

The Pharmacokinetic Responses of Humans to 20 g of Alanyl-Glutamine Dipeptide Differ with the Dosing Protocol but Not with Gastric Acidity or in Patients with Acute Dengue Fever

Petra Klassen,¹ Manolo Mazariegos,* Noel W. Solomons* and Peter Fürst

*Institute for Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany and the *Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala*

ABSTRACT Pharmacokinetic responses to oral doses of the dipeptide, L-alanyl-glutamine (Ala-Gln), were evaluated after a single, bolus load or an intermittent dosing in normal healthy subjects ($n = 8$) to find the optimal mode of oral administration. In a subgroup ($n = 4$) of the healthy subjects, the influence of a gastric acid suppressor (Omeprazole) was investigated. The influence of an acute episode of classic Dengue fever was examined in eight patients. All modes of administration to healthy subjects significantly increased free plasma Gln and alanine concentrations. Peak increments of plasma Gln concentration were $794 \pm 107 \mu\text{mol/L}$ (mean \pm SEM) after bolus intake of 20 g of Ala-Gln and $398 \pm 61 \mu\text{mol/L}$ after intermittent intake of the same cumulative dosage of the dipeptide ($P < 0.01$). After intermittent dosing, the maximum peak increase appeared significantly later ($P < 0.01$). Areas under the curve (AUC), expressing the integrated responses over time of plasma free Gln and alanine concentrations, did not differ after bolus and intermittent loads of Ala-Gln. Pretreatment with the acid suppressor, Omeprazole, did not influence Gln ($P = 0.79$) or alanine ($P = 0.90$) plasma increment. Dengue patients manifested the same pharmacokinetic responses to a 20 g Ala-Gln bolus as healthy controls. In general, on a micromolar concentration basis, Gln and alanine followed parallel tracks in terms of plasma appearance, clearance and elimination after the oral administration of 20 g of the Ala-Gln dipeptide through the range of conditions and dosing protocols explored here. *J. Nutr.* 130: 177–182, 2000.

KEY WORDS: • glutamine • alanine • humans • pharmacokinetics • oral administration

Glutamine (Gln) is the most abundant free amino acid in the human body, constituting $> 60\%$ of the muscle free amino acid pool (Bergström et al. 1974). Together with alanine (Ala), it accounts for $\sim 80\%$ of the amino acids released from skeletal muscle (Felig 1975). Glutamine plays a major role in various metabolic pathways and is essential to normal immune responses (Calder 1994 and 1995, Newsholme et al. 1988). There is a growing body of evidence that Gln, but not glutamate, is easily transported across the cell membrane, hence serving as the glutamate precursor in glutathione synthesis (Cao et al. 1998, Hong et al. 1992, Welbourne et al. 1993).

Hypercatabolic and hypermetabolic situations are accompanied by marked depressions in muscle intracellular Gln and Ala (Jepson et al. 1988, MacLennan et al. 1988). It has thus been postulated that Gln supplements might be beneficial in the treatment of stressed and malnourished patients (Fürst 1998). The constraints in administering free Gln, at least parenterally, are its limited solubility of $\sim 3 \text{ g}/100 \text{ mL}$ (Stehle and Fürst 1995), which leads to the need for large fluid volumes to administer the requisite amounts of Gln, and its instability in aqueous solutions (Fürst et al. 1990a). This problem can be overcome by the use of synthetic, stable,

highly soluble (568 g/L) Gln-containing dipeptides (Stehle and Fürst 1995). They are rapidly cleared from the circulation without accumulation in tissues and with inconsequential losses in urine (Albers et al. 1988 and 1989). Enteral (oral) utilization of Gln or Gln-containing dipeptides is a controversial issue at present. On the one hand, there are numerous negative reports related to nitrogen utilization, protein synthesis and plasma Gln concentrations (Jebb et al. 1994, Long et al. 1995). On the other hand, beneficial effects have been claimed after administration of high doses of free Gln to victims of multiple trauma (Houdijk et al. 1998).

A proper evaluation of the efficacy of Gln requires knowledge of its pharmacokinetics from the various alternative dosing forms and protocols. About 50% of orally administered Gln is extracted by the splanchnic bed in healthy humans (Hankard et al. 1996, Matthews et al. 1993, Ziegler et al. 1990). Moreover, the increase in circulating concentrations of free Gln after varying oral loads is dose related (Ziegler et al. 1990).

In this work, we present the pharmacokinetic response to bolus and intermittent oral doses of the Gln dipeptide, L-alanyl-glutamine (Ala-Gln), in healthy adults, living in sanitary environments in Guatemala City, as the standard for the pharmacokinetic response. A fixed amount of 20 g of L-Ala-Gln, containing $\sim 13 \text{ g}$ Gln, was administered both as a bolus and intermittently. This is approximately half the amount of

¹ To whom correspondence should be addressed.

² Abbreviations used: Ala-Gln, L-alanyl-glutamine; AUC, area under the curve; Cl, total clearance; $t_{1/2}$, terminal half-life time; Vd, volume of distribution.

Gln that was recently estimated to be needed as an exogenous supplement in disease (Silk 1999). Bolus dosing is a model for a conventional regimen of episodic oral doses, and intermittent dosing was chosen to mimic an enteral-drip delivery. In Guatemala and many other developing countries, gastritis is an endemic problem; thus the use of antacids and acid-secretion blockers is rising. Moreover, the spreading endemicity of *Helicobacter pylori* (Castro and Coelho 1998) may produce premature gastric atrophy in large segments of developing societies. For both reasons, it was of interest to determine whether induced hypochlorhydria influenced the response to the Ala-Gln dipeptide. Because amino acids are electronically charged molecules when dissociated in solution, the intraluminal transit, which is dependent on pH, may be influenced by the pH change induced by the antacid. This, in turn, may have an effect on pharmacokinetic variables such as maximum peak height or time of appearance of the maximum peak height. Most of the recent studies in humans have been done in patients suffering from severe illness. Our interest, however, was to evaluate the pharmacokinetic characteristics in subcritical acute disease, and specifically in tropical febrile disease. Therefore, we assessed the pharmacokinetics of the dipeptide in patients with classical Dengue fever.

SUBJECTS AND METHODS

Materials. Solutions of Ala-Gln (Degussa, Coubevoie, France) were prepared <24 h before administration, at 20 g/20 mL purified water for bolus and 4 g/20 mL purified water for intermittent administration, and stored at 4°C until used. The dipeptide is highly soluble in water (568 g/L H₂O at 20°C) (Fürst and Stehle 1994).

Healthy subjects and patients. Eight normal volunteers, aged 22–36 y, were recruited in Guatemala City among the staff and students affiliated with CeSSIAM (Table 1). They were healthy, and without known metabolic or gastrointestinal diseases. All were free of gastroenteritis episodes within 3 wk of the study. None were taking antibiotics, antacids or gastric acid-suppressive medications.

Eight patients in the age range from 16 to 54 y (Table 1), suffering from classical Dengue fever, were recruited in the public hospital in Tiquisate, Escuintla Province, on the tropical, coastal plain of Guatemala where the vectors, *Aedes aegypti* and *Aedes albopictus* mosquitoes, are endemic. Patients with a maximum duration of 5 d of the disease were eligible. Dengue fever was diagnosed on the basis of clinical symptoms, including high fever (>40°C), severe frontal headache, pain behind the eyes, muscle and joint pain, nausea and vomiting. The absence of a malarial parasitemia on a thick blood smear strongly suggested a diagnosis of Dengue. Dengue infection was confirmed by one or both of virus isolation using a CC-36 cell culture line of *Aedes albopictus* cells and serological testing by detecting immunoglobulin M using a capture ELISA. These determinations were made in the laboratories of the Division on Malaria and Vector Diseases of the Ministry of Health of the government of Guatemala within 24 h after blood drawing. The clinical information obtained from each patient was sent with the blood specimen as part of the surveillance system in Guatemala.

All subjects gave their informed consent after receiving an explana-

tion of the nature, purpose, inconveniences and discomforts. The survey was approved by the Human Subjects Committee of CeSSIAM.

Experimental design. The individual regimen of Ala-Gln administration consisted of three protocols (A, B and C). Protocol A was designed to determine how oral bolus or intermittent doses of Ala-Gln, administered on two different study days to eight healthy subjects, would influence plasma Gln and Ala concentrations compared with their baseline concentrations. The baseline concentrations were obtained on a third study day from seven of the eight healthy volunteers (one subject did not appear on the study day when baseline concentrations were determined). After an overnight fast, a basal 2-mL blood sample was drawn at 0800 h for measurement of baseline plasma concentrations of Gln and Ala. Additional, identical blood samples of 2 mL each were drawn every 30 min during the study period of 3 or 5 h, yielding a total of 7 and 11 samples per subject per day, respectively, after the bolus or intermittent doses. Blood samples were drawn using an indwelling scalp-vein needle and cannula system, which remained in the vein until the end of the absorption study. The system was kept open between blood collections with sterile heparin solution. The blood samples were transferred from a syringe into EDTA-containing vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), immediately placed on ice and subsequently centrifuged at 2800 × g for 7 min to obtain plasma. The plasma was separated into screw-cap plastic vials and stored at -40°C. Sampling and timing of the experiments are depicted in the figures, illustrating Gln and Ala concentration changes after bolus and intermittent administration of Ala-Gln (Fig. 1B, C). Hematocrit was determined every hour. For packed cell volume (hematocrit), a heparinized capillary was filled with whole blood and centrifuged in a hematocrit microcentrifuge for 5 min at 4192 × g. Hematocrit was measured using an International Microcapillary Reader (International Equipment Company, Boston, MA) and recorded as a percentage of packed red blood cells.

Protocol B was designed to evaluate whether the intake of a gastric acid blocker, (Omeprazole, "Losec," Hoechst Marion Roussel, Sweden) as a model of achlorhydria (Maton 1993), before Ala-Gln administration would influence the absorption of the dipeptide (Maton 1993) and thus alter the pharmacokinetics. Volunteers were asked to take a pretreatment dose of 20 mg of Omeprazole three times, at 24, 12 and 2 h before the scheduled dipeptide intake. Four of the healthy volunteers from protocol A underwent two additional applications (bolus and intermittent) using the Omeprazole regimen. The sampling and sample handling were as described in protocol A.

Protocol C was designed to assess how an oral bolus dose of Ala-Gln influences plasma Gln and Ala concentrations in eight Dengue patients. A further objective was to compare the response to the bolus dose in patients with that in healthy volunteers. The patients were admitted to a hospital in the coastal area of Guatemala during the febrile phase of the disease, and the study was carried out in the morning in fasting subjects. Sampling and sample handling were identical as described in protocol A.

Analytical methods. Free plasma Gln and Ala were determined using an automated on-line reversed-phase HPLC system with precolumn derivatization (*o*-phthaldialdehyde/β-mercapto propionic acid) (Fürst et al. 1990b). The samples were precipitated with 5-sulfosalicylic acid (300 g/L), incubated at 4°C for 1 h and centrifuged; the supernatant was collected in screw-cap cryovials (Greiner, Kremsmünster, Austria) before placement on dry ice. The samples were stored at -40°C until transport to the University of Hohenheim, Stuttgart, Germany, and stored at -80°C until analyzed.

Pharmacokinetic evaluation. For each subject, pharmacokinetic variables, such as areas under the concentration-time curve (AUC),³ terminal half-life time (*t*_{1/2}), total clearance (Cl) and volume of distribution (Vd) were calculated from the individual plasma concentration vs. time profiles according to a noncompartmental model, using the computer program TopFit (Heinzel et al. 1993). Areas under the concentration vs. time curves were calculated according to the linear trapezoid method.

Statistical analysis. All results are expressed as means ± SEM. The SPSS program (SPSS, Chicago, IL) was used for statistical analyses. To compare the distinct response variables (AUC, peak rise, time of peak increment and total clearance) for main effects of bolus

TABLE 1

Characteristics of the study population¹

	Healthy subjects	Dengue fever patients
Age, y	27 ± 6 (25)	30 ± 14 (25)
Height, cm	—	162 ± 10 (160)
Weight, kg	75 ± 11 (75)	57 ± 7 ² (55)

¹ Values are means ± SEM and (medians), *n* = 8.

² Significantly different from controls, *P* < 0.05.

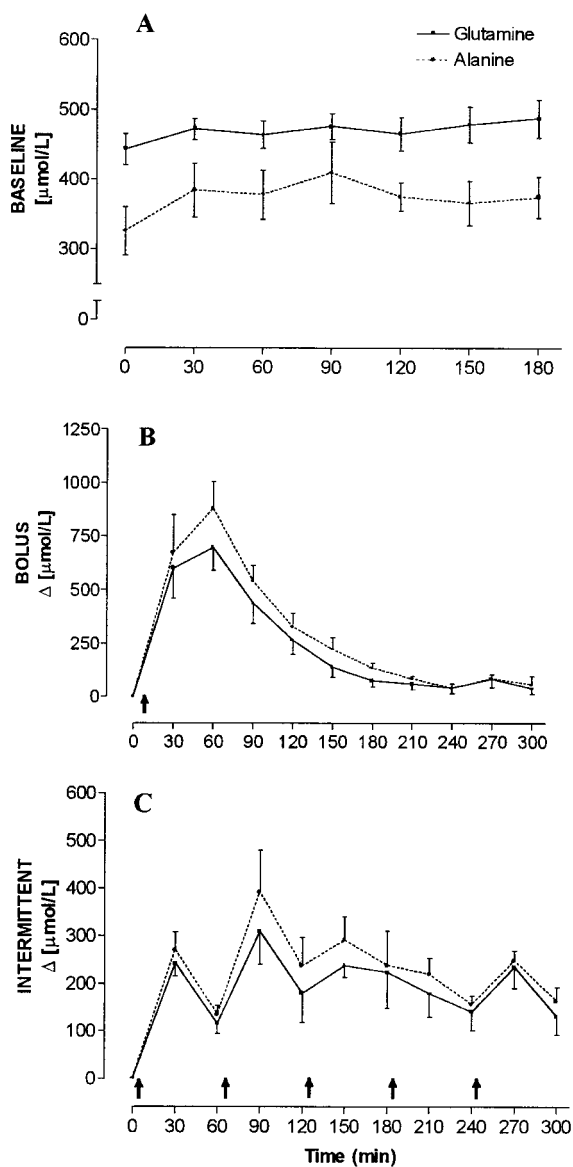


FIGURE 1 Composite curves of Gln and Ala in plasma of healthy humans after single, bolus or intermittent dosing. (A) Composite curves of serial changes in the circulating concentration of Gln and Ala ($n = 7$); (B) composite curves of the mean changes in Gln and Ala with respect to individual, respective baseline concentrations after a bolus dose of 20 g of Ala-Gln ($n = 8$); and (C) composite curves of mean changes in the index amino acids after intermittent dosing of a cumulative dose of 20 g of dipeptide ($n = 8$). Values are expressed as means \pm SEM; (\uparrow) denotes the timing of administration of oral Ala-Gln doses.

or intermittent dosing and the influence of pretreatment with Omeprazole, paired t test was used. To test for differences in pharmacokinetic responses between healthy subjects and Dengue patients, independent t test was performed. Results were considered significant if the P -value was <0.05 .

RESULTS

Protocol A: pharmacokinetics of oral Ala-Gln dipeptide in healthy humans. The baseline concentrations of Gln and Ala are illustrated in Figure 1A. Plasma Gln concentrations were borderline low (Fürst et al. 1990b), whereas Ala concentrations were normal (Divino Filho et al. 1997) and remained essentially unchanged throughout the study. The pharmaco-

kinetic responses to bolus intake are shown in Figure 1B as the increment of Gln and Ala concentrations over the individual baseline levels. The peak rise was calculated as the maximum increase of Gln or Ala plasma concentrations above the basal, individual concentration, measured at the beginning of each study day. The peak increment in Gln concentration in response to the 20-g bolus dose of dipeptide was $794 \pm 107 \mu\text{mol/L}$ (Table 2), representing a 1.4-fold increase above the baseline concentration. The peak increment occurred from 30 to 120 min postdose, with a mean of 49 ± 8 min. Plasma values of Gln and Ala were normal 180 min after dipeptide administration. The average peak rise in Ala concentration was $981 \pm 147 \mu\text{mol/L}$, representing a 2.7-fold increase above baseline concentration. The peak rise occurred between 30 to 150 min postdose, with a mean of 64 ± 13 min. Plasma Ala concentrations returned to baseline values at the completion of the study. In Figure 1C, the composite curves of the individual changes in plasma concentrations of Gln and Ala in response to the intermittent administration are shown. The average peak rise in Gln concentration was $398 \pm 61 \mu\text{mol/L}$ (Table 2), representing a 0.8-fold increase. The peak increment occurred from 30 to 210 min postdose, with a mean of 146 ± 28 min. The average peak rise in Ala concentration in response to the dose was $462 \pm 85 \mu\text{mol/L}$ (Table 2), which is a 1.4-fold increase above baseline values. The maximum peak rise occurred between 30 to 150 min postdose, with a mean of 101 ± 14 min. The results of the pharmacokinetic calculations are shown in Table 3. There was a wide variation in the AUC for both Gln and Ala. After the intermittent administration of Ala-Gln, a steady-state plasma concentration of Gln and Ala was reached within 120 min after the first dose. The average $\text{AUC}\tau$ in the steady-state phase was multiplied by five as a correction for the intermittent dosing. All subjects had normal hematocrits (Lee et al. 1989). Packed cell volume remained stable throughout each study period, indicating that no dehydration or fluid shifts arose during the study.

In statistical comparisons within the same eight individuals, the distinct response variables were compared for the bolus and intermittent-dosing studies using the paired t test. In terms of the AUC, the average response for Gln after the bolus dose ($\text{AUC}_{0 \rightarrow \infty}$) was not significantly different from that following the intermittent dosing ($\text{AUC}\tau \cdot 5$) (Table 3). The average peak rise for individual Gln concentrations for the former was significantly greater than that for the latter ($P = 0.003$). The time from baseline to attainment of the peak increment, as mentioned above, was 49 ± 8 min for the bolus and 146 ± 28 min for the intermittent dose ($P = 0.009$).

For Ala, the accompanying amino acid in the dipeptide, the within-individual differences between the bolus and intermittent administration followed an identical pattern to that seen with Gln with respect to peak rise and time course (data not shown). Areas under the curve ($\text{AUC}_{0 \rightarrow \infty}$) were significantly larger after the bolus dose than in the steady-state phase after intermittent dosing ($\text{AUC}\tau \cdot 5$) ($P < 0.01$, using paired t test).

Protocol B: influence of Omeprazole-induced gastric acid inhibition on the pharmacokinetics of Ala-Gln. The integrated response curves for Gln and Ala were not significantly different after the bolus dose, when subjects received the acid blocker regimen before ingestion of the dipeptide (Fig. 2). Furthermore, no significant differences for peak increment across these two situations of gastric acid secretion were observed when Ala-Gln was administered as intermittent loads. Comparing the responses to bolus and intermittent administration of Ala-Gln after Omeprazole intake, $\text{AUC}_{0 \rightarrow 180}$, peak height and time course were not significantly different (data

TABLE 2

Peak responses of plasma glutamine (Gln) and alanine (Ala) after oral bolus and intermittent L-alanyl-glutamine (Ala-Gln) loading with and without pretreatment with antacid Omeprazole in healthy Guatemalan adults and patients with classical Dengue fever¹

Ala-Gln administration	n	Gln		Ala	
		Baseline, ²	Peak height, ³ Δ	Baseline, ²	Peak height, ³ Δ
		μmol/L			
Healthy ⁴					
Bolus, 20 g	8	548 ± 26	794 ± 107	366 ± 26	981 ± 147
Intermittent, 5 × 4 g	8	530 ± 43	398 ± 61	394 ± 45	462 ± 85
Bolus + Omeprazole	4	435 ± 31	779 ± 106	376 ± 24	954 ± 192
Intermittent + Omeprazole	4	448 ± 31	439 ± 183	313 ± 29	620 ± 239
Dengue ⁵					
Bolus, 20 g	8	520 ± 25	942 ± 177	285 ± 44	923 ± 194

¹ Values are means ± SEM.

² Plasma concentrations before administration of dipeptide on each study day.

³ Maximum plasma Gln/Ala increment over baseline concentration.

⁴ In healthy subjects, comparison of peak heights was performed using paired *t* test for the following matching pairs: Bolus vs. Intermittent (*P* < 0.01 for Gln and Ala); Bolus + Omeprazole vs. Intermittent + Omeprazole (NS); Bolus vs. Bolus + Omeprazole (NS); and Intermittent vs. Intermittent + Omeprazole (NS). NS, not significant, *P* > 0.05.

⁵ Peak heights after bolus dose in Dengue patients were compared with those in healthy subjects (NS).

not shown). There were no differences in Cl, *t*_{1/2} and Vd with or without gastric blocker intake.

Protocol C: influence of Dengue fever on pharmacokinetics of Ala-Gln. Figure 3 depicts the changes in concentrations of the two index amino acids derived from the dipeptide; the individual, averaged AUC for the same analytes are shown in Table 3. The peak increment of Gln was 942 ± 177 μmol/L in Dengue patients compared with 794 ± 107 μmol/L in the group of healthy subjects (Fig. 1A). The time to peak increment of 71 ± 10 min in the Dengue patients tended to be longer than the 49 ± 8 min observed in controls (*P* = 0.09). Pharmacokinetic variables were not different in healthy controls and Dengue patients (data not shown).

The comparison of the behavior of the Ala component of the dipeptide across groups as applied to the same variables also failed to disclose any intergroup differences. The average AUC over 180 min in Dengue patients was not different from the response for the first 180 min in the healthy controls. No differences were seen when comparing the Ala peak increment and time of peak increment. Pharmacokinetic variables were not different between the two groups (data not shown).

DISCUSSION

Ingestion of Ala-Gln was well tolerated by all subjects, and no complaints were recorded. Glutamine is absorbed in the

TABLE 3

Integrated responses over time (AUC), terminal half-life time (*t*_{1/2}), total clearance (Cl) and volume of distribution (Vd) of plasma glutamine (Gln) and alanine (Ala) after oral bolus or intermittent L-alanyl-glutamine (Ala-Gln) loading with or without pretreatment with the antacid, Omeprazole, in healthy Guatemalan adults and patients with classical Dengue fever¹

Ala-Gln administration	n	AUC, μmol · min/L		<i>t</i> _{1/2} , min		Cl, mL/min		Vd, L	
		Gln	Ala	Gln	Ala	Gln	Ala	Gln	Ala
Protocol A: 300 min ²									
Bolus ³	8	73361 ± 9293	89560 ± 10323	28.4 ± 2.8	30.0 ± 2.1	1458 ± 259	1147 ± 164	56.3 ± 7.4	47.4 ± 4.2
Intermittent ⁴	8	58782 ± 10040	180472 ± 12484	—	—	—	—	—	—
Protocol B: 180 min ⁵									
Bolus + Omeprazole ³	4	84595 ± 24714	91958 ± 22514	36.6 ± 9.2	29.5 ± 5.1	1155 ± 320	1212 ± 310	59.6 ± 15.4	45.7 ± 5.5
Protocol C: 180 min ⁶									
Bolus Dengue ³	8	84782 ± 14414	91682 ± 15694	32.9 ± 3.4	33.1 ± 4.4	1374 ± 265	1421 ± 258	70.1 ± 22.8	63.1 ± 13.2

¹ Values are means ± SEM.

² Pharmacokinetic variables of Gln and Ala after bolus dose were compared with those after intermittent dose using paired *t* test. Significant difference was found in AUC for Ala.

³ Values are expressed as AUC_{0→∞}.

⁴ Values are expressed as mean AUC_τ in the steady-state phase × 5, as correction for the intermittent dose of 4 g compared with the 20-g bolus dose; τ = 60 min.

⁵ Pharmacokinetic variables of Gln and Ala after bolus dose were compared with bolus dose and pretreatment with Omeprazole using paired *t* test. All comparisons were not significant.

⁶ Pharmacokinetic variables of healthy subjects (Gln and Ala) after bolus dose were compared with responses in Dengue patients using independent *t* test. All comparisons were not significant.

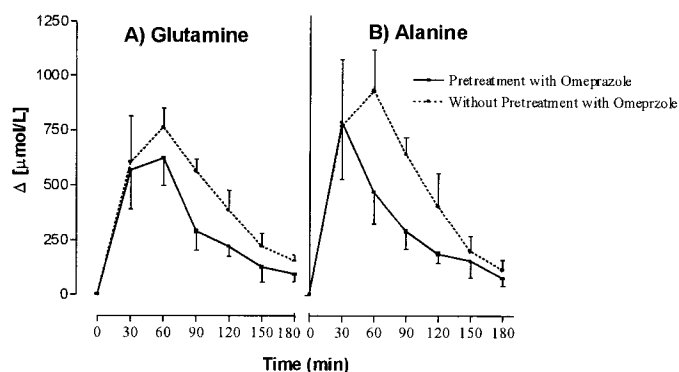


FIGURE 2 The composite mean curves of individual changes with respect to baseline concentrations of plasma Gln (*panel A*) and Ala (*panel B*) after a bolus oral dose of 20 g Ala-Gln dipeptide in the same four healthy volunteers on two occasions as follows: 1) with pretreatment of Omeprazole to reduce acid secretion; and 2) without pretreatment. The variance is expressed as SEM.

intestinal tract after an oral load (Welbourne 1995). The majority of enterally or orally administered Gln is absorbed and subsequently extracted by the splanchnic bed. We observed a significant increase in free plasma Gln. This is in good agreement with previous studies in which the effect of an enteral Gln load was evaluated (Hankard et al. 1995, Welbourne 1995, Ziegler et al. 1990), although there are also studies in which little or no increase of plasma Gln concentrations was observed (Darmaun et al. 1994, Long et al. 1995).

The evaluation of AUC is the usual method to appraise *in vivo* kinetics. Because the major interest in the present evaluation was to study the increment of Gln over time, we disregarded the extracellular Gln pool. Hence, plasma baseline concentrations of Gln and Ala ($t = 0$) on each study day were subtracted from the observed concentrations throughout the study (t_1, t_2, \dots, t_{11}). This means that the average baseline concentrations (Fig. 1A) in the fasting state were not used for this correction to avoid probable effects of fasting (Darmaun 1995) and to allow adjustment for individual, day-to-day variations in plasma baseline levels. Differences in extracellular Gln might be due to varying amounts, routes of administration and the timing of the Gln load.

It is interesting to address the question of how comparable are the differences in circulating Gln concentrations in the two Guatemalan populations, healthy and infected, with those reported in a previous study in the U.S. in healthy volunteers who received oral Gln at two dosage levels (0.1 and 0.3 g/kg) (Ziegler et al. 1990). Those authors assessed whole-blood concentration vs. time profiles of 240 min. The body weight of the healthy population in this study and in the North American investigation was the same (75 kg); the mean dosage of Gln in Guatemala was 0.18 g/kg, which falls between the dosages used in the North American study. To compare the two sets of data, we assumed a bioavailability factor (F) for Gln and Ala derived from Ala-Gln dipeptide of 1.0. Indeed, this assumption is justified, considering the prompt *in vivo* hydrolysis of Ala-Gln (Albers et al. 1988 and 1989), indicating that after oral administration, the appearance of Gln from the dipeptide might be practically identical with that of free Gln. This deduction allows the calculation of V_d per kg body weight, which is 761 vs. 512 mL/kg with 0.1 g/kg and 1254 mL/kg with 0.3 g/kg dosage in the North American study. Nevertheless, by extrapolating the dosage of the North American study to 0.18 g/kg as given in this study, the resultant V_d is comparable (809 mL/kg). Similarly, V_d derived from the

Dengue patients was 1199 mL/kg compared with 1031 mL/kg in the North American study when adjusted to the 0.24 g/kg given to the patients. After the dose of 0.18 g/kg, we observed an average maximal plasma concentration of 1342 $\mu\text{mol/L}$, whereas in the North American study with a dosage of 0.3 g/kg, a mean whole-blood concentration of 1328 $\mu\text{mol/L}$ was reported. Because the transmembrane gradient is 1.1, we may assume that there was an equal volume of distribution, and thus the concentrations obtained in plasma or in whole blood are directly comparable.

In our study, Gln $t_{1/2}$ was 28 min, which is more rapid than the half-life time reported in the North American study of 117 min. This finding is interesting in the face of the classical studies from the late 1960s (Adibi and Phillips 1968, Matthews et al. 1968), demonstrating a specific dipeptide carrier in the intestine. These early reports emphasized that the transport of amino acids is more rapid from dipeptides than from free amino acids. Indeed, this notion has been confirmed repeatedly during the years (Abumrad and Miller 1983, Ganapathy and Leibach 1986, Gardner 1975, Gardner et al. 1983), and the present finding may indicate the importance of the favorable physiologic handling of the Gln dipeptide.

Various tissues, such as muscle, are the major store of intracellular Gln; the concentration is ~ 20 mmol/L intracellular water (Bergström et al. 1974), with an intra-/extracellular transmembrane gradient of 30. Consequently, a change in intracellular concentration after supplemental oral Gln-dipeptide might considerably influence and complicate pharmacokinetic calculations. We assume that the healthy adults in our study were not Gln depleted, and thus the oral Gln administered would not be taken up by the muscle. This line of reasoning implies that changes occurring in the extracellular compartment may reflect postabsorptive Gln handling fairly well. Indeed, we employed a noncompartmental pharmacokinetic model to avoid all possible influences of a large intracellular Gln pool.

As shown in Figure 1A, baseline plasma concentrations of Ala were lower than those of Gln. Because the constituent amino acids of the dipeptide were given in equimolar amounts, it is interesting to note that increments in Ala concentrations are higher than those of Gln in both relative (fold-increase) and absolute terms. After intravenous administration of Ala-Gln to healthy adults (Albers et al. 1988), this pattern was not observed. Reduced splanchnic extraction of Ala compared with Gln might serve as an explanation. Alternatively, we may hypothesize that Gln and Ala have different metabolic handling as far as uptake, initial clearance, and secondary clearance are concerned. Indeed, these factors may define the course of plasma Gln and Ala after the dipeptide administra-

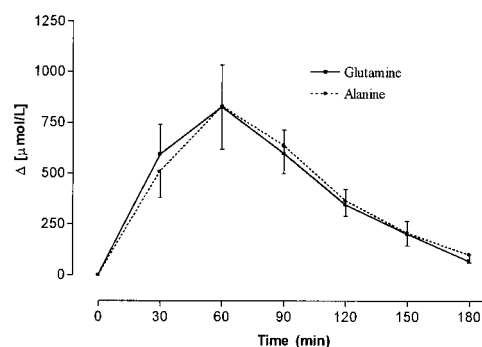


FIGURE 3 Composite curves of changes in plasma Gln and Ala concentrations after bolus dose of Ala-Gln in Dengue patients ($n = 8$). The variance is expressed as SEM.

tion, although the principal response of the two amino acids is similar.

Despite the within-subject, repeated-measure design, the sample size of only four subjects completing all five components of the Ala-Gln pharmacokinetic study was insufficient to resolve whether lower gastric acidity is a modulating factor. The data, however, showed no significant difference between AUC with and without gastric acid blockade.

Relating the amount of administered Ala-Gln to weight, Dengue fever patients had a significantly higher intake (0.18–0.26 g/kg) than healthy volunteers (0.13–0.20 g/kg). This can be explained by the significantly lower body weight of Dengue patients. Despite the greater uptake, no greater peak increments of the constituent amino acids or greater AUC were observed in Dengue patients. Because Dengue fever is a systemic disease, without specific localization in the gut, it is not surprising that neither digestion of the dipeptide nor uptake of the constituent amino acids was different from that in healthy adults.

Both bolus and intermittent oral administration of the dipeptide Ala-Gln led to similar time vs. concentration responses in healthy adults; only the maximum increment was higher with the bolus dosing. In this study, neither the intake of a gastric acid blocker nor acute febrile Dengue infection affected the pharmacokinetic responses of the constituent amino acids in plasma compared with healthy adults.

LITERATURE CITED

- Abumrad, N. N. & Miller, B. (1983) The physiologic and nutritional significance of plasma-free amino acid levels. *J. Parent. Enteral Nutr.* 7: 163–170.
- Adibi, S. A. & Phillips, E. (1968) Evidence for greater absorption of amino acid from peptide than from free form by human intestine. *Clin. Res.* 16: 446.
- Albers, S., Wernerman, J., Stehle, P., Vinnars, E. & Fürst, P. (1988) Availability of amino acids supplied intravenously in healthy man as synthetic dipeptides: kinetic evaluation of L-alanyl-L-glutamine and glycyl-L-tyrosine. *Clin. Sci. (Lond.)* 75: 463–468.
- Albers, S., Wernerman, J., Stehle, P., Vinnars, E. & Fürst, P. (1989) Availability of amino acids supplied by constant intravenous infusion of synthetic dipeptides in healthy man. *Clin. Sci. (Lond.)* 76: 643–648.
- Bergström, J., Fürst, P., Noree, L. O. & Vinnars, E. (1974) Intracellular free amino acid concentration in human muscle tissue. *J. Appl. Physiol.* 36: 693–697.
- Calder, P. C. (1994) Glutamine and the immune system. *Clin. Nutr.* 13: 2–8.
- Calder, P. C. (1995) Fuel utilization by cells of the immune system. *Proc. Nutr. Soc.* 54: 65–82.
- Cao, Y., Feng, Z., Hoos, A. & Klimberg, V. S. (1998) Glutamine enhances glutathione production. *J. Parent. Enteral Nutr.* 22: 224–227.
- Castro, L. D. & Coelho, L. G. (1998) *Helicobacter pylori* in South America. *Can. J. Gastroenterol.* 12: 509–512.
- Darmaun, D. (1995) Response of glutamine metabolism to enteral and parenteral nutrition. In: *Pharmacological Nutrition Immune Nutrition* (Cynober, L., Fürst, P. & Lawin, P., eds.), pp. 59–67. International Symposium, Nice, 1995. W. Zuckerschwerdt Verlag, Munich, Germany.
- Darmaun, D., Just, B., Messing, B., Rongier, M., Thuillier, F., Koziat, J. & Grasset, E. (1994) Glutamine metabolism in healthy adult men: response to enteral and intravenous feeding. *Am. J. Clin. Nutr.* 59: 1395–1402.
- Divino Filho, J. C., Bergström, J., Stehle, P. & Fürst, P. (1997) Simultaneous measurements of free amino acid patterns of plasma, muscle and erythrocytes in healthy human subjects. *Clin. Nutr.* 16: 299–305.
- Felig, P. (1975) Amino acid metabolism in man. *Annu. Rev. Biochem.* 44: 933–955.
- Fürst, P. (1998) Old and new substrates in clinical nutrition. *J. Nutr.* 128: 789–796.
- Fürst, P., Albers, S. & Stehle, P. (1990a) Glutamine-containing dipeptides in parenteral nutrition. *J. Parent. Enteral Nutr.* 14: 118S–124S.
- Fürst, P., Pollack, L., Graser, T. A., Godel, H. & Stehle, P. (1990b) Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. *J. Chromatogr.* 499: 557–569.
- Fürst, P. & Stehle, P. (1994) Are intravenous amino acid solutions unbalanced? *New Horiz.* 2: 215–223.
- Ganapathy, V. & Leibach, F. H. (1986) Carrier-mediated reabsorption of small peptides in renal proximal tubule. *Am. J. Physiol.* 251: F945–F953.
- Gardner, M.L.G. (1975) Absorption of amino acids and peptides from a complex mixture in the isolated small intestine of the rat. *J. Physiol.* 253: 232–256.
- Gardner, M. G., Lindblad, B. S., Burston, D. & Matthews, D. M. (1983) Transmucosal passage of intact peptides in the guinea-pig small intestine in vivo: a re-appraisal. *Clin. Sci. (Lond.)* 64: 433–439.
- Goringe, A. P., Brown, S., O'Callaghan, U., Rees, J., Jebb, S., Elia, M. & Poynton, C. H. (1998) Glutamine and vitamin E in the treatment of hepatic veno-occlusive disease following high-dose chemotherapy. *Bone Marrow Transplant.* 21: 829–832.
- Hankard, R. G., Darmaun, D., Sager, B. K., D'Amore, D., Parsons, W. R. & Haymond, M. (1995) Response of glutamine metabolism to exogenous glutamine in humans. *Am. J. Physiol.* 269: E663–E670.
- Hankard, R. G., Haymond, M. W. & Darmaun, D. (1996) ESPEN Research Symposia, presented at ESPEN 1995: effect of enteral glutamine on glutamine and leucine metabolism in humans. *Clin. Nutr.* 15: 84–85.
- Heinzel, G., Woloszczak, R. & Thomann, P. (1993) TopFit Version 2.0: pharmacokinetic and pharmacodynamic data analysis for the PC. New York, NY.
- Hong, R. W., Rounds, J. D., Helton, W. S., Robinson, M. K. & Wilmore, D. W. (1992) Glutamine preserves liver glutathione after lethal hepatic injury. *Ann. Surg.* 215: 114–119.
- Houdijk, A. P., Rijnsburger, E. R., Jansen, J., Wesdorp, R. I., Weiss, J. K., McCamish, M. A., Teerlink, T., Meuwissen, S. G., Haarman, H. J., Thijs, L. G. & van Leeuwen, P. A. (1998) Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. *Lancet* 352: 772–776.
- Jebb, S. A., Osborne, R. J., Maughan, T. S., Mohideen, N., Mack, P., Mort, D., Shelley, M. D. & Elia, M. (1994) 5-Fluorouracil and folic acid-induced mucositis: no effect of oral glutamine supplementation. *Br. J. Cancer* 70: 732–735.
- Jepson, M. M., Bates, P. C., Broadbent, P., Pell, J. M. & Millward, D. J. (1988) Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. *Am. J. Physiol.* 255: E166–E172.
- Lee, R., Bithell, T., Foerster, J., Athens, J. W., Lukens, J. N. & Kushner, J. (1989) *Wintrobe's Clinical Hematology*. Lea and Febiger, Philadelphia, PA.
- Long, C. L., Nelson, K. M., DiRienzo, D. B., Weis, J. K., Stahl, R. D., Broussard, T. D., Theus, W. L., Clark, J. A., Pinson, T. W. & Geiger, J. W. (1995) Glutamine supplementation of enteral nutrition: impact on whole body protein kinetics and glucose metabolism in critically ill patients. *J. Parent. Enteral Nutr.* 19: 470–476.
- MacLennan, P. A., Smith, K., Weryk, B., Watt, P. W. & Rennie, M. J. (1988) Inhibition of protein breakdown by glutamine in perfused rat skeletal muscle. *FEBS Lett.* 237: 133–136.
- Maton, P. (1993) Omeprazole administration as a model for atrophic gastritis. CRC Press, Boca Raton, FL.
- Matthews, D. E., Marano, M. A. & Campbell, R. G. (1993) Splanchnic bed utilization of glutamine and glutamic acid in humans. *Am. J. Physiol.* 264: E848–E854.
- Matthews, D. M., Craft, I. L., Geddes, D. M., Wise, I. J. & Hyde, W. C. (1968) Absorption of glycine and glycine peptides from the small intestine of the rat. *Clin. Sci. (Lond.)* 35: 415–424.
- Newsholme, E. A., Newsholme, P., Curi, R., Challoner, E. & Ardawi, S. M. (1988) A role for muscle in the immune system and its importance in surgery, trauma, sepsis and burns. *Nutrition* 4: 261–268.
- Silk, D.B.A. (1999) Formulation of enteral diets. *Nutrition* 15: 626–632.
- Stehle, P. & Fürst, P. (1995) Glutamine and the gut. In: *Pharmacological Nutrition Immune Nutrition* (Cynober, L., Fürst, P. & Lawin, P., eds.), pp. 59–67. International Symposium, Nice, 1995. W. Zuckerschwerdt Verlag, Munich, Germany.
- Welbourne, T. C. (1995) Increased plasma bicarbonate and growth hormone after an oral glutamine load. *Am. J. Clin. Nutr.* 61: 1058–1061.
- Welbourne, T. C., King, A. & Horton, K. (1993) Enteral glutamine supports hepatic glutathione efflux during inflammation. *J. Nutr. Biochem.* 4: 236–242.
- Ziegler, T. R., Benfell, K., Smith, R. J., Young, L. S., Brown, E., Ferrari-Baliviera, E., Lowe, D. K. & Wilmore, D. W. (1990) Safety and metabolic effects of L-glutamine administration in humans. *J. Parent. Enteral Nutr.* 14: 137S–146S.