Plasma free amino acid concentrations in healthy Guatemalan adults and in patients with classic dengue[1,2]

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ABSTRACT

Background: Plasma free amino acid patterns in health and disease have been reported. However, amino acid concentrations in adult populations in developing countries and in patients with dengue, as a model for an acute infectious viral disease endemic to the tropics, have not been reported.

Objective: The purpose of this study was to determine the amino acid profile in both healthy Guatemalan adults from different socioeconomic backgrounds and at 3 time points during the course of classic dengue.

Design: The study was carried out in Guatemala and included measurement of plasma free amino acids in 22 healthy control subjects (14 low income, 8 middle class) and 17 febrile patients. Measurements of amino acids were repeated within a 48-h interval in 20 of the healthy Guatemalans. In 9 patients with dengue, amino acids were assayed 3 times: on admission to a local hospital in the coastal plain of Guatemala, on hospital discharge, and 7 d after hospital discharge.

Results: Branched-chain amino acid concentrations in healthy adults and dengue patients in Guatemala were lower than normal values reported in the literature for healthy Swedish adults. With the exception of increased phenylalanine concentrations and an increased ratio of phenylalanine to tyrosine, all amino acids as well as the Fischer molar ratio were decreased in the acute phase of dengue.

Conclusions: Healthy Guatemalans have different amino acid patterns than do Swedish subjects independent of socioeconomic status. The systemic viral disease dengue is associated with changes in the plasma free amino acid pattern, reflecting infection-related alterations in amino acid metabolism. *Am J Clin Nutr* 2001;73:647–52.

KEY WORDS Plasma amino acids, Guatemala, dengue, infection, Fischer molar ratio, phenylalanine-to-tyrosine ratio, glycine-to-valine ratio

INTRODUCTION

Plasma free amino acid (PFAA) profiles have been reported over the past decades for healthy subjects and for patients with various diseases (1–8). The free amino acid pool represents only a small fraction of the total-body free amino acid content; concentrations in the intracellular space are considerably higher than plasma concentrations (9) and most free amino acids are located in muscle tissue. However, PFAA concentrations might be of great value in reflecting changes in organ nitrogen handling and altered amino acid metabolism.

During inflammation and infection, protein metabolism is distinctly altered (9, 10; PJ Reeds, A Kurpad, A Opekun, et al, unpublished observations, 1994). This alteration is directly due to a flux of amino acids from peripheral tissues, primarily skeletal muscle, into the liver (11; PJ Reeds et al, unpublished observations, 1994). Amino acids are utilized in the synthetic pathways of arginine and glutathione and in lymphocyte and acute-phase proteins (9, 10, 12; PJ Reeds, et al, unpublished observations, 1994). In fact, a decrease in almost all PFAA concentrations is observed during acute infection, the exception being concentrations of phenylalanine (11).

The relations between the concentrations of some PFAAs, such as the molar ratio of phenylalanine to tyrosine (Phe:Tyr; 13) and the Fischer molar ratio [(valine + leucine + isoleucine)/(phenylalanine + tyrosine); (14, 15)], may prove to be important informative indexes for distinguishing malnutrition from infectious processes. The glycine-to-valine index is used to distinguish malnutrition from catabolic stress (16).

Throughout the 1950s and 1970s, major advances took place in amino acid chromatography (4, 9) to yield computer-automated HPLC. Amino acid concentrations have been measured in plasma, muscle, and erythrocytes by using highly sensitive, automated, online HPLC (17).

Few data are available on PFAA concentrations in adults in developing countries. Differences in dietary habits and a wide range of socioeconomic classes in these populations might be associated with great variations in nutritional status. The interaction between human nutrition and acute infections was mentioned as early as 3 decades ago in the classic treatise *Interactions of Nutrition and Infection* (18). With respect to this interaction, the postulation is that individuals with poor nutritional status are more susceptible to infections or their adverse consequences, or that

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TABLE 1
Population characteristics[1]

	Age	Weight	Height
	y	*kg*	*m*
Low-income Guatemalans (*n* = 9 F, 5 M)	30 ± 10	56 ± 6	1.53 ± 0.08
Guatemalan professionals (*n* = 2 F, 3 M)	30 ± 6	77 ± 13	—
Non-Guatemalan professionals (*n* = 2 F, 1 M)	23 ± 2	73 ± 5	—
Guatemalans and professionals combined (*n* = 13 F, 9 M)	31 ± 9	63 ± 12	—
Patients with dengue (*n* = 8 F, 9 M)	30 ± 14	59 ± 12	1.61 ± 0.08
Swedish control group (*n* = 11 F, 16 M)[2]	39 ± 3	70 ± 2	1.73 ± 0.02

[1] $\bar{x}$ ± SD.
[2] Data from Divino-Filho et al (17).

nutritional status will be adversely affected in those with infections (19). Dengue is an acute viral infectious disease endemic to the tropics that is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. It is characterized by a sudden onset of high fever (39–41°C), with the major symptoms being severe frontal headache, bone or joint and muscular pain, and retroorbital pain. Treatment is symptomatic. In the absence of antipyretic control, the course of fever usually lasts 3–5 d. The purpose of this study was to investigate PFAA patterns in healthy Guatemalan adults from different socioeconomic strata, potentially reflecting different nutritional status, and among patients with classic dengue at different stages of their disease and recovery.

SUBJECTS AND METHODS

Subjects

Twenty-two healthy adults were enrolled in the study. Subjects were eligible if they were asymptomatic and free of any systemic, metabolic, or gastrointestinal disease at the time of the study. Fourteen subjects lived in a low-income suburb of Guatemala City. Five subjects were Guatemalan professionals from a middle-class background who worked at CeSSIAM and lived in the capital and 3 subjects were of non-Guatemalan origin but resided in the capital while studying at CeSSIAM. Twelve of the low-income subjects were studied on 2 occasions within a 3-d interval. The 8 healthy professionals were studied on 2 occasions within 2 d. The characteristics of the population studied are shown in **Table 1**. All subjects gave their informed consent after the nature, purpose, and possible inconveniences and discomforts of the study were explained to them. The study was approved by the Human Subjects Committee of CeSSIAM.

Seventeen patients with classic dengue (Table 1) were recruited from the public hospital in Tiquisate, in the province of Escuintla on the coastal plain of Guatemala, an area in which the mosquito vectors *A. aegypti* and *A. albopictus* are endemic. Patients were first assessed within 5 d of the onset of the febrile disease. Dengue was suspected on the basis of clinical symptoms and the absence of malarial parasitemia on the thick blood smear performed at admission. Dengue was confirmed in the laboratories of the Division on Malaria and Vector Diseases of the Guatemalan Ministry of Health by virus isolation (by using a CC-36 cell culture line of *A. albopictus* cells), serologic testing (detecting immunoglobulin M by using a capture enzyme-linked immunosorbent assay method), or both. In 9 of the patients with dengue, serial measurements of PFAA were made at 3 time points: *1)* on admission to a local hospital, *2)* on hospital discharge after defervescence at ≈5 d postadmission, and *3)* 7 d after hospital discharge. After subjects had fasted overnight, a 2-mL blood sample was drawn at 0800 and transferred into an EDTA-containing Vacutainer (Becton Dickinson, Franklin Lakes, NJ) and placed immediately on ice. The samples were subsequently centrifuged at 4000 × *g* for 7 min at 4°C to obtain plasma, which was stored at −40°C until analyzed at the University of Hohenheim, Stuttgart, Germany.

Methods

PFAAs were assessed by using an automated, online, reversed-phase HPLC system with precolumn derivatization (*o*-phtaldialdehyde 3-mercaptopropionic acid) (20). The samples were precipitated with 5-sulfosalicylic acid, incubated at 4°C for 1 h, and centrifuged at 6000 × *g* for 1 min at room temperature. The supernate was collected in screw-top cryovials (Greiner, Kremsmünster, Austria) and placed on dry ice. Hematocrit was measured by using an International Microcapillary Reader (International Equipment Company, Boston). Albumin and cholesterol were measured by using commercial Menagent test kits (Menarini Diagnostics, Firenze, Italy). Triacylglycerol was analyzed on a COBAS MIRA autoanalyzer (La Roche, Grenzach, Germany) with a commercial test kit (La Roche).

Statistical analysis

Results are expressed as means ± SDs or as means ± SEMs. The computer programs SPSS (version 6.0; SPSS Inc, Chicago) and EXCEL 97 (Microsoft, Redwood, WA) were used for statistical analyses. Significance of differences between control subjects and patients was determined by using analysis of variance (ANOVA) and Dunnett's post hoc test with use of the healthy Guatemalans as a control group. Differences within the group of patients with dengue during the course of disease were determined by using repeated-measures ANOVA. Pearson's product-moment correlation coefficients were calculated to assess the reproducibility or correspondence of amino acid measurements over time. The Lin concordance coefficient (21, 22), which evaluates the intraclass correlation in relation to its deviation from the line of identity, was also applied to the test-retest assessment. Differences were considered statistically significant if the *P* value was ≤0.05.

RESULTS

Compared with the community group, patients with dengue had lower plasma albumin concentrations and hematocrits throughout the course of the disease. Hematologic variables for the low-income Guatemalan control subjects and the patients

TABLE 2
Biochemcial variables of control subjects (low-income Guatemalans) and of patients with dengue during the course of disease[1]

		Patients with dengue (n = 9)[2]		
	Low-income Guatemalans (n = 12)	Time point 1	Time point 2	Time point 3
Hematocrit	0.46 ± 0.05	0.41 ± 0.04[3]	0.42 ± 0.04[3]	0.41 ± 0.05[3]
Triacylglycerol (mmol/L)	2.59 ± 1.29	1.08 ± 0.36[4]	1.72 ± 0.44	1.45 ± 0.56[4]
Cholesterol (mmol/L)	4.3 ± 0.6	4.2 ± 0.9	4.2 ± 0.9	4.5 ± 1.2
HDL (mmol/L)	0.99 ± 0.27	0.82 ± 0.21	0.59 ± 0.19[4]	0.65 ± 0.17[4]
Albumin (g/L)	53 ± 6	42 ± 6[4]	42 ± 5[4]	42 ± 4[4]
Urea (mmol/L)	6.5 ± 2.5	8.6 ± 3.2	6.9 ± 1.4	7.1 ± 2.6
Total protein (g/L)	77 ± 4	72 ± 7	79 ± 7	72 ± 5

[1] $\bar{x}$ ± SD.
[2] Time point 1, admission to the hospital (acute phase); time point 2, discharge from the hospital (>24 h without fever); time point 3, 7 d after discharge.
[3,4] Significantly different from low-income Guatemalans (ANOVA and Dunnett's test): [3] $P < 0.05$, [4] $P < 0.01$.

with dengue are shown in **Table 2**. Compared with the low-income Guatemalans, patients with dengue had significantly lower plasma albumin concentrations and hematocrit values throughout the course of disease and lower triacylglycerol and HDL-cholesterol concentrations at 2 time points.

Comparative perspective of amino acid profiles

Plasma from the 2 populations sampled in Guatemala were analyzed in the same laboratory as was the control material from Sweden. Because no significant differences between the healthy Guatemalan subgroups were found, we combined these into one group for comparison with the patients with dengue. For comparison of the Guatemalan control subjects with either the Swedish adults or the patients with dengue, we used data for the Guatemalan control group from the first examination day. Compared with the healthy Guatemalan adults, patients with dengue had lower total PFAA concentrations and lower nonessential amino acids concentrations at time point 1 (**Table 3**). Of the essential amino acids, histidine was lower in patients with dengue than in Guatemalan control subjects, whereas phenylalanine concentrations were higher at time points 1 and 2 (**Figure 1**); alanine was lower in patients with dengue than in the Guatemalan control group.

Intersampling reproducibility of individual amino acids in healthy adults

The sample-resample correlation coefficients for each of the 20 amino acids measured in 12 of the healthy, low-income Guatemalan control subjects are shown in **Table 4**. The highest concordance between amino acid measurements was found for glycine, citrulline, tyrosine, histidine, and lysine with Pearson's product-moment correlation coefficient and for glycine, citrulline, histidine, lysine, and tyrosine with the Lin concordance coefficient. The lowest r values were found for taurine, ornithine, isoleucine, asparagine, and alanine with Pearson's product-moment correlation coefficient and for taurine, ornithine, asparagine, isoleucine, and alanine with the Lin concordance coefficient.

Comparison of amino acid concentrations during the course of dengue

Variations in the plasma amino acid profile of patients with dengue in the acute phase and of healthy Guatemalan control subjects are shown in Figure 1A. The significant changes in amino acid concentrations during the course of dengue are illustrated in Figure 1B. During the course of the disease, most of the amino acid concentrations changed toward normal ranges as evaluated by multivariate ANOVA.

Molar amino acid ratios and interactions

Relevant amino acid ratios are shown in Table 3. Phe:Tyr was higher in patients with dengue than in any other group. This ratio decreased significantly during the course of disease (time point 1 compared with time point 3: 1.8 compared with 1.0; $P = 0.002$). The Fischer molar ratio was lowest in patients with dengue at time point 1 and increased significantly at recovery (time point 2: 2.65; time point 3: 2.87). The ratio of glycine to valine increased significantly during the course of dengue (time point 1 compared with time point 3: 0.93 compared with 1.57; $P < 0.05$).

DISCUSSION

Amino acid concentrations in plasma have been quantified for decades and various methods have been compared repeatedly over the years (20, 23). Nevertheless, because the standardization of methods may vary from one laboratory to the next, great caution should be exercised in any direct comparison of individual amino acid concentrations across centers.

The present study is a first attempt to determine PFAA patterns in healthy adults and of patients with acute, classic dengue in Guatemala. The pattern of amino acids is known to be influenced by numerous factors, such as age, sex, physical activity, and dietary intake (24, 25). As shown in Table 1, the healthy Guatemalans and the patients with dengue were comparable with respect to age, weight, and height, allowing an appropriate comparison between groups. Differences between healthy Guatemalans and patients with dengue were found for hematocrit (Table 2) and albumin, although these values were within the normal range. The higher hematocrit values in the control subjects may have been due to microcytosis because these subjects lived 1500 m above sea level whereas the patients lived in the coastal plain.

In general, the healthy Guatemalans in our study had lower PFAA concentrations than did the Swedish reference group. Concentrations of branched-chain amino acids were significantly lower in the Guatemalans (Table 3). The amino acid pattern reported in the present study is comparable with that reported in Mexicans (26, 27). It is known that the composition of dietary protein may alter plasma amino acid concentrations (26) and consumption of diets rich in complex carbohydrates

TABLE 3
Plasma free amino acid concentrations of healthy Guatemalan subjects, of Guatemalan patients with dengue at admission to the hospital (time point 1), and of a Swedish reference group[1]

Amino acid	Swedish reference group[2] (n = 27)	Guatemalans: Healthy (n = 22)	Guatemalans: Patients with dengue (n = 17)
		μmol/L	
Essential			
Histidine	87 ± 3	87 ± 6	67 ± 3[3]
Isoleucine	63 ± 3[3]	53 ± 3	49 ± 3
Leucine	120 ± 5[3]	105 ± 6	95 ± 6
Lysine	195 ± 9	150 ± 8	126 ± 10
Methionine	25 ± 1[3]	24 ± 1	25 ± 2
Phenylalanine	53 ± 2	50 ± 3	75 ± 8[3]
Threonine	128 ± 5[3]	108 ± 5	80 ± 6
Tyrosine	60 ± 4	54 ± 2	50 ± 3
Tryptophan	46 ± 3[3]	34 ± 2	28 ± 3
Valine	220 ± 8[3]	182 ± 11	162 ± 8
Nonessential			
Alanine	316 ± 17	340 ± 17	274 ± 24[4]
Arginine	86 ± 3[4]	69 ± 3	63 ± 5
Asparagine	47 ± 2[4]	40 ± 2	36 ± 2
Citrulline	34 ± 1	24 ± 2	21 ± 2
Glutamic acid	32 ± 4	36 ± 20	37 ± 3
Glutamine	655 ± 17[3]	511 ± 4	467 ± 23
Glycine	248 ± 13	210 ± 17	171 ± 13
Ornithine	66 ± 4[3]	41 ± 3	38 ± 3
Serine	114 ± 4[4]	102 ± 5	86 ± 7
Taurine	49 ± 3[4]	36 ± 22	41 ± 9
BCAA	438 ± 21[3]	337 ± 20	312 ± 17
Σ EAA	857 ± 27	841 ± 38	726 ± 65
Σ NEAA	1446 ± 38	1435 ± 49	1121 ± 57[4]
Σ TAA	2303 ± 58	2342 ± 88	1847 ± 116[4]
Ratios			
Phe:Tyr	0.96	0.94	1.54[3]
FR	3.57	3.26	2.46[3]
Gly:Val	1.13	1.20	0.93

[1] $\bar{x}$ ± SEM. BCAA, branched-chain amino acids; EAA, essential amino acids; NEAA, nonessential amino acids; TAA, total amino acids; FR, Fischer molar ratio.
[2] Data from Divino-Filho et al (17).
[3,4] Significantly different from healthy Guatemalan control subjects (ANOVA and Dunnett's test): [3] $P < 0.01$, [4] $P < 0.05$.

results in low concentrations of branched-chain amino acids (28). The diet consumed in the present study consisted mainly of corn tortillas and black beans, a traditional Mesoamerican diet, and was rich in complex carbohydrates and dietary fiber. Of the total protein intake, 38% was provided by black beans, 31% by corn tortillas, and only 12% by animal sources (cheese and eggs) (29). Because of normal plasma albumin and total protein concentrations (Table 2), it is unlikely that the lower amino acid concentrations observed in the Guatemalan control subjects were due to underlying protein malnutrition.

Pearson correlation coefficients and Lin concordance coefficients for amino acids, calculated twice in the same subset of 12 healthy Guatemalans within 48 h, showed a fair within-individual stability in PFAA concentrations. This may reflect the repeatedly described homeostatic mechanisms that control plasma amino acid concentrations under physiologic conditions (24, 26, 30).

Infection with the dengue virus influenced the PFAA pattern, as shown by differences in the PFAA pattern between the patients and the healthy Guatemalan control subjects. Although healthy Guatemalans had a different amino acid pattern than did the Swedish population, the influence of viral disease on the amino acid pattern was evident as 3 major changes: elevated phenylalanine concentrations and decreased values for plasma histidine and alanine (Table 3). These findings nicely confirm the classic studies of Wannemacher et al (31, 32) regarding the changes in plasma amino acids during infectious disease.

The increased phenylalanine concentrations were the reason for the elevated Phe:Tyr in the patients with dengue. Other investigators showed that increases in Phe:Tyr during infection are related to a release of phenylalanine from muscle tissue that exceeds its utilization by other cells of the viscera (31). The changes found in this study were in close agreement with those reported in patients with other infectious diseases (32) and injury and sepsis (7). Our group reported earlier a Phe:Tyr of 0.90 during injury and a Phe:Tyr of 1.28 during sepsis (7). The range for Phe:Tyr in viral diseases is reported to be 1.20–1.99 (31). Dengue seems to be another viral disease in which Phe:Tyr seems to be a good clinical indicator of the catabolic effect of the disease.

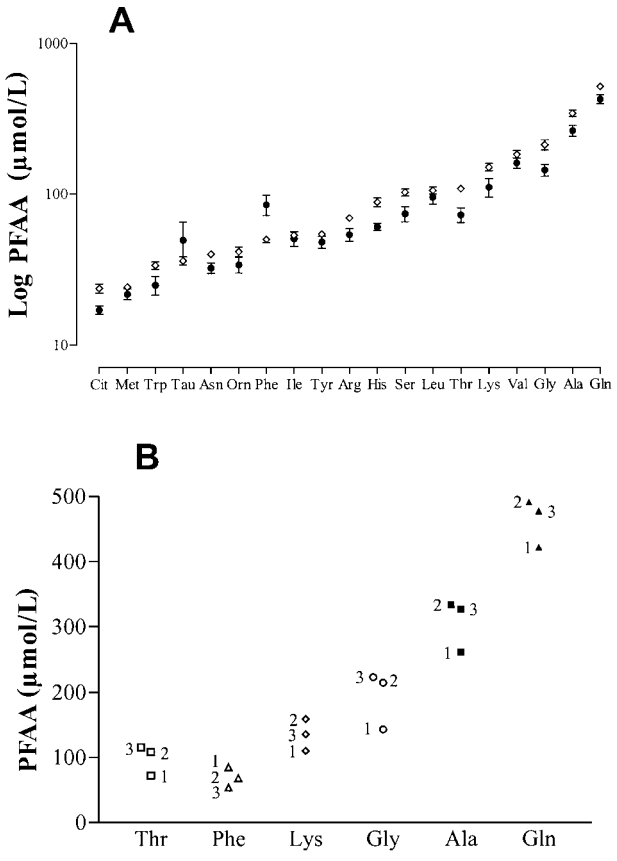

FIGURE 1. A: Mean (±SEM) plasma free amino acid (PFAA) concentrations in healthy Guatemalan adults (◇; n = 22) and in patients with dengue (●; n = 17) at admission to the hospital. B: The concentrations of PFAAs that varied significantly (multivariate ANOVA, $P < 0.05$) during the course of dengue. Time point 1, admission to the hospital; time point 2, discharge from the hospital; time point 3, 7 d after discharge.

TABLE 4
Pearson's product-moment correlation coefficients and Lin concordance coefficients of amino acids measured at a 48-h interval in 12 healthy, low-income Guatemalan control subjects

Amino acid	Pearson correlation	Lin concordance
Essential		
Histidine	0.602[1]	0.509
Isoleucine	0.333	0.292
Leucine	0.471	0.358
Lysine	0.590[1]	0.488
Methionine	0.437	0.324
Phenylalanine	0.419	0.337
Threonine	0.426	0.330
Tyrosine	0.677[1]	0.453
Tryptophan	0.501[1]	0.451
Valine	0.491[1]	0.428
Nonessential		
Alanine	0.408	0.305
Arginine	0.581[1]	0.429
Asparagine	0.402	0.284
Citrulline	0.698[1]	0.599
Glutamine	0.521[1]	0.400
Glycine	0.806[1]	0.651
Ornithine	0.284	0.219
Serine	0.518[1]	0.420
Taurine	−0.056	−0.039

[1] $P < 0.05$.

The Fischer molar ratio for healthy adults is reported to be 3.0–3.5 (33) and can be decreased by liver disorders to values between 1 and 1.5 (7, 34). The healthy control subjects in the present study had a normal Fischer molar ratio, whereas the patients with dengue had lower ratios throughout the course of their disease that remained low during convalescence (Table 3). This suggests an impairment of liver function in these patients (34, 35).

The ratio of glycine to valine is a sensitive indicator of moderate protein-energy malnutrition (36). In the present study, this ratio was not altered because both valine and glycine concentrations decreased. The results show changes in the amino acid pattern indicative of infection-related—and, conclusively, not starvation- or malnutrition-induced—alterations in amino acid metabolism (11).

We conclude that low-income Guatemalans consuming a traditional Mesoamerican diet have lower PFAA concentrations than do healthy Swedish adults. Systemic dengue infection alters this amino acid profile even further, but in patterns consistent with those seen in infected patients in developed countries. The pattern of response seen in this study further confirms the observed increases in the phenylalanine concentration and Phe:Tyr in other infected populations.

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