A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants[1–3]

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ABSTRACT

Background: Growth faltering during infancy is a characteristic of life in developing countries. Previous studies have shown that small-intestine mucosal enteropathy, accompanied by endotoxemia and a persistent systemic inflammatory response, accounts for up to 64% of the growth faltering in Gambian infants.

Objective: The objective was to test whether glutamine, with its putative trophic effects on enterocytes, immune cells, and intestinal integrity, can accelerate the repair of the intestine, lower immunostimulation, and reduce growth faltering.

Design: Ninety-three infants aged 4–10 mo from the West Kiang region of The Gambia were studied in a double-blind, double-placebo, controlled trial. Glutamine (0.25 mg/kg body wt) or a placebo that contained an isonitrogenous, isoenergetic mix of nonessential amino acids was orally administered twice daily throughout the 5-mo rainy season. Anthropometric measurements were made monthly during the supplementation period and for 6 mo after supplementation. Intestinal permeability was measured monthly (by determining the ratio of lactulose to mannitol), and finger-prick blood samples were collected for the analysis of plasma proteins on 3 occasions.

Results: Gambian infants showed a seasonal deterioration in growth and persistently elevated acute phase protein concentrations and intestinal permeability. Oral supplementation with glutamine did not improve growth ($\bar{x} \pm$ SE: weight gain, 60 ± 19 and 69 ± 20 g/mo; length gain, 1.01 ± 0.05 and 0.95 ± 0.03 cm/mo) or intestinal permeability [lactulose:mannitol ratio: 0.29 (95% CI: 0.23, 0.35) and 0.26 (95% CI: 0.21, 0.32)] in the glutamine and placebo groups, respectively. It also had no effect on infant morbidity or on plasma concentrations of immunoglobulins or acute phase proteins.

Conclusion: Glutamine supplementation failed to improve growth or intestinal status in malnourished Gambian infants. *Am J Clin Nutr* 2007;86:421–7.

KEY WORDS Glutamine, growth, intestinal permeability, infants, malnutrition, immune response, randomized control trial, The Gambia

INTRODUCTION

Growth faltering during infancy is frequently seen in developing countries, including The Gambia (1). Growth is good during the first 3 mo of life but then decreases sharply during the remainder of the first year; weight reaches ≈75% of standard at 12–15 mo. The severity of growth faltering cannot be explained in terms of dietary inadequacies (2), which has led investigators to look for other causes. Up to 43% of this growth faltering can be explained in terms of intestinal mucosal damage, as measured by intestinal permeability (3). Moreover, elevated permeability has been shown to be associated with persistently elevated plasma concentrations of immunoglobulins and acute phase proteins, which suggests the presence of a chronic inflammatory enteropathy and was later confirmed by biopsy (4). Two mechanisms that could lead to growth faltering have been described (5): *1*) partial villus atrophy, which reduces digestion and absorption of nutrients (6), and *2*) damage to the mucosal barrier, which allows translocation of macromolecules into the body and triggers both local and systemic immune and inflammatory mechanisms (7). Once the enteropathy is established, it is very slow to resolve (5). This may be due to continued ingestion of pathogens in unhygienically prepared food, but it is also possible that these children, who suffer from frequent minor illnesses, may be at risk of glutamine deficiency (8).

Glutamine is the most abundant free amino acid in the body (9). Traditionally considered a nonessential amino acid, evidence now suggests that it may become conditionally essential during times of injury or illness (8). During catabolic stress, endogenous synthesis of glutamine is impaired and mobilization of glutamine from muscle stores may fail to meet the increased metabolic requirements of rapidly proliferating cells, particularly those of the small intestinal mucosa and the immune system (10–13). Turnover rates may be compromised, which leads to reduced physiologic function (14). Many studies in both animals and humans have shown that both enterally and parenterally administered glutamine improves gastrointestinal function in many clinical situations (16). Thus, glutamine has been shown to stimulate the regeneration of the mucosa after prolonged parenteral feeding (17); increase the rate of mucosal healing after damage caused by radio- or chemotherapy (18, 19); improve villus height, intestinal permeability, and bacterial translocation

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in trauma patients (17, 20, 21); improve mucosal and systemic immune function; and modulate inflammatory processes (13, 22, 23). However, the quality of much of the human data has recently been criticized and caution is recommended when interpreting the results (24, 25). Nevertheless, it seems likely that an adequate supply of glutamine is essential for normal activity and for the maintenance of the small intestinal mucosa.

In Gambian infants, the glutamine requirement may be high because of frequent disease, but because of malnutrition, availability may be low. It therefore seems possible that the persistent intestinal mucosal damage and resulting growth faltering may benefit from the reported restorative capacity of glutamine.

SUBJECTS AND METHODS

Subjects

The study took place during 2 consecutive rainy seasons (July to October) in the West Kiang region of The Gambia, West Africa. Over the 2 y of the study, 93 subjects (40 boys, 53 girls) between the ages of 4 and 11 mo were recruited ≥1 mo before the onset of the rainy season from 6 villages (Jouli, Jula Kunda, Kemoto, Karantaba, Kanton Kunda, and Kuli Kunda) in the West Kiang region. One infant died of suspected meningitis during the study, but all others completed the study period. No selection criteria were used and all suitably aged children in the villages entered the study. The study was granted ethical approval by the Gambian Government/Medical Research Council Laboratories Joint Ethics Committee. Informed consent was given by the infants' parents, who were free to withdraw their children from the study at any time.

Supplement and placebo

The study was a double-blind, double-placebo controlled investigation. To comply with the double-placebo protocol, at the start of the study each child was assigned by restricted randomization (balanced for age) to 1 of 4 groups. Two of these groups received 0.5 g glutamine $\cdot kg^{-1} \cdot d^{-1}$ and 2 groups were given 0.5 g $\cdot kg^{-1} \cdot d^{-1}$ of an isonitrogenous, isoenergetic mix of nonessential amino acids and fructose [18% alanine, 15.2% glycine, 21.3% serine, 22.8% asparagine (monohydrate), and 22.7% fructose by weight]. However, none of the investigators knew which groups received glutamine and which the placebo. Because of concerns regarding possible toxic effects of glutamine administration in infants, the safety of a dose of 0.5 g glutamine $\cdot kg^{-1} \cdot d^{-1}$ was confirmed before the main study by clinical and biochemical evaluation in a pilot investigation (26). Because glutamine is unstable in aqueous solution, both glutamine and placebo were manufactured in powder form and identically packaged in 0.25- and 0.5-g sachets (Special Hospital Services International, Liverpool, United Kingdom). Immediately before the supplement or placebo was administered, the contents of the preweighed sachets were mixed in the appropriate dose with expressed breast milk, or, occasionally if the mother was not available, with distilled water. Records show a high compliance with this method of delivery. The supplement or placebo was given daily to each child in 2 doses (morning and evening), except on the weekend, when the supplement was given in a single dose in the morning. Trained fieldworkers gave the supplement or placebo and recorded whether the dose had been successfully taken. The supplement and placebo were well received by most of the infants. The fieldworkers collected infant morbidity data daily from the mothers.

Infants were maintained on the supplement or placebo for 5–6 mo, which included the whole of the rainy season (late July to early October). A delay in the delivery of the supplement to The Gambia meant that supplementation did not begin until early June in the first year. Each subject attended the clinic every 4 wk during the study. A maximum of 6 clinic visits were made, although those infants delayed by the late arrival of the glutamine attended clinic on only 5 occasions.

Anthropometric measures

Infant weight, length, and midupper arm circumference (MUAC) were measured at each clinic visit. Weight was measured with a Seca (CMS, London, United Kingdom) electronic baby scales to within 0.01 kg, and length was measured with a Kiddimeter length board (Raven Equipment, Essex, United Kingdom) to within 0.1 cm. Both pieces of apparatus were checked and calibrated before use. MUAC of the left arm was measured with a nonstretchable plastic measuring tape correct to 0.1 cm. All measurements were made in triplicate by the same investigator throughout the supplementation period (May to October) and were independently checked by a trained fieldworker. Anthropometric data collection continued for the 6-mo period after supplementation (November to April) by 1 of 2 trained fieldworkers and was independently checked by a third. Infant weight-for-age and height-for-age were expressed as *z* scores (WAZ and HAZ, respectively) based on the 1990 British growth curves (27). The reference data were used to express these data corrected for both age and sex. *Z* scores were not used to express MUAC for which the actual values are given.

Subjects were clinically examined at each clinic visit, and treatment was provided when required. Medical attention was also given to the subjects during the course of the study whenever required, and clinical examinations took place on request. At 3 time points during the supplementation period (start, midway, and end), finger-prick blood samples were collected from the subjects. Blood was used to check for the presence of malaria parasites and to determine hemoglobin concentrations. Malaria and anemia were treated appropriately when detected. An additional 200 μL blood was collected into heparinized plasma separator tubes (BD Microtainers; Direct Medical Supplies, Hants, United Kingdom). Blood was kept on ice until centrifugation at 1500 × *g* for 10 min at 4 °C (between 1 and 3 h after collection), and the plasma was stored at −20 °C before transfer to the United Kingdom for analysis.

Intestinal permeability

An intestinal permeability test was performed on each infant at each clinic visit to assess the integrity of the small intestine. Subjects were given an oral dose of lactulose and mannitol, and the excretion of these markers in the urine was used as an index of intestinal permeability (28). The dose contained 200 mg lactulose and 50 mg mannitol per mL water, and the subjects received 2 mL of the dosing solution per kg body weight. The time at which the dose was given was recorded, and a urine bag fitted with a drainage tube was attached (Hollister U-bag; Abbott Labs, Queensborough, Kent, United Kingdom). Urine was collected from the subjects for 5 h after the oral dose of lactulose mannitol was given. Urine was drained as soon as it was produced into

TABLE 1
Anthropometric and intestinal permeability variables of Gambian infants in the glutamine and placebo groups at the beginning and end of the supplementation period[1]

	First visit		Last visit	
	Glutamine	Placebo	Glutamine	Placebo
Age (mo)	7.9 ± 0.3[2]	7.8 ± 0.3	11.7 ± 0.3	11.6 ± 0.3
Weight (kg)	7.08 ± 0.99	7.07 ± 1.07	7.31 ± 0.96[3]	7.33 ± 0.97[3]
Height (cm)	66.4 ± 3.2	66.1 ± 3.6	69.5 ± 3.0[3]	69.4 ± 3.47[3]
Weight-for-age *z* score	−1.70 ± 0.19	−1.69 ± 0.18	−2.69 ± 0.17[3]	−2.58 ± 16[3]
Height-for-age *z* score	−1.23 ± 0.14	−1.33 ± 0.14	−1.73 ± 0.15[3]	−1.76 ± 0.14[3]
MUAC	13.5 ± 0.2	13.5 ± 0.2	12.8 ± 0.2[3]	13.0 ± 0.15[3]
L:M ratio[4]	0.33 (0.25, 0.43)	0.33 (0.26, 0.41)	0.29 (0.23, 0.35)	0.26 (0.21, 0.32)
Mannitol recovery (%)[4]	2.65 (2.02, 3.48)	2.50 (1.87, 3.36)	2.48 (1.99, 3.11)	2.14 (1.62, 2.82)
Lactulose recovery (%)[4]	0.21 (0.16, 0.28)	0.20 (0.15, 0.26)	0.17 (0.13, 0.21)	0.14 (0.11, 0.18)

[1] For the anthropometric data, $n = 45$ for both groups; for the intestinal permeability data, $n = 42$ and 40 for the glutamine and placebo groups, respectively. MUAC, midupper arm circumference; L:M, lactulose:mannitol. Statistical analysis was by ANOVA, with adjustment for village, sex, and year. There were no significant differences between the glutamine and placebo groups at the beginning or the end of the supplementation period.
[2] $\bar{x}$ ± SE (all such values).
[3] Significantly different from first visit, $P < 0.001$.
[4] Values are geometric $\bar{x}$; 95% CIs in parentheses.

collecting bottles containing a few drops (50–100 μL) of 20% (wt:vol) chlorhexidine gluconate as preservative. The total urine volume was recorded, and an aliquot was stored at −20 °C for subsequent analysis. It was not practical (or ethical) to fast the infants before the permeability test; however, the subjects were not given the permeability dose until they had been at the clinic for ≥30 min. After the dose of lactulose and mannitol was given, these infants were not allowed food or breast milk for 2 h, but were encouraged to drink water.

Morbidity

Trained fieldworkers collected daily details of any episodes of illness suffered by the infants during the course of supplementation. The mother was the usual respondent, although, when she was not available, the nursemaid or other member of the compound (usually a cowife or grandmother) responded. When an episode of diarrhea was reported, the bowel habits of the infants were also recorded. For the purposes of analysis, diarrhea was defined as ≥3 watery stools/d.

Analysis of biological samples

Urinary lactulose and mannitol were measured by using automated enzymatic assays (29, 30). Urinary creatinine was assayed by using an automated Jaffé method using a kit (Sigma, Poole, United Kingdom). The urinary results were expressed as the ratio of lactulose to mannitol (L:M) and as the percentage recovery of each of the probe molecules. Plasma albumin, α_1-antichymotrypsin (ACT), and immunoglobulins A, G, and M (IgA, IgG, and IgM) were measured turbidimetrically from DakoCytomation (Ely, Cambs, United Kingdom). Plasma C-reactive protein (CRP) was assayed by enzyme-linked immunosorbent assay with the use of antibodies purchased from DakoCytomation.

Statistical analysis

Data for L:M, the percentage recovery of lactulose and mannitol, and plasma CRP concentration were skewed and were therefore log transformed before statistical analysis. A summary mean was calculated for each variable measured throughout the period of supplementation. The effects of supplement or placebo were analyzed by using analysis of variance, correcting for village, year, sex, and age. Three infants were excluded from the analyses, one died midproject, and 2 had a major change in social circumstance when their parents went to work and live in a newly built tourist hotel adjacent to their village. Valid urine collections were obtained for 423 of 462 (91.5%) intestinal permeability tests performed. Failed tests were usually the result of fecal contamination of urine or detachment of the urine bag. Eight subjects (5 in the placebo group and 3 in the glutamine group) who failed to produce acceptable samples on more than one occasions were omitted from any statistical analysis involving this variable.

A power calculation ($\alpha = 0.05$ and $\beta = 0.80$) was performed to determine the number of subjects required to show a significant response to glutamine. Data from previous observations showed that the mean change in weight for age z-score for 4–10-mo-old Gambian infants during the 5-mo rainy season period was −1.31 ± 0.5. A 33% improvement in WAZ over the 5-mo period of treatment therefore required a cohort of 46 infants. Previous data suggested that an increase in growth performance would be associated with a change in log L:M permeability ratio of 0.5 units with a mean pooled SD of 0.67; 40 infants would be required to see on improvement of this magnitude.

RESULTS

Ninety-three infants were recruited into the study: 46 (20 boys, 26 girls) were randomly allocated to receive the glutamine supplement and 47 (22 boys, 25 girls) to receive placebo. The mean (±SD) age at recruitment was 7.9 ± 2.0 mo in the glutamine infants and 7.8 ± 2.0 mo in the placebo group.

Initial (before supplementation) and final (after supplementation) mean values for growth and intestinal permeability markers in both groups of infants are shown in **Table 1**. No differences between the glutamine and placebo groups were observed in any variable at the start of the investigation, and none were seen at the

TABLE 2
Plasma protein concentrations in Gambian infants in the glutamine and placebo groups at the beginning and end of the supplementation period[1]

	First visit (n = 45)		Last visit (n = 45)	
	Glutamine	Placebo	Glutamine	Placebo
Albumin (g/L)	36.6 ± 0.8[2]	35.4 ± 0.7	34.3 ± 0.9	34.5 ± 0.9
ACT (g/L)	0.33 ± 0.02	0.29 ± 0.02	0.39 ± 0.03	0.39 ± 0.02
IgA (g/L)	0.49 ± 0.03	0.45 ± 0.04	0.73 ± 0.05[3]	0.62 ± 0.04[3]
IgG (g/L)	6.62 ± 0.31	6.78 ± 0.39	10.48 ± 0.34[3]	10.51 ± 0.38[3]
IgM (g/L)	0.91 ± 0.05	0.87 ± 0.06	0.98 ± 0.07	0.96 ± 0.06
CRP (mg/L)[4]	3.45 (2.28, 5.36)	2.62 (1.76, 3.46)	1.70 (1.01, 2.85)	2.16 (1.33, 2.85)

[1] ACT, α_1-antichymotrypsin; Ig, immunoglobulin; CRP, C-reactive protein. Statistical analysis was by ANOVA, with adjustment for village, sex, and year. There were no significant differences between the glutamine and placebo groups at the beginning or the end of the supplementation period and no significant time-by-treatment interactions (repeated-measures ANOVA).
[2] $\bar{x}$ ± SE (all such values).
[3] Significantly different from first visit, $P < 0.001$.
[4] Values are geometric $\bar{x}$; 95% CIs in parentheses.

end of the supplementation period. Mean (±SE) growth in both the weight and length of the infants was poor: 60 ± 19 g and 69 ± 20 g/mo in the glutamine group and 1.01 ± 0.05 and 0.95 ± 0.03 cm/mo for the placebo group, respectively, but growth rates did not differ significantly between the groups. This poor growth was reflected by marked reductions in WAZ, HAZ, and MUAC during the supplementation period; again, there were no significant differences between the glutamine and placebo groups. L:M intestinal permeability ratios did not differ between infant groups at the onset of the study or at the end of the supplementation period, nor were there differences between initial and final values (Table 1). Similarly, no significant differences were observed in mannitol and lactulose recovery values

None of the plasma markers showed significant differences between the groups either at the start or the end of supplementation (**Table 2**). The concentration of IgA and IgG increased during the study ($P < 0.001$), but plasma albumin, IgM, ACT, and CRP values showed no change. In both groups, plasma CRP concentrations were often elevated, which indicated the frequent presence of an active inflammatory process. The accepted upper limit of normality for this acute phase protein is 5 mg/L. At the first blood collection, 37 of 90 infants had values above this limit; at the second collection, 32 of 89 infants had values above this limit; and at the third collection, 27 of 90 had values above this limit. Five infants had persistently high CRP concentrations, whereas 30 infants never had a CRP value >5 mg/L. Glutamine supplementation, however, did not have any effect on the numbers of children with elevated plasma CRP.

Mean (±SE) WAZ and HAZ scores of the 2 groups of infants for both the period of supplementation and for the 6-mo period after supplementation are shown in **Figure 1** and **Figure 2**. There were no significant differences between the groups in HAZ (−1.24 ± 0.14 and −1.33 ± 0.14) or WAZ (−1.70 ± 0.20 and 1.69 ± 0.18) in the glutamine and placebo groups, respectively, at the start of the supplementation period, and both z scores decreased in both groups of infants during the supplementation period ($P < 0.001$ and $P = 0.03$, respectively). Z scores continued to decrease after supplementation, and were lowest in November when the mean WAZ was −2.91 ± 0.19 and −2.74 ± 0.18 and the mean HAZ was −2.04 ± 0.12 and −2.08 ± 0.17 in the glutamine and placebo groups, respectively. There was some improvement in WAZ ($P = 0.008$) but not in HAZ during the period December to April; however, neither group achieved their starting values within the period of study. There were no significant differences in the WAZ or HAZ scores between the 2 groups overall or at any time point during the data collection after correction for sex, year, village, and age. Similarly, MUAC did not differ significantly between the groups at the start of the study (13.5 ± 0.2 and 13.5 ± 0.2 cm in the glutamine and placebo infants, respectively) (**Figure 3**). During the supplementation period, MUAC decreased in both groups of infants and was lowest in October ($P = 0.006$); from November onward, values increased in both groups of infants ($P = 0.005$), but at no time were there significant differences between the glutamine and placebo groups.

Monthly variation in intestinal permeability is shown in **Table 3**. At the onset of the supplementation period, L:M ratios were 0.33 (geometric mean) in both the glutamine and placebo groups. During supplementation, repeated-measures ANOVA

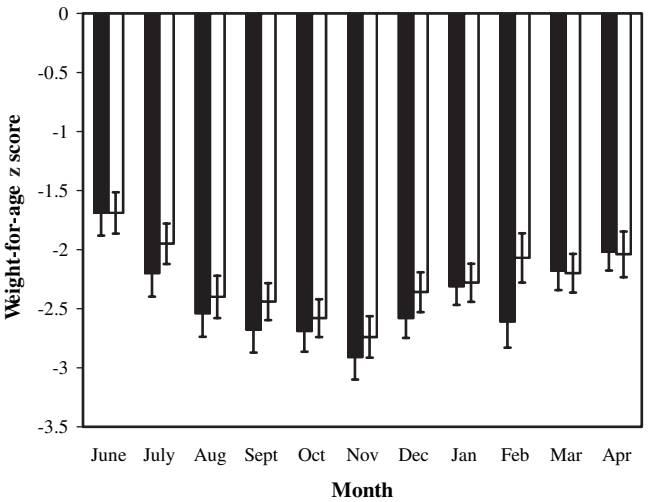

FIGURE 1. Monthly variation in mean (±SE) weight-for-age z scores in glutamine-supplemented (■) and placebo-supplemented (□) Gambian infants (n = 45 for each). ANOVA analysis, after adjustment for village, age, sex, and year, showed no statistically significant differences between the glutamine and placebo groups at the onset of the study, at any time during the study period, or overall. Monthly variation was significant ($P < 0.001$) after adjustment for village, sex, and year.

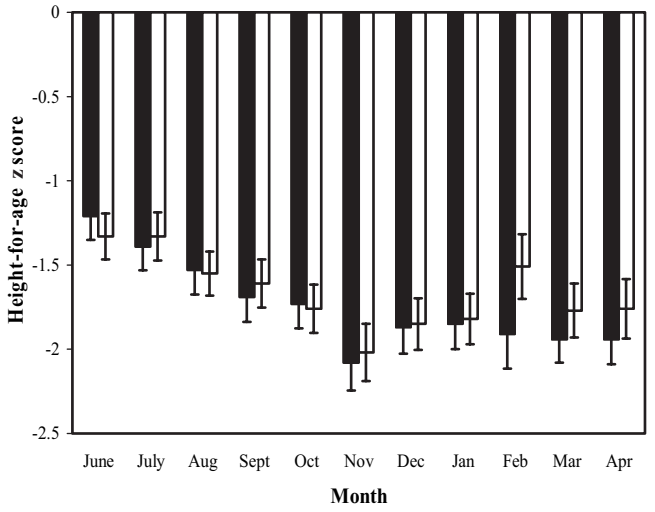

FIGURE 2. Monthly variation in mean (±SE) height-for-age *z* scores in glutamine-supplemented (■) and placebo-supplemented (□) Gambian infants ($n = 45$ for each). ANOVA analysis, after adjustment for village, age, sex, and year, showed no statistically significant differences between the glutamine and placebo groups at the onset of the study, at any time during the study period, or overall. Monthly variation was not significant after adjustment for village, sex, and year.

showed that, overall, glutamine-treated infants had marginally higher permeability values than did the placebo controls ($P = 0.05$). This difference is on the borderline of significance, but the trend was opposite to that expected, ie, the mucosa of the glutamine-treated infants tended to be in a poorer state than that of the children receiving the placebo. An additional correction of the data for plasma concentrations of the acute phase proteins ACT and CRP (as indexes of illness and inflammation) slightly increased the significance of this effect (to $P = 0.035$). Monthly variation in intestinal permeability values, however, showed no

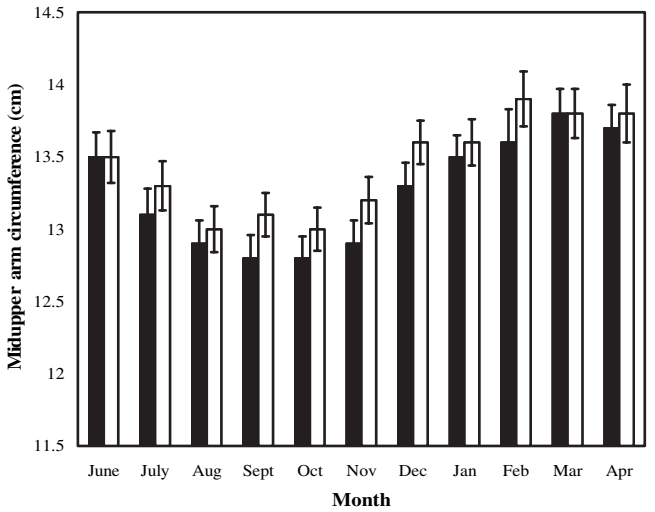

FIGURE 3. Monthly variation in mean (±SE) midupper arm circumference in glutamine-supplemented (■) and placebo-supplemented (□) Gambian infants ($n = 45$ for each). ANOVA analysis, after adjustment for village, age, sex, and year, showed no statistically significant differences between the glutamine and placebo groups at the onset of the study, at any time during the study period, or overall. Monthly variation was significant ($P < 0.001$) after adjustment for village, sex, and year.

TABLE 3
Monthly variation in intestinal permeability data in Gambian infants in the glutamine and placebo groups[1]

	Glutamine ($n = 42$)	Placebo ($n = 40$)
Lactulose:mannitol ratio[2]		
June	0.33 (0.25, 0.43)	0.33 (0.26, 0.41)
July	0.35 (0.29, 0.43)	0.37 (0.30, 0.46)
August	0.39 (0.32, 0.47)	0.28 (0.24, 0.33)
September	0.31 (0.25, 0.38)	0.26 (0.21, 0.32)
October	0.29 (0.23, 0.35)	0.26 (0.21, 0.32)
Recovery of mannitol (%)		
June	2.65 (2.02, 3.48)	2.50 (1.87, 3.36)
July	2.52 (2.09, 3.03)	2.56 (2.08, 3.15)
August	2.07 (1.68, 2.53)	2.16 (1.75, 2.66)
September	2.13 (1.68, 2.71)	2.34 (1.84, 2.97)
October	2.48 (1.99, 3.11)	2.14 (1.62, 2.82)
Recovery of lactulose (%)		
June	0.21 (0.16, 0.28)	0.20 (0.15, 0.26)
July	0.21 (0.17, 0.27)	0.23 (0.17, 0.30)
August	0.19 (0.15, 0.26)	0.15 (0.12, 0.19)
September	0.14 (0.10, 0.18)	0.16 (0.12, 0.21)
October	0.17 (0.13, 0.21)	0.13 (0.11, 0.18)

[1] All values are geometric $\bar{x}$; 95% CIs in parentheses. No significant variation in the recovery variables was observed between groups.

[2] Repeated-measures ANOVA showed that the lactulose:mannitol ratio was higher in glutamine-treated-children ($P = 0.05$) than in those given the placebo, but there was no significant time-by treatment interaction.

significant differences between groups. The percentage recovery of mannitol was very low throughout and showed no variation between the groups. Lactulose recovery was also not different overall between the glutamine-supplemented and placebo infants, nor were there differences at any monthly measurement. No significant effects of time-by-treatment interaction were observed for any of the variables.

Morbidity data are expressed as the mean percentage of time with a particular illness as reported by the mother. The glutamine-supplemented infants had diarrhea 6.2% of the time compared with 7.7% of the time in the placebo group, vomiting 3.6% compared with 3.1%, fever 16.8% compared with 15.7%, cough 14.7% compared with 12.2%, skin infections 7.6% compared with 7.3%, and other 5.4% compared with 4.4%. There was no significant difference between the groups in the percentage of time reported with a particular illness or illness overall.

DISCUSSION

In common with most parts of the developing world, growth faltering during the first 2 y of life is a characteristic feature of Gambian infants. In this country, although growth is poor throughout the year, it is most severely restricted during the rainy season, from July to October (1, 31). This study was initiated before this time of substantial growth faltering, that is at a time when any improvement brought about by the intervention would be most likely to be detected.

A reduction in WAZ, HAZ, and MUAC occurred in both the glutamine- and placebo-supplemented infants and coincided with the duration of the rainy season, the time of year typically associated with increased prevalence of infection overall, particularly diarrhea. During this 5-mo period, the WAZ and HAZ scores decreased by 0.99 and 0.39 units to reach −2.65 and

−1.75, respectively ($P < 0001$ for both), by October. MUAC data behaved in a similar manner. During the ensuing dry season (November to April), these indexes of growth improved, although all remained well below reference standards. However, at no time were there any differences in any growth variable between the glutamine- and placebo-supplemented groups.

Previous studies of Gambian infants have shown that much of their growth faltering can be explained by the presence of a persistent, small intestinal, inflammatory mucosal enteropathy (7). It was postulated that the trophic action of glutamine on enterocytes and its antiinflammatory properties might attenuate the mucosal damage and allow better infant growth. Intestinal permeability measurements in these infants were confirmed as being much higher than those measured in the United Kingdom (32) at the start of the study and remained elevated throughout the study. However, the effect of glutamine supplementation on the L:M permeability test data were not as expected. Overall, infants given glutamine tended to have a higher L:M value, which indicated a more damaged mucosa in the glutamine group than in the placebo group ($P = 0.05$). An additional correction of the data for plasma concentrations of ACT and CRP (as indicators of current infection or inflammation) slightly exaggerated the statistical significance of the detrimental effect of glutamine on intestinal permeability. However, a month-by-month breakdown of the data showed that L:M values did not differ significantly between the groups at any one time. No significant changes in mannitol or lactulose recovery were found, so it is not possible to say whether this change was due to greater villus damage or to barrier function leakiness. Because the worsening of mucosal status was on the borderline of statistical significance, it is possible that this may have been a chance finding. Alternatively, however, it may reflect a true difference between the groups, which would imply that, in certain circumstances, supplementary glutamine could have a detrimental effect on the mucosa.

Low mannitol recovery is considered to reflect a reduced small intestinal absorptive surface and partial villus atrophy (28). However, there was no significant difference in mannitol absorption between the 2 groups, which suggested that glutamine supplementation did not exert its reputed trophic effect on the mucosal architecture of these children. This was despite the finding that <3% of the oral dose of mannitol was recovered in the urine of both groups of infants, which indicated substantial mucosal atrophy and scope for improvement. This finding is similar to the lack of improvement observed with glutamine administration in both adults and children with Crohn disease (33, 34).

There was also no effect of glutamine on the plasma markers of immunostimulation. The plasma concentration of all 3 immunoglobulins increased during the study, probably because of a combination of increased age and increased exposure to pathogens during the rainy season (7). Although mean values were within the wide normal range, plasma IgG, IgA, and IgM concentrations exceeded age-matched reference values by 79%, 76%, and 27%, respectively (35). Plasma CRP concentrations showed evidence of active inflammation (>5 mg/L) in 94 of 263 measurements (36%), which reflected the frequent episodes of illness observed in these children but no change in values occurred with glutamine administration.

Similarly, there was also no evidence of an improvement in the morbidity of the glutamine-supplemented infants; infants were ill for ≈50% of the time in both groups. The proportion of time that infants suffered from the various illnesses detailed in the results was also not significantly different between the 2 groups.

Despite their apparent sensitivity and fluidity in respect of environmental changes, none of the variables measured responded as expected to oral supplementation with glutamine. Given the numerous claims in the literature of a trophic effect on intestinal structure and function and inflammatory activity, it is difficult to understand why this substantial glutamine supplement was without effect on these infants. There is no doubt that these children suffered a persistent mucosal enteropathy and a chronic stimulation of their immune and inflammatory mechanisms by their frequent episodes of illness. The ensuing increased demand for glutamine by the gut and immune cells, coupled with a diet that may be marginal during illness, could be expected to limit glutamine availability within the body. In such circumstances, some improvement in intestinal permeability or in morbidity might be expected with supplementation, even if other restrictions, such as the marginal diet, prevented an improvement in growth.

It is possible that the oral route of glutamine administration may not have been appropriate. For practical reasons, glutamine was given orally twice daily, although many clinical studies that have shown a beneficial effect of glutamine typically provided a constant supply of glutamine using either parenteral or enteral feeding (16). Such an approach was obviously not feasible in the free-living environment in which this study was conducted. However, several human and many animal studies have reported improved gut function and other beneficial effects after oral or dietary enrichment with glutamine (36). The daily dose of glutamine (0.5 mg/kg body weight) may not have been ideal, but it is certainly within the range in which beneficial effects have been reported (16). Glutamine may be a double-edged sword in respect to its action on the immune and inflammatory mechanisms. Although glutamine is reported to reduce the expression of proinflammatory cytokines in the gut (37), the fact that it is a preferred fuel for immune cells gives it the potential to increase gut inflammatory activity (15). Thus, the more abnormal permeability of the mucosa of glutamine-treated infants may be a reflection of greater inflammatory activity caused by more active immune cells. A study by Shinozaki at al (38) showed that glutamine in an experimental model of colitis exacerbated colonic ulcerations compared with animals receiving lower amounts of glutamine or no glutamine. Glutamine has also been shown to increase acute phase protein concentrations, which indicates increased mucosal leakiness, when given to rats with experimental colitis (39). However, until more is known about the mechanisms by which this amino acid elicits its beneficial activities, it is difficult to speculate on why it failed to do so in this trial.

Finally, it is possible that the constant daily exposure to pathogenic organisms brought about by living in unhygienic conditions precludes any improvement short of changing the living conditions of the infants. Although this "blanket" approach to supplementation failed to improve infant health and growth, the question remains whether glutamine would benefit severely malnourished infants in a hospital situation, where enteral feeding would be more practical. A small study from Brazil reported that intestinal permeability in severely malnourished hospitalized children was improved by the addition of glutamine to standard rehabilitation formula, but no effect on catch-up growth or disease status was seen (40). Very-low-birth-weight infants also may benefit from enteral glutamine supplementation; Neu et al

(41) showed reduced morbidity in such infants with a glutamine-supplemented enteral feed. Nevertheless, there is little doubt that the results of this study strongly suggest that mass glutamine supplementation of malnourished infants in developing countries is unlikely to improve their growth or health.

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