P, Mertas N, Albers S, et al. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. *Lancet* 1989;1:231–3.
- Ziegler TR, Young LS, Benfell K, et al. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. *Ann Intern Med* 1992;116:821–8.
- Neu J, Roig JC, Meetze WH, et al. Enteral glutamine supplementation for very low birth weight infants decreases morbidity. *J Pediatr* 1997;131:691–9.
- Rhoads M. Glutamine signaling in intestinal cells. *JPEN J Parenter Enteral Nutr* 1999;23(Suppl.):38–40.
- Islam S, Mahalanabis D, Chowdhury AKA, et al. Glutamine is superior to glucose in stimulating water and electrolyte absorption across rabbit ileum. *Dig Dis Sci* 1997;42:420–3.
- Silva AC, Santos-Neto MS, Soares AM, et al. Efficacy of a glutamine-based oral rehydration solution on the electrolyte and water absorption in a rabbit model of secretory diarrhea induced by cholera toxin. *J Pediatr Gastroenterol Nutr* 1998;26:513–9.
- Nath SK, Dechelotte P, Darmaun D, et al. [<sup>15</sup>N] and [<sup>14</sup>C] glutamine fluxes across rabbit ileum in experimental bacterial diarrhea. *Am J Physiol* 1992;262:G312–8.
- Argenzio RH, Rhoads JM, Armstrong M, et al. Glutamine stimulates prostaglandin-sensitive Na<sup>+</sup>-H<sup>+</sup> exchange in experimental porcine cryptosporidiosis. *Gastroenterology* 1994;106:1418–28.
- Rhoads JM, Keku EO, Quinn J, et al. L-Glutamine stimulates jejunal sodium and chloride absorption in pig rotavirus enteritis. *Gastroenterology* 1991;100:683–91.
- Duggan C. Glutamine-based oral rehydration solutions: the magic bullet revisited? *J Pediatr Gastroenterol Nutr* 1998;26:533–5.
- van Loon FP, Banik AK, Nath SK, et al. The effect of L-glutamine on salt and water absorption: a jejunal perfusion study in cholera in humans. *Eur J Gastroenterol Hepatol* 1996;8:443–8.
- Punjabi NH, Kumala S, Rasidi C, et al. Glutamine supplemented ORS is superior to standard citrate glucose ORS for the maintenance therapy of adult cholera patients in Jakarta (abstract). *Am J Trop Med Hyg* 1991;45(Suppl):114.
- Riberio H, Riberio T, Mattos A, et al. Treatment of acute diarrhea with oral rehydration solutions containing glutamine. *J Am Coll Nutr* 1994;13:251–5.
- Farthing MJG. Oral rehydration: An evolving solution. *J Pediatr Gastroenterol Nutr* 2002;34(Suppl 1):64–7.
- World Health Organization (WHO). *A Manual for the Treatment of Acute Diarrhea for Use by the Physicians and Other Senior Health Workers*. Geneva: WHO; 1984, WHO/CDD/SER 80.2 REV 1.
- Mistchenko AS, Diez RA, Mariani AL, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. *J Pediatr* 1994;124(5 Pt 1):714–20.
- O'Riordian MG, De Beaux A, Fearon KCH. Effect of glutamine on immune function in the surgical patient. *Nutrition* 1996;12(Suppl):S82–4.
- de Beaux AC, O'Riordain MG, Ross JA, et al. Glutamine-supplemented total parenteral nutrition reduces blood mononuclear

- cell interleukin-8 release in severe acute pancreatitis. *Nutrition* 1998;14:261–5.
26. Li J, King BK, Janu PG, et al. Glycyl-L-glutamine-enriched total parenteral nutrition maintains small intestine gut-associated lymphoid tissue and upper respiratory tract immunity. *JPEN J Parenter Enteral Nutr* 1998;22:31–6.
  27. Hamill PV, Drizd TA, Johnson CL, et al. Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr* 1979;32:607–29.
  28. Miller AL. Therapeutic considerations of L-glutamine: a review of the literature. *Altern Med Rev* 1999;4:239–48.
  29. Ziegler TR, Benfell K, Smith RJ, et al. Safety and metabolic effects of L-glutamine administration in humans. *JPEN J Parenter Enteral Nutr* 1990;14(Suppl.):137–46.
  30. Hurley SB, Cummings SR. *Designing clinical Research*. Baltimore: Williams & Wilkins; 1988.
  31. Griffiths RD, Jones C, Palmer TE. Six-month outcome in critically ill patients given glutamine-supplemented parenteral nutrition. *Nutrition* 1997;13:293–302.
  32. van der Hulst RRWJ, Van Kreel BK, Von Meyenfeldt MF, et al. Glutamine and the preservation of gut integrity. *Lancet* 1993;334:1363–5.
  33. Ameho CK, Adjei AA, Harrison EK, et al. Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. *Gut* 1997;41:487–93.
  34. Eckmann L, Kagnoff MF, Fierer J. Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. *Infect Immun* 1993;61:4569–74.
  35. Sheth R, Anderson J, Sato T, et al. Rotavirus stimulates IL-8 secretion from cultured epithelial cells. *Virology* 1996;221:251–9.
  36. Sansonetti PJ, Arondel J, Huerre M, et al. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect Immun* 1999;67:1471–80.
  37. Marcotte H, Lavoie M. Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol Mol Biol Rev* 1998;62:71–109.
  38. Kudsk KA, Wu Y, Fukatsu K, et al. Glutamine-enriched total parenteral nutrition maintains intestinal interleukin-4 and mucosal immunoglobulin A levels. *JPEN J Parenter Enteral Nutr* 2000;24:270–4.
  39. Conejero R, Bonet A, Grau T, et al. Effect of a glutamine-enriched enteral diet on intestinal permeability and infectious morbidity at 28 days in critically ill patients with systemic inflammatory response syndrome: a randomized, single-blind, prospective, multicenter study. *Nutrition* 2002;18:716–21.
  40. Potz B, Holliday N, Lewis P, et al. Glutamine supplementation and deprivation: effect on artificially reared rat small intestinal morphology. *Pediatr Res* 2002;52:430–6.
  41. Powell-Tuck J, Jamieson CP, Bettany GEA, et al. A double blind, randomised, controlled trial of glutamine supplementation in parenteral nutrition. *Gut* 1999;45:82–8.
  42. Buchman AL. Glutamine: commercially essential or conditionally essential? A critical appraisal of the human data. *Am J Clin Nutr* 2001;74:25–31.
  43. Kozelsky TF, Meyers GE, Sloan JA, et al. Phase III double-blind study of glutamine versus placebo for the prevention of acute diarrhea in patients receiving pelvic radiation therapy. *J Clin Oncol* 2003;21:1669–74.
  44. Scolapio JS. Effect of growth hormone and glutamine on the short bowel: five years later. *Gut* 2000;47:164–7.
  45. Novak F, Heyland DK, Avenell A, et al. Glutamine supplementation in serious illness: a systemic review of the evidence. *Crit Care Med* 2002;30:2022–9.