

Diarrhea and Reduced Levels of Antiretroviral Drugs: Improvement with Glutamine or Alanyl-Glutamine in a Randomized Controlled Trial in Northeast Brazil

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The effects of therapy with glutamine and alanyl-glutamine on diarrhea and antiretroviral drug levels in patients with acquired immune deficiency syndrome (AIDS) were examined in a randomized, double-blinded, placebo-controlled study in northeast Brazil. Patients with AIDS and with diarrhea and/or wasting were randomized into 4 groups to determine the efficacy of glutamine or high- or low-dose alanyl-glutamine given for 7 days, compared with isonitrogenous glycine given to control subjects. All patients in whom baseline antiretroviral drug levels were determined had low levels 2 h after dosing. Gastrointestinal symptom scores improved with receipt of high-dose alanyl-glutamine ($P < .05$) or glutamine ($P < .01$). Antiretroviral drug levels increased in patients given alanyl-glutamine ($P = .02$) or glutamine ($P = .03$) by 113% ($P = .02$) and 14% ($P = .01$), respectively. Antiretroviral drug resistance mutations were common in all groups. The dose-related efficacy of alanyl-glutamine and glutamine in treating diarrhea and in increasing antiretroviral drug levels shows that these supplements may help to improve therapy for patients with AIDS who have diarrhea and/or wasting in developing, tropical areas.

Persistent diarrhea and wasting are predominant manifestations of HIV infection in developing areas [1, 2]. Both intestinal infections and HIV enteropathy cause malabsorption, histologically seen as villus blunting and atrophy [3]. We have previously documented antiretroviral (ARV) drug malabsorption in patients with chronic diarrhea and wasting [4]. This malabsorption also leads to progressive deterioration in nutritional status [5]. Individuals with AIDS, chronic diarrhea, and wasting have a worse prognosis than do individ-

uals living in the same conditions with the same CD4 cell counts who do not have diarrhea [6–9].

Glutamine (Gln) is the primary energy source for the enterocyte [10, 11]. It preserves intestinal mucosal structure, boosts immune function, and offsets deleterious changes in gut permeability in patients receiving total parenteral nutrition [12, 13]. Gln is poorly soluble and is unstable in solution. Alternatively, the dipeptide alanyl-Gln (Ala-Gln) has been shown to be well tolerated and safe [14]. In animal studies and in tissue culture studies, both Gln and Ala-Gln have been shown to speed injury repair [15, 16] (R.L.G. et al., unpublished data), and both Gln and Ala-Gln drive sodium absorption better than does glucose [17, 18]. We therefore examined whether oral Gln therapy or therapy with its stable derivative Ala-Gln increases ARV drug absorption in patients with AIDS.

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PATIENTS, MATERIALS, AND METHODS

Patients with HIV infection who were receiving therapy and who presented to the Hospital São Jose (Ceará, Brazil) during the period from March 2001 through April 2002 with diarrhea (defined as ≥ 3 stools per day for ≥ 14 days) and wasting (defined as the loss of $\geq 10\%$ of body weight) in the absence of active tuberculosis, disseminated fungal infection, or malignancy were invited to participate in the study. Patients with renal insufficiency (defined as a creatinine level of >2.5 mg/dL), patients with hepatic dysfunction, and patients who were unable to give informed consent or to receive oral therapy were excluded from the study.

After informed consent was received, patients were assigned to 1 of 4 equinitrogenous blinded study groups (figure 1). The control group received 46 g of oral glycine per day (Spectrum). It has previously been shown that the addition of glycine to a therapeutic regimen has no added benefit in treating diarrhea [19]. The second group (the Gln group), received 30 g of oral Gln (Cambridge Neutraceuticals) and 15 g of glycine per day. The third group (the low-dose Ala-Gln group) received 4 g of Ala-Gln (Degussa/Creanova) and 42 g of glycine per day. The fourth group (the high-dose Ala-Gln group) received 44 g of Ala-Gln per day. The 44 g Ala-Gln dose was calculated to be equimolar to 30 g of Gln. Glycine was added to the regimens of the Gln and low-dose Ala-Gln groups to equalize the amount of nitrogen received by all participants.

Once enrolled, patients received 2 days of observed ARV drug therapy. On day 3 of therapy, blood samples were obtained and used for viral genotyping and to determine 2-h drug levels

(i.e., drug levels for each of the ARV drugs 2 h after dosing). Urine samples were also obtained to determine lactulose and mannitol excretion levels for intestinal permeability studies. Blood and urine samples were obtained again in an identical fashion after 7 days of concomitant administration of the study drug and ARV drug treatment. ARV 2-h drug levels were obtained from matched control patients who were receiving ARV drugs but who did not have diarrhea or wasting.

Stool samples were examined for ova and parasites, and cultures for *Salmonella*, *Shigella*, and *Campylobacter* species were performed. Specific tests for *Escherichia coli* and *Clostridium difficile*, which are not widely available for patient care, were not used in this study. Treatment was provided for patients who had treatable pathogens.

Patients were asked daily about the frequency and consistency of their diarrhea and about other gastrointestinal complaints, including anorexia, vomiting, and abdominal cramping. Changes in the frequency of diarrhea was scored as follows: 0 if the total number of stools in the second half of the 9-day study was <2 fewer (or greater) than the number in the first half of the study (including the 2 days before study treatment was started); +1 or -1 if stool frequencies were reduced by 2-3 (i.e., if the diarrhea improved) or increased by 2-3, respectively; and +2 or -2 if the frequencies were reduced or increased by >3 , respectively. In addition, consistencies were graded as liquid, soft, or formed and were scored as 0, 1, or 2, respectively. Scores for changes in consistency were added to the frequency scores (table 1).

Intestinal permeability was measured by urinary excretion

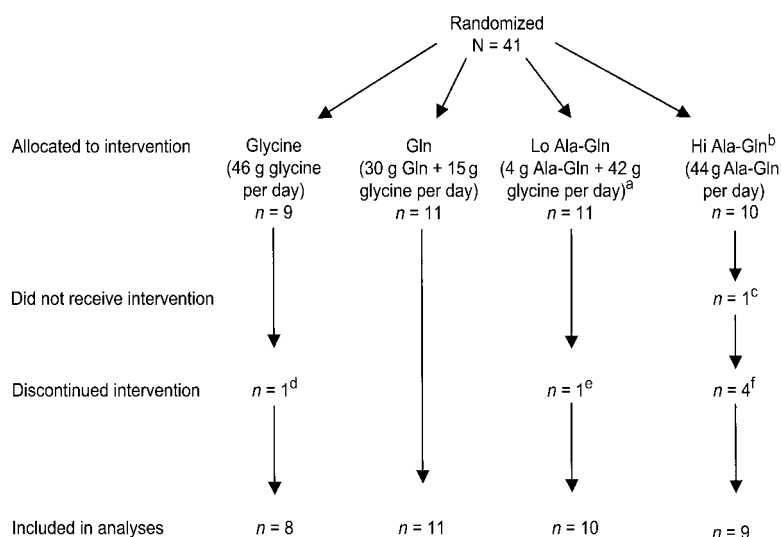


Figure 1. Participant flow in a study of diarrhea and reduced antiretroviral drug levels in patients with AIDS and wasting that examined improvements associated with receipt of glutamine (Gln) or alanyl-Gln (Ala-Gln) therapy. ^aGlycine was added to Gln and Ala-Gln to equalize the amount of nitrogen given. ^bThe high dose of Ala-Gln was calculated to be equi-molar to 30 g of Gln per day. ^cPatient did not tolerate study drug and experienced nausea and vomiting. ^dPatient died of apparent pulmonary embolism within 2 days after starting therapy with the study drug. ^ePatient refused blood draw. ^fFour patients experienced improvement and were discharged from the hospital before the study was completed. Hi, high dose; Lo, low dose.

Table 1. Clinical response of patients with AIDS and diarrhea or wasting to 7 days of therapy with glycine, glutamine (Gln), low-dose alanyl-Gln (Ala-Gln), or high-dose Ala-Gln, by frequency of diarrhea and consistency of stool.

Drug (no. of patients), score	No. of patients, by score					
	-2	-1	0	+1	+2	+3
Glycine (8)						
Consistency	2	2	1	1	2	...
Frequency	1	3	1	1	1	1
Gln (11)						
Consistency	5	3	3	...
Frequency	4	5	2
Low-dose Ala-Gln (10)						
Consistency	...	2	5	2	1	...
Frequency	3	5	2	...
High-dose Ala-Gln (5) ^a						
Consistency	1	1	3	...
Frequency	1	1	1	2

NOTE. For scoring, see Patients, Materials, and Methods.

^a Four additional patients in this group left the hospital on days 3–5 of therapy because of improvements in their conditions.

of lactulose and mannitol and by the ratio of lactulose to mannitol levels, as previously described [20]. Lactulose and mannitol levels in urine samples were determined by high-performance liquid chromatography at Federal University of Ceará, (Fortaleza, Brazil) and at Great Smokies Diagnostic Laboratory (Asheville, NC).

ARV drug levels were determined at Tibotec-Virco (Mechelen, Belgium). Two-hour drug levels were determined for each drug, and drug level ranges supplied by the manufacturer were used to define low levels. Viral genotype was determined by DNA sequencing at Visible Genetics (Suwanee, GA). All genotypes were identified as clade B subtypes by their reverse transcriptase and protease regions.

Two-tailed statistical analyses for comparison of paired values included Student's *t* test and analysis of variance. They are expressed as mean \pm SD. The χ^2 test or Fisher's exact test were used to compare proportions. Human investigation committees at the University of Virginia and the Federal University of Ceará, Fortaleza, Brazil approved this study.

RESULTS

Forty-one patients, 29% of whom were women, (age, 23–52 years; median age, 36 years) were enrolled in the study and randomized as shown in table 2. Seven patients did not complete the study (figure 1); 1 patient left before starting treatment with the study drug (high-dose Ala-Gln) because he declined to have blood drawn. One patient, assigned to receive low-dose Ala-Gln, died of an apparent pulmonary embolism within 2

days after initiating treatment with the study drug. Only 1 patient did not tolerate a study drug (glycine); all others seemed to tolerate the study drugs well. Four patients were discharged from the hospital or left the hospital improved before the study period was completed; all 4 were in the high-dose Ala-Gln group.

Cultures were negative for *Salmonella*, *Shigella*, and *Campylobacter* species for all patients. The 4 patients with helminths and 1 patient with *Isospora* species were treated; otherwise, no antibiotics were used.

ARV drug levels were determined for 33 study patients with diarrhea and wasting. Regimens included zidovudine (AZT) and lamivudine (3TC) or stavudine (d4T) and didanosine (ddI) with either a nonnucleoside reverse-transcriptase inhibitor (NNRTI; either efavirenz or nevirapine) or a protease inhibitor (PI; in most cases, nelfinavir). Compared with patients without diarrhea or wasting, study patients had significantly lower ARV drug levels of ddI (mean \pm SD, 397 \pm 151 ng/mL [*n* = 12] vs. 737 \pm 226 ng/mL [*n* = 3]; *P* < .007) and 3TC (mean, 1503 ng/mL [*n* = 18] vs. 2813 ng/mL [*n* = 9]; *P* = .02). All patients who were receiving ddI (12 patients), 3TC (18), ritonavir (3), saquinavir (1), and 12 (85%) of the 14 patients receiving efavirenz had drug levels below the expected range. Four (44%) of 9 patients receiving nelfinavir and 3 (20%) of 15 patients receiving stavudine also had lower than expected 2-h drug levels.

Improved clinical symptom scores with Gln/Ala-Gln.

Clinical responses among the 38 evaluable patients are summarized in table 1. Eight (89%) of 9 patients in the high-dose Ala-Gln group improved, compared with 3 (38%) of 8 control subjects (*P* < .05). Twenty-six (87%) of 30 patients receiving any study drug improved (*P* < .01 vs. control subjects receiving glycine), and 19 (95%) of 20 patients receiving Gln or high-dose Ala-Gln improved (*P* < .003 vs. control subjects receiving glycine).

Improved ARV drug absorption with Gln/Ala-Gln.

Nine (75%) of 12 2-h paired nucleoside reverse-transcriptase inhibitor (NRTI) levels increased in patients given high-dose Ala-Gln (*P* = .009); 9 (64.3%) of 14 drug levels increased in patients in the Gln group (*P* = .028) and 11 (55%) of 20 levels increased in patients in the low-dose Ala-Gln group (*P* = .056), compared with 2 (15.4%) of 13 levels in patients in the glycine control group (figure 2A). Among patients receiving Gln or either dose of Ala-Gln, 29 (63%) of 46 paired 2-h drug levels increased (*P* = .006 vs. patients in the glycine control group). Similar significant increases with high-dose Ala-Gln (*P* = .02) and Gln (*P* = .03) were seen when data for all ARV drugs (including 17 NNRTI and 14 PI levels) were combined. Figure 2B shows the mean percentage increases in ARV drug levels. The greatest increase occurred with high-dose Ala-Gln (113%; *P* = .02), the second-greatest increase occurred with

Table 2. Baseline demographic and clinical characteristics of patients with AIDS and diarrhea or wasting enrolled in a randomized controlled trial of the effects of therapy with glutamine (Gln) or alanyl-Gln (Ala-Gln) on antiretroviral drug levels, by randomized groups.

Characteristic	All patients	Gly group	Gln group	Low-dose Ala-Gln group	High-dose Ala-Gln group
Age, mean years \pm SD	36 \pm 6	36 \pm 6	38 \pm 6	36.6 \pm 6	36.9 \pm 3
Weight, mean kg \pm SD	47.9 \pm 9.54	46 \pm 9.23	54 \pm 11.4	44 \pm 7.88	46 \pm 7.5
Glutamine level					
Before treatment, mean μ mol/L \pm SD	23.16 \pm 10.4	23.04 \pm 11.48	20.53 \pm 9.91	24.16 \pm 12.07	23.96 \pm 9.23
After treatment, mean μ mol/L \pm SD	23.29 \pm 12.61	22.36 \pm 10.13	21.83 \pm 12.84	20.88 \pm 14.93	26.12 \pm 7.59
Mean increase, μ mol/L	0.13	-0.68	1.29	-3.29	2.17
Stool sample culture, no. or no. (%) of patients positive					
Lactoferrin	24 (59)	6	8	3	7
<i>Cryptosporidium</i> species	4 (10)	1	3
<i>Strongyloid stercoralis</i>	3	1	2
<i>Trichuris trichiura</i>	1	...	1
<i>Isospora belli</i>	1	...	1
Plasma HIV RNA level, mean copies/mL	...	101,000	485,000	400,000	270,000
CD4 cell count					
Mean cells/mm ³ \pm SD	98 \pm 121	97 \pm 131	123 \pm 175	86 \pm 68	84 \pm 101
Median cells/mm ³ (IQR)	48 (20-111)	48 (32-87)	100 (17-109)	66 (33-135)	27 (19-117)
<50 cells/mm ³ , no. of patients	14	4	3	3	4
50-200 cells/mm ³ , no. of patients	10	2	3	3	2
>200 cells/mm ³ , no. of patients	3	1	1	...	1

NOTE. The glycine (Gly) group served as the control group. IQR, interquartile range.

Gln (14%; $P = .01$), and the third-greatest increase occurred with low-dose Ala-Gln (8%; $P = .06$, compared with the glycine control group, which experienced a mean decrease of 32%). Taken together, all 3 treatment groups had a mean increase in ARV drug levels of 45% ($P = .02$ vs. the glycine control group).

Eighteen patients received 3TC. The drug levels of 10 (56%) of the patients increased with therapy; 6 were receiving Ala-Gln (4 in the low-dose group and 2 in the high-dose group), 2 were receiving Gln, and 2 were receiving glycine. Of the 8 patients whose 3TC levels did not increase, 3 were receiving low-dose Ala-Gln, 3 were receiving glycine, and 2 were receiving Gln. None of the 8 patients whose 3TC levels failed to increase were in the high-dose Ala-Gln group. Fifteen patients were receiving d4T. Seven (47%) had an increase in their drug levels. Six were receiving Ala-Gln (3 in the high-dose and 3 in the low-dose group), and the remaining patient received Gln. Of the 8 patients whose d4T levels did not increase, 3 were receiving Gln, 2 were receiving low-dose Ala-Gln, 1 was receiving high-dose Ala-Gln, and 2 were receiving glycine. Among the 14 patients who were receiving AZT, 6 (43%) had increased drug levels with therapy, and all were in the Gln and Ala-Gln groups ($P = .07$). Of the 8 patients who did not improve, one-half were in the control group, 3 were receiving low-dose Ala-Gln, and 1 was receiving high-dose Ala-Gln.

Increased intestinal absorptive capacity with Gln/Ala-Gln.

Intestinal permeability studies show graded trends for the percentage increase in mannitol excretion that match the clinical and drug-absorption responses of the treatment groups (figure 3). The mean percentage increase in mannitol excretion for all treatment groups was 25.4% ($P = .071$ vs. the glycine control group). Lactulose absorption was not affected.

Viral genotyping. HIV genotyping results for 9 of 16 patients showed 65 resistance mutations. Forty-five (69.2%) of these 65 mutations were associated with low levels of a drug to which resistance was conferred. Four patients had viruses with mutations conferring resistance to all 13 of the drugs being administered (46.2% of these drugs being malabsorbed); 3 patients had viruses with mutations conferring resistance to 2 of 3 drugs being given (6 drugs being malabsorbed, resistances to 4 of which were among the 6 resistance mutations); 1 patient had a virus with mutations conferring resistance to 3 of 4 drugs being administered (1 drug being malabsorbed); and 1 patient had a virus with mutations conferring resistance to 1 of 3 drugs being administered. These 9 patients were receiving a total of 29 ARV drugs. Sixteen (55%) of the drugs were being malabsorbed, and 11 (69%) of these were associated with a resistance genotype in these patients. Overall, in the 16 patients whose HIV genotypes were determined, 26 (52%) of the 50 ARV drug levels were low, and 11 (42%) of these 26 ARV drug levels were associated with a resistance genotype.

The most common mutations seen were at codon 184 (in 5

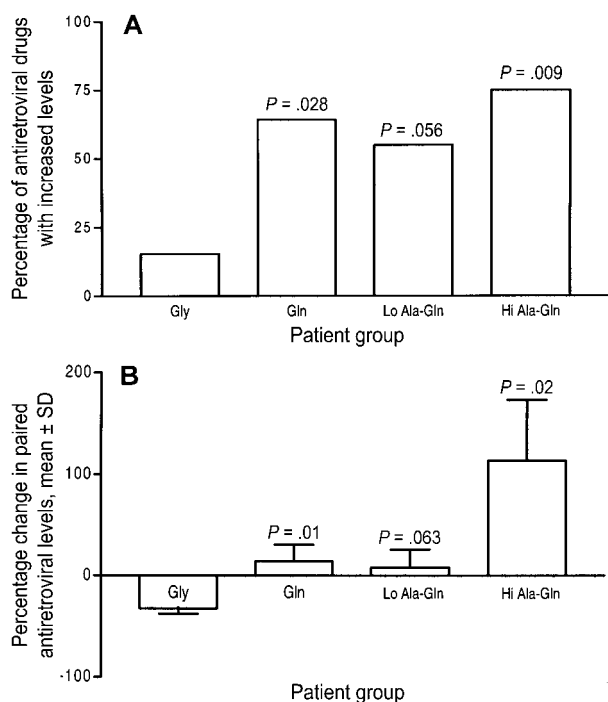


Figure 2. Improvements in the absorption of nucleoside reverse-transcriptase inhibitors (NRTIs) in patients with AIDS and diarrhea and wasting who received 7 days of therapy with glutamine (Gln), low-dose alanyl-Gln (Lo Ala-Gln), or high-dose Ala-Gln (Hi Ala-Gln), compared with control patients who received glycine (Gly). *A*, percentage of antiretroviral drugs with increased levels after 7 days of therapy, by patient group. In the Gly group, 2 of 13 levels increased. In the Gln group, 9 of 14 levels increased. In the Lo Ala-Gln group, 11 of 20 levels increased. In the Hi Ala-Gln group, 9 of 12 levels increased. For all treatment groups, including patients receiving NRTIs, non-NRTIs, or protease inhibitors, the results were as follows (data not shown): 12 (63%) of 19 drug levels increased in the Hi Ala-Gln group ($P = .02$), 12 (57%) of 21 drug levels increased in the Gln group ($P = .03$), and 15 (50%) of 30 drug levels increased in the Lo Ala-Gln group (not significant), compared with increases in 4 (20%) of 20 drug levels in the Gly group. *B*, Mean (\pm SD) percentage change in levels of paired antiretroviral drugs, by patient group. Bars, SD.

patients), conferring resistance to 3TC in all cases. Other NRTI-associated mutations found were M41L (3 patients), D67N (3 patients), K70R (3 patients) and T215F (3 patients). Multi-NRTI resistance with codon 151 mutation complex was noted in 2 patients, and 1 patient had 69 insertion complex. Overall, 6 of 7 patients who were receiving AZT and 3TC had a virus that was resistant to ≥ 1 of the drugs in their treatment regimen; 4 of 6 patients who were receiving NNRTIs had a mutation at codon 103 [21].

DISCUSSION

In Brazil, >60% of all patients with HIV initially present with diarrhea [22, 23], often associated with malabsorption [20]. Our findings that treatment with oral Gln or its more stable

and more soluble derivative Ala-Gln significantly improves diarrheal symptoms as well as ARV drug absorption suggest that specific therapy to increase absorption may provide an important, novel approach to improving ARV drug efficacy, especially in tropical, developing regions.

Gln supplementation has been shown to improve intestinal mucosal structure and function after injury by chemotherapy, radiotherapy [13, 25], or prolonged parenteral nutrition [26]. In addition, it is an effective oral rehydration and nutrition therapy (ORNT) [12, 17, 18, 27]. Consistent with this, significant clinical improvements in the frequency and consistency of stool were noted in the Gln and Ala-Gln groups ($P < .01$ vs. the glycine control group), with the greatest improvements seen in the high-dose Ala-Gln group ($P < .003$, when combined with the data from the Gln group). Although the isonitrogenous amino acid supplements used in this study had different osmolarities, all are rapidly absorbed in the upper small bowel. Therefore, in this study, we feel that these (likely transient) differences in osmolarity are outweighed by the importance of equal components of nitrogen.

Tepper et al. [24] have described impaired mannitol absorption, an indicator of intestinal absorptive area, in patients with AIDS. We have reported a 3-fold increase in the ratio of lactulose level to mannitol level (reflecting intestinal barrier disruption and absorptive capacity, respectively) in HIV-infected patients with diarrhea, compared to ratios in HIV-infected patients without diarrhea. A 10-fold increase in ratios of lactulose to mannitol is seen when patients with AIDS who

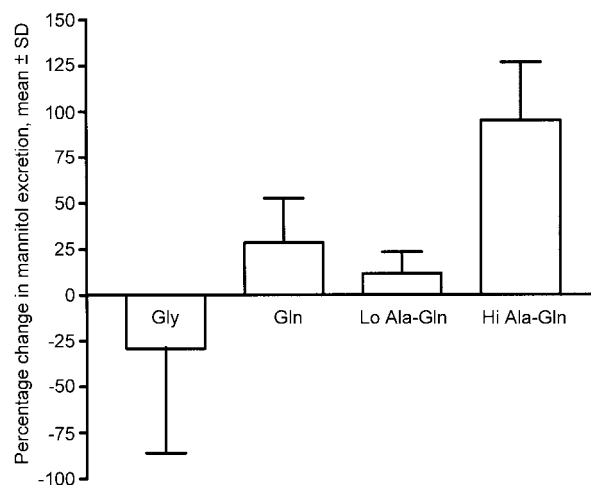


Figure 3. Percentage change (mean \pm SD) in mannitol excretion in patients with AIDS and diarrhea and wasting who received 7 days of therapy with glutamine (Gln), low-dose alanyl-Gln (Lo Ala-Gln), or high-dose Ala-Gln (Hi Ala-Gln), compared with control patients who received glycine (Gly). The percentage change in mannitol excretion is expressed in relation to a mean (\pm SD) mannitol excretion percentage of 4.73 ± 3.18 , based on 26 mannitol excretion values determined before initiation of the study. Bars, SD.

have diarrhea are compared with healthy control subjects, again predominantly due to decreased mannitol absorption [20]. These findings, documenting impaired intestinal function in HIV-positive patients with diarrhea [20, 24], prompted Noyer et al. [31] to study the effect of Gln on intestinal mucosal function. They demonstrated that receipt of 4 g of Gln per day resulted in less worsening of intestinal permeability. At a dosage of 8 g of Gln per day, intestinal permeability stabilized, and mannitol excretion increased, suggesting a dose-response effect.

Consistent with these studies, our study showed that higher doses of Gln and equinigenous doses of Ala-Gln increased mannitol excretion. However, this increase did not reach statistical significance after only 1 week of supplementation. These trends suggest that, during the first week, the primary effect of Gln and Ala-Gln is on rebuilding absorptive surface area, rather than on changes in barrier function.

We measured ARV drug levels 2 h after administration as an indicator of ARV drug absorption because most ARV drugs peak in the plasma at that time. Although 2-h drug levels are a reasonable surrogate for peak levels, trough levels that are affected by clearance and drug adherence before hospitalization could also influence peak drug levels and should be tested in future studies. It is also possible that some patients could have had delayed absorption, with their peak level occurring 4–6 h after dosing.

Low levels of ARV drugs occurred in all 33 patients with diarrhea and wasting, significantly so for those receiving ddI ($P < .007$ vs. the control group) and those receiving 3TC ($P < .02$ vs. the control group). Trends from this and other studies [4] reveal that certain drugs are more prone to malabsorption than others. These low drug levels may drive viral resistance. Several studies have shown that concentration-managed therapy with NRTIs is feasible and is associated with better outcomes [28–30], although the utility of routine therapeutic drug monitoring for this class of drugs is debated.

We found that NRTI levels increased significantly after Gln- or Ala-Gln-based ORNT, whether assessed as the percentage of patients with increased levels or as the mean change in serum drug concentrations. Both assessments showed that the greatest effects were seen with high-dose Ala-Gln (75% of patients had increased NRTI levels; mean increase, 113%), the second-greatest effects were seen with Gln, and the third-greatest effects were seen with low-dose Ala-Gln. Determining whether receipt of Gln or Ala-Gln improves NNRTI or PI absorption requires further study.

We found a high frequency of resistance mutations, including mutations giving resistance to ARV drugs that were being malabsorbed. This may be related to prior experience with ARV drugs, poor adherence to therapy, or advanced HIV disease, which we have not addressed. The potential importance of the malabsorption of ARV drugs and its relation to the develop-

ment of viral resistance requires further study. Similarly, the question of whether Gln- or Ala-Gln-based ORNT prevents or delays the emergence of ARV drug resistance deserves exploration.

This pilot study has several limitations, including the small number of patients enrolled, the subjective measures used in quantifying diarrhea, and the question of optimal timing of measurements for peak ARV drug levels. Nevertheless, significant improvements in symptoms and in ARV drug absorption occurred with supplementation of Gln or its analogue Ala-Gln. Improvements in all of the clinical and laboratory parameters measured were greatest in patients who received 44 g of Ala-Gln per day, second-greatest in those who received 30 g of Gln per day, and third-greatest in those who received 4 g of Ala-Gln per day. All patients in the Gln analogue groups had better results than did those in the control group, who received 46 g of glycine per day. The dose-related effect of Ala-Gln and its apparent superiority to other agents, in addition to its greater stability and solubility and its superior ability to drive water and electrolyte absorption, demonstrate its promise as a therapy for improving diarrhea and malabsorption. These improvements may enhance ARV drug therapy and reduce the emergence of drug resistance in patients with HIV/AIDS in tropical, developing areas.

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Conflict of interest. R.L.G. is a cofounder of Al Glutamine, which licensed his United States patent (patent 5,561,111) on the use of Ala-Gln as a novel oral rehydration and nutrition therapy. All other authors: No conflict.

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