Arginine flux and intravascular nitric oxide synthesis in severe childhood undernutrition[1–3]

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ABSTRACT

Background: Although nutritionally dispensable amino acids are not essential in the diet, adequate synthesis is necessary for maintenance of good health. Whereas children with edematous severe childhood undernutrition (SCU) can maintain production rates of glycine and serine despite a slower body protein breakdown rate, it is unknown whether the same is true for the semidispensable amino acid arginine.

Objective: We aimed to measure arginine flux and intravascular nitric oxide synthesis in children with SCU.

Design: Arginine flux and the fractional and absolute synthesis rates of plasma nitrite plus nitrate were measured postabsorptively by using a 6-h infusion of [$^{15}N_2$]-arginine in 2 groups of children with edematous ($n = 14$) or nonedematous ($n = 7$) SCU when they were infected and malnourished (postadmission day ≈3; clinical phase 1), when they were no longer infected (postadmission day ≈15; clinical phase 2), and when they were recovered (postadmission day ≈55; clinical phase 3).

Results: Arginine flux was slower ($P < 0.01$) and plasma arginine concentrations were lower in the edematous group than in the nonedematous group at clinical phase 1. At clinical phase 2, flux doubled to a value that was not significantly different from the value at clinical phase 3. There were no significant differences in the plasma concentration or fractional or absolute synthesis rate of plasma nitrite plus nitrate between the groups at any clinical phase and among clinical phases within each group.

Conclusion: Whereas children with nonedematous SCU can maintain arginine flux at the same rate as when recovered, children with edematous SCU cannot. The slower arginine flux was not, however, associated with slower nitric oxide synthesis. *Am J Clin Nutr* 2007;86:1024–31.

KEY WORDS Arginine kinetics, nitric oxide, edematous severe childhood undernutrition, nonedematous severe childhood undernutrition, marasmus, kwashiorkor

INTRODUCTION

In severe childhood undernutrition (SCU), less amino acids of dietary origin are available for metabolic purposes because of the reduced protein intake associated with chronic food deprivation. Because breakdown of body proteins is the major contributor to the overall flux of amino acids (1), our finding that the whole-body protein breakdown rate is slower in children with the edematous form of SCU than in children with the nonedematous form of SCU (2) suggests that a more severe shortage in the availability of dietary essential amino acids will exist in children with edematous SCU. In the case of the dietary nonessential amino acids, it is possible for de novo synthesis to fill the gap created by any reduction in the amount released from protein breakdown. Whereas we found this to be true for glycine and serine (3, 4), this was not the case for cysteine, which requires sulfur from the essential amino acid methionine for its synthesis (5). These findings suggest that in children with edematous SCU, the availability of a particular nonessential amino acid may depend on whether it is derived from an essential amino acid or not.

An amino acid that occupies a unique position is arginine, a semi-essential amino acid, because it is produced in adequate quantities to meet the requirements of healthy adults but not of growing children or persons stressed by severe trauma or sepsis (6). Because the production of arginine depends on the availability of citrulline, a nonprotein amino acid synthesized mostly in the gut from glutamine and proline, it is highly likely that arginine will be in short supply in children with SCU, especially those with edematous SCU because of decreased release from a slower protein breakdown (2).

In addition to its role as a substrate for protein synthesis, arginine serves several important physiologic functions related to ammonia detoxification to urea, wound healing, and immune function. As a precursor for the synthesis of creatine and nitric oxide (NO), arginine serves important roles in energy metabolism and regulation of blood pressure plus the numerous other regulatory roles attributed to NO (6). Low plasma arginine has been shown to be correlated with a worse prognosis in septic patients (6), which suggests that there may be an overall increase in the requirement for arginine that is not met by endogenous production in stressed states. The situation will be worse in severely malnourished children stressed by concurrent infections. In a recent study, we reported a marked reduction in plasma

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TABLE 1
Age and physical characteristics of the subjects[1]

	Age[2,3]	Weight[2,3]	Length[2]	Weight-for-age[2,4]	Weight-for-length[2,4]
	mo	*kg*	*cm*	*%*	*%*
Clinical phase 1					
Marasmus ($n = 7$)	11.9 ± 1.5[5]	5.4 ± 0.3[5,6]	65.6 ± 0.2	57.1 ± 1.6[5,6]	76.5 ± 3.0[5]
Kwashiorkor ($n = 7$)	12.9 ± 1.6[5]	7.0 ± 0.5[5]	70.1 ± 2.4	70.5 ± 2.4[5]	84.2 ± 2.8[5]
Marasmic Kwashiorkor ($n = 7$)	9 ± 1.3[5,7]	4.0 ± 0.3[5,6]	58.6 ± 2.4	46.0 ± 3.2[5,6]	77.1 ± 4.3[5]
Clinical phase 2					
Marasmus ($n = 7$)	12.9 ± 1.6[5]	6.6 ± 0.3[5]	67.8 ± 1.8	67.5 ± 2.3[5]	85.4 ± 1.8[5]
Kwashiorkor ($n = 7$)	13.7 ± 1.6[5]	7.0 ± 0.5[5]	70.0 ± 2.4	70.6 ± 2.7[5]	84.6 ± 2.9
Marasmic Kwashiorkor ($n = 7$)	9.9 ± 1.3[5,7]	4.9 ± 0.4[5,6]	59.9 ± 2	53.5 ± 3[5,6]	87.0 ± 3.5
Clinical phase 3					
Marasmus ($n = 7$)	14.3 ± 1.5	8.0 ± 0.5	70.0 ± 1.8	78.5 ± 4.5	95.6 ± 3.6
Kwashiorkor ($n = 7$)	14.5 ± 1.5	9.1 ± 0.6	72.1 ± 1.8	87.4 ± 3.9	101.3 ± 3
Marasmic Kwashiorkor ($n = 7$)	10.8 ± 1.3[7]	6.7 ± 0.6[6]	61.9 ± 1.6	69.9 ± 3.4[6]	106.8 ± 2.8

[1] All values are $\bar{x}$ ± SEM. Cell means were compared by repeated-measures ANOVA. Clinical phase 1,≈ 3 d after admission when the subjects were infected and malnourished; clinical phase 2, ≈15 d after admission when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, ≈55 d after admission when the subjects were recovered.
[2] Main effect of clinical phase, $P < 0.001$.
[3] Diagnosis × clinical phase interaction, $P < 0.05$.
[4] Main effect of diagnosis, $P < 0.05$.
[5] Significantly different from corresponding clinical phase 3 value, $P < 0.001$ (post hoc comparison by Bonferroni method).
[6] Significantly different from kwashiorkor within clinical phase, $P < 0.01$ (post hoc comparison by Bonferroni method).
[7] Significantly different from kwashiorkor and marasmus within clinical phase, $P < 0.05$ (post hoc comparison by Bonferroni method).

arginine concentrations in children with edematous SCU compared with the value of their nonedematous counterparts and the value at recovery (3), which suggests that its availability as a precursor molecule in anabolic reactions may be limiting. This does not seem to be the case with respect to NO synthesis, however, because Fechner et al (7) reported that children with edematous SCU have plasma nitrite plus nitrate (NOx) concentrations, products of NO metabolism, that are twice those of children with nonedematous SCU. Together, these observations suggest an increased conversion of arginine to NO in the face of decreased arginine availability in children with edematous SCU. Hence, we propose to test the hypothesis that at the time they are admitted to the hospital for treatment, children with edematous SCU will have a slower arginine flux but faster NO synthesis than do children with nonedematous SCU. We also hypothesized that arginine flux would be slower in the malnourished state than in the recovered state in the edematous group but not in the nonedematous group.

SUBJECTS AND METHODS

Subjects

Twenty-one children who were admitted to the Tropical Metabolism Research Unit (TMRU), University of the West Indies, for treatment of SCU participated in the study. During their hospitalization, the children were managed according to a standard treatment protocol as previously described by us (2–5). As shown in **Table 1**, each subject had a deficit in body weight–for-age of >20%, which indicated severe undernutrition. Seven of the children had nonedematous and 14 had edematous SCU; 7 had kwashiorkor and 7 had marasmic kwashiorkor (Table 1 and **Table 2**). The diagnosis of type of SCU, ie, marasmus, kwashiorkor, or marasmic kwashiorkor, was based on the Wellcome Classification (8). All except one of the children had evidence of one or more infections at admission.

This study was approved by the Medical Ethics Committee of the University Hospital of the West Indies and the Baylor Affiliates Review Board for Human Subject Research of Baylor College of Medicine. Written informed consent was obtained from at least one parent of each child before enrollment.

Study design

Arginine and NO kinetics were measured 3 times during the children's hospitalization by using a constant intravenous infusion of [$^{15}N_2$]-guanidino arginine at ≈3 d after admission when

TABLE 2
Clinical characteristics of the subjects at admission[1]

Parameter	Edematous subjects	Nonedematous subjects
No. of subjects (n)	14	7
Sex (M/F)	9/5	3/4
Diagnosis	7 K, 7 MK	7 M
Type of infection	3 B, 9 C, 3 D, 2 LRTI, 1 OM, 1 P, 6 URTI, 2 UTI	2 C, 2 D, 1 LRTI, 1 OM, 1 S, 4 URTI, 1 UTI
Infections/subject (n)	3/2, 2/10, 1/2	3/2, 2/2, 1/2, 0/1
Hemoglobin (g/L)	82 ± 3.7[2]	99 ± 7[3]
WBC (1×10^9 cells/L)	11.2 ± 0.88	15.1 ± 1.8[3]
Temperature (°C)	37.19 ± 0.14	37.4 ± 0.38

[1] K, kwashiorkor; MK, marasmic kwashiorkor; M, marasmus; WBC, white blood cells; B, bacteremia; C, Candidiasis; D, infective diarrhea; LRTI, lower respiratory tract infection; OM, otitis media; P, pneumonia; S, scabies; URTI; upper respiratory tract infection; UTI, urinary tract infection.
[2] $\bar{x}$ ± SEM (all such values).
[3] Significantly different from edematous group, $P < 0.05$ (unpaired t test).

the subjects were both infected and undernourished but clinically stable as indicated by blood pressure, pulse, and respiration rates (clinical phase 1); at ≈15 d after admission when the subjects were still severely undernourished (anthropometrically) but no longer infected (ie, all clinical features of the infective episode had resolved), they had lost edema, and had improved affect and appetite (clinical phase 2); and at ≈55 d after admission when the rate of catch-up growth had reached a plateau and weight-for-length was ≥90% of expected (clinical phase 3).

Treatment and diets

During their hospitalization, the children were managed according to a standard protocol that divided their treatment into phases. The acute resuscitation and maintenance phases of treatment extended from admission until the child's appetite returned, infection was cleared, and edema was lost in those children with the edematous forms of severe undernutrition. The mean duration of this phase was 15.4 d. During this period, fluid and electrolyte imbalances were first corrected, and infections were treated with broad-spectrum antibiotics, usually parenteral penicillin and gentamicin, plus oral metronidazole. The children were fed a resuscitative diet made by using a commercial milk powder (61 g Nan; Nestlé SA, Vevey, Switzerland), 81 g glucose, and 858 g water. The energy content of the feed was 2633 kJ/kg with a macronutrient composition per kg feed of 7.6 g protein, 14.6 g lipid, and 116.7 g carbohydrate. The energy distribution of the feed was 74% from carbohydrate, 21% from fat, and 5% from protein. The amount offered aimed to provide ≈418 $kJ \cdot kg^{-1} \cdot d^{-1}$ and ≈1.2 g protein $\cdot kg^{-1} \cdot d^{-1}$. The feed was given as boluses every 3 h throughout the day or as smaller 2-hourly boluses when the child was having problems tolerating the feed.

The next phase in the clinical care of the children was the rapid catch-up growth phase. In this phase of treatment, the children were fed an energy-dense, milk-based formula until their growth rate plateaued and weight-for-length was ≥90% of expected. The high-energy feed given during rapid catch-up growth was made from the same commercial milk powder (NAN; Nestlé SA) plus additional carbohydrate. The energy content was 6071 kJ/kg, and the macronutrient composition per kg feed was 64.8 g lipid, 183 g carbohydrate, and 33.75 g protein. The energy distribution of the feed was 40.2% from fat, 50.5% from carbohydrate, and 9.3% from protein. The children were fed every 4 h ad libitum. During this phase, energy intake may have been as high as 626–750 $kJ \cdot kg^{-1} \cdot d^{-1}$ and protein as high as 3.5–4 $g \cdot kg^{-1} \cdot d^{-1}$.

In addition, both diets were supplemented with vitamins (Tropivite; Federated Pharmaceuticals, Kingston, Jamaica) and a mineral mix prepared in the TMRU metabolic kitchen. Each child received 2 mL/d of the vitamin solution, which contained 6000 IU vitamin A (palmitate), 1600 IU vitamin D (calciferol), 2 mg thiamine HCL, 3.2 mg riboflavin, 120 mg vitamin C (ascorbic acid), 4 mg vitamin B-6 (B-6 HCL), and 28 mg nicotinamide. They also received 5 mg folic acid/d and 2 mL of the mineral mix $\cdot kg^{-1} \cdot d^{-1}$. The mineral mix consisted of 37.28 g KCl + 50.84 mg $MgCl_2O.6H_2O$ + 3.36 g $(CH_3COO)_2Zn.2H_2O$/L H_2O (BDH Chemicals, Poole, United Kingdom). During the rapid catch-up growth phase but not in the maintenance phase, the children also received 60 mg $FeSO_4$. Weight and length were monitored throughout the hospitalization, the former daily with an electronic balance (model F150S; Sartorius, Göttingen, Germany) and the latter weekly with a horizontally mounted stadiometer (Holtain Ltd, Crymych, United Kingdom).

Experimental protocol

The subjects were studied in the postabsorptive state. The isotope infusion started 3 h after the subject's last bolus of feed, and the first blood sample used to measure arginine and NO kinetics was taken 4 h after the infusion started. Hence, arginine kinetics were calculated by using isotopic data obtained from blood samples taken during the 7–9-h postabsorptive period. To avoid possible hypoglycemia during the experimental period, a 0.278-mol/L glucose solution was infused intravenously at 3 $mg \cdot kg^{-1} \cdot min^{-1}$ starting 1 h after the last bolus feed, that is, 2 h before the isotope infusion started.

The rate of synthesis of NO from arginine in the plasma compartment was estimated by determining the fractional rate of synthesis of plasma NOx from arginine and the concentration of NOx in plasma. This approach is feasible on the basis of the fact that ^{15}N -labeled nitric oxide is a product of the reaction in which [$^{15}N_2$]-guanidino arginine is converted to [^{15}N-ureido]citrulline (9). Hence, [$^{15}N_2$]-guanidino arginine is the only possible precursor of ^{15}N-labeled NO. Also, ^{15}N-labeled NOx can only be made from ^{15}N-labeled NO. Therefore, ^{15}NOx is an excellent surrogate measurement of ^{15}NO. By administering [$^{15}N_2$]-guanidino arginine and measuring the isotopic enrichment of plasma arginine at steady state (precursor pool) and the plasma isotopic enrichment of NOx (product), one can calculate the fractional rate of synthesis of nitrite and nitrate, and hence NO, by using the standard precursor-product equation.

Infusion protocol

Sterile solutions of [$^{15}N_2$]-guanidino arginine (98%; Cambridge Isotope Laboratories, Woburn, MA) were prepared in 9 g NaCl/L. Two intravenous access sites were established in opposite arms by the insertion of 24 G catheters after preparation of the access sites with a topical anesthetic (EMLA cream; Astra Pharmaceuticals Ltd, Langley, United Kingdom). One intravenous catheter was used for infusion of the labeled arginine and the other for blood sampling.

After 2 h of continuous glucose infusion, a 3-mL blood sample was drawn for baseline measurements, and a bolus injection of 100 mg of ^2H_2O/kg (99.9%; Cambridge Isotope Laboratories, Woburn, MA) was given intravenously. This was immediately followed by administration of a priming dose of 5 μmol/kg of [$^{15}N_2$]-guanidino arginine, which was followed immediately by a continuous infusion at 5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for 6 h. One-milliliter blood samples were drawn hourly for the first 3 h, and 3-mL blood samples were drawn hourly from hours 3 to 6 of the infusion. The infusion and blood sampling protocols were the same for the 2 subsequent experiments performed at clinical phases 2 and 3.

Sample analyses

The blood samples were centrifuged immediately at 1000 × *g* for 15 min at 4 °C, and the plasma was removed and stored immediately at −70 °C for later analyses. The isotopic enrichment of plasma arginine was measured by negative chemical ionization gas chromatography–mass spectrometry (NCI GC-MS) with a Hewlett-Packard HP 5989B quadrupole mass

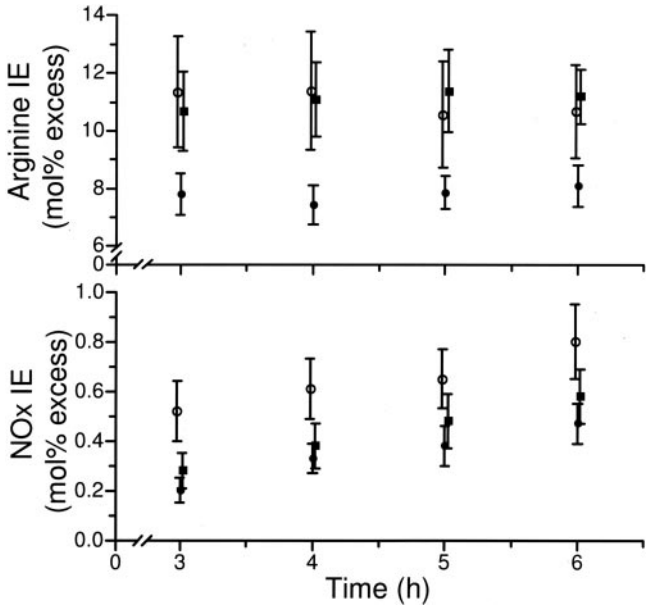

FIGURE 1. Mean (±SEM) isotopic enrichment (IE) of plasma arginine and nitrite plus nitrate (NOx) during a primed-constant intravenous infusion of [$^{15}N_2$]-guanidino arginine in 7 children with marasmus (●), 7 with marasmic kwashiorkor (■), and 7 with kwashiorkor (○) at clinical phase 1, ≈2 d after admission when the subjects were infected and malnourished.

spectrometer (Palo Alto, CA). Plasma arginine was extracted by cation-exchange chromatography, and the trifluoroacetyl ester derivative was prepared by adding 0.4 mL of a 4:1 mixture of dichloroethane:trifluoroacetic anhydride to the dried eluant containing arginine and heating at 100 °C for 2 h. Isotope ratios were measured by selectively monitoring ions at mass-to-charge (*m/z*) ratios of 444 to 446.

The isotopic enrichment of plasma NOx was also determined by NCI GC-MS with the method described by Tsikas (10). Briefly, the nitrate in 0.2 mL of plasma was reduced to nitrite by adding 25 mg cadmium. The mixture was acidified with 0.1 mL of 20% acetic acid and shaken at room temperature for 15 min to reduce nitrate to nitrite. After centrifugation at 1000 × *g* for 5 min, the supernatant fluid was removed, further extracted with acetone, and the nitrite converted to its pentafluorobenzyl derivative by adding 25 μL of 2,3,4,5,6-pentafluorobenzyl bromide and heating at 50 °C for 1 h. The isotope ratio was measured by selectively monitoring ions at *m/z* ratios 46 to 47. The $^2H_2$ content of plasma water was determined by reducing water extracted from 10 μL of plasma with zinc in quartz vessels and determining the $^2H_2$ abundance of the resulting hydrogen gas by gas isotope-ratio mass spectrometry (δ-E; Finnigan MAT, San Jose, CA).

Plasma arginine concentrations were measured by standard ion-exchange chromatography. Plasma NOx concentrations were measured by in vitro isotope dilution as described by Tsikas (10). Briefly, 0.2 mL of the baseline plasma sample was spiked with a known quantity of $Na^{15}NO_3$, the internal standard, the nitrate was reduced to nitrite, and the isotopic enrichment of the nitrite was measured as described above.

Calculations

As shown in **Figure 1**, the isotopic enrichment of arginine reached a plateau in plasma in all 3 groups of subjects during the final 3 h of the infusion, which permitted use of the steady state equation described below to calculate flux.

Arginine flux was calculated from the equation

$$\text{Flux} = [(\text{IE}_{\text{Inf}}/\text{IE}_{\text{pl}}) - 1] \times i \quad (1)$$

where IE_{Inf} and IE_{pl} are the isotope enrichments of the tracer in the infusate and in plasma at isotopic steady state, *i* is the rate of infusion of [$^{15}N_2$]arginine in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (or $\mu\text{mol} \cdot \text{kg}$ fat-free $\text{mass}^{-1} \cdot \text{h}^{-1}$), and the units of flux are $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.

Total body water (TBW) was calculated as follows:

$$\text{TBW (mL)} = (E_{\text{D}_2\text{O}} \times \text{dose})/(E_{\text{pD}_2\text{O}} \times 1.04) \quad (2)$$

where E_{D2O} is the enrichment of the deuterium oxide dose, E_{pD2O} is the plasma water plateau enrichment, and 1.04 is the factor that converts the deuterium dilution space to total water (11).

Fat-free mass (FFM) was calculated as follows:

$$\text{FFM (kg)} = \text{TBW}/K \quad (3)$$

where *K* is the age- and sex-specific hydration constant for FFM as reported by Fomon et al (12). In the children with edematous SCU, total body water measured in the malnourished edematous state (ie, clinical phase 1 measurement) was corrected by subtracting the contribution from edema fluid. Edema fluid was estimated as the difference between body weight on the day of the clinical phase 1 experiment, ≈3 d after admission, and the lowest postexperiment weight observed before the clinical phase 2 measurement, ≈15 d after admission.

The fractional synthesis rate (FSR) of nitric oxide was calculated according to the precursor-product equation as previously described by us (13). Essentially, when a labeled amino acid is given by constant infusion, the precursor pool enrichment (arginine) reaches a constant value with time; thus, by measuring the rate of incorporation of label into a product (NOx) after a plateau is reached in the precursor pool, the FSR can be calculated. As shown in Figure 1, the isotopic enrichment of arginine reached a plateau after 3 h of infusion and NOx enrichment increased linearly during this time. Hence, FSR was calculated from

$$\text{FSR (\%pool/h)} = [(\text{IE NOx}_{t6} - \text{IE NOx}_{t3})/\text{IEarg}_{\text{pl}}] \times (24 \times 100)/t_6 - t_3 \quad (4)$$

where $\text{IE NOx}_{t6} - \text{I.E NOx}_{t3}$ is the increase in isotopic enrichment of NOx over the period from 3 to 6 h ($t_6 - t_3$) of the infusion and $\text{IE arg}_{\text{pl}}$ is the plateau isotopic enrichment of plasma arginine. In this calculation, the plateau enrichment of arginine in plasma is assumed to represent the isotopic enrichment of all the arginine pools from which nitric oxide and hence nitrite and nitrate are synthesized. The absolute synthesis rate (ASR) of plasma NOx in the intravascular compartment was calculated as the product of plasma NOx concentration and the FSR. The units of intravascular ASR are expressed as $\mu\text{mol} \cdot \text{L plasma}^{-1} \cdot \text{h}^{-1}$. Plasma volumes were based on historical values previously reported by us (14)

Statistics

Data are expressed as means ± SEMs. Differences between the nonedematous and edematous groups at clinical phase 1 were determined by use of unpaired 2-tailed *t* tests to test our primary hypothesis that at the time they are admitted to the hospital for

treatment, children with edematous SCU have a slower arginine production rate but faster NO synthesis than do children with nonedematous SCU. To determine the response to treatment by each group and whether differences due to effects of treatment depend on diagnosis, 2-factor repeated-measures analysis of variance (RMANOVA) was used with diagnosis as the between factor and clinical phase as the repeated factor. If the interaction terms from the repeated-measures ANOVA were significant, pair-wise comparisons were made by the Bonferroni method. For the clinical characteristic variables, an unpaired *t* test was used to compare values between the edematous and the nonedematous subjects in the undernourished state. Inferential tests were considered statistically significant if $P < 0.05$. Data analysis was performed with GRAPHPAD PRISM version 4 software (GraphPad Software, San Diego, CA).

RESULTS

There were significant differences in the subjects' mean ages at the time they participated in the clinical phase 1 experiment, with the mean age of the marasmic kwashiorkor group being the lowest. The children had a mean weight-for-age between ≈46% of expected in the marasmic kwashiorkor subjects to ≈71% in the kwashiorkor subjects (Table 1). As expected in the undernourished state, there were significant differences in weight-for-age and weight-for-length by diagnosis. At clinical phase 1, the marasmic and marasmic kwashiorkor children were lighter than the children with kwashiorkor. This difference persisted at clinical phase 2 between the marasmic kwashiorkor and kwashiorkor groups. When the children had recovered at clinical phase 3, all anthropometric measurements except for length increased significantly compared with the values at clinical phase 1.

The clinical characteristics of the subjects at admission are shown in Table 2. All subjects but one were anemic. Twenty of the 21 subjects had one or more infections, but white blood cells were elevated in only 14 subjects. Mean hemoglobin and white blood cell concentrations were significantly higher ($P < 0.05$) in the nonedematous group than in the edematous group.

There was a significant interaction between clinical phase (nutritional status) and diagnosis. Thus, the mean arginine flux of all subjects was significantly slower ($P < 0.001$) at clinical phase 1 than at clinical phases 2 and 3 (**Table 3**). This slower arginine flux at clinical phase 1 was associated with a markedly lower plasma arginine concentration ($P < 0.001$) compared with the values at clinical phases 2 and 3. At clinical phase 2, arginine flux increased by 62% to a value that was almost identical to the value at clinical phase 3. Concurrently, plasma concentrations increased by 77% but remained significantly lower ($P < 0.05$) than the clinical phase 3 value. When the subjects were separated into different groups according to diagnosis, arginine flux remained significantly slower ($P < 0.001$) at clinical phase 1 than at clinical phases 2 and 3 in the marasmic kwashiorkor and kwashiorkor

TABLE 3
Endogenous arginine production and plasma concentration in children with edematous and nonedematous severe undernutrition[1]

	Clinical phase 1	Clinical phase 2	Clinical phase 3
Endogenous arginine flux			
All subjects ($n = 21$)			
(μmol · kg^{-1} · h^{-1})	47 ± 4[2]	77 ± 4	74 ± 5
(μmol · kg FFM^{-1} · h^{-1})	52 ± 4[2]	84 ± 4	85 ± 4
Nonedematous ($n = 7$)[3,4]			
(μmol · kg^{-1} · h^{-1})	65 ± 8	71 ± 6	68 ± 6
(μmol · kg FFM^{-1} · h^{-1})	71 ± 7	78 ± 6	78 ± 6
All edematous ($n = 14$)[3,4]			
(μmol · kg^{-1} · h^{-1})	38 ± 3[2,5]	79 ± 5	80 ± 6
(μmol · kg FFM^{-1} · h^{-1})	42 ± 3[2]	87 ± 6	91 ± 6
Marasmic Kwashiorkor ($n = 7$)[3,4]			
(μmol · kg^{-1} · h^{-1})	42 ± 5[2]	83 ± 9	89 ± 9
(μmol · kg FFM^{-1} · h^{-1})	46 ± 4[2]	92 ± 8	103 ± 9
Kwashiorkor ($n = 7$)[3,4]			
(μmol · kg^{-1} · h^{-1})	35 ± 4[2]	75 ± 7	70 ± 5
(μmol · kg FFM^{-1} · h^{-1})	38 ± 4[2]	83 ± 7	80 ± 5
Plasma arginine concentration (μmol/L)			
All subjects ($n = 21$)	69 ± 6.4[2]	122 ± 6.6[6]	143 ± 6.7
Nonedematous ($n = 7$)[3,4]	91 ± 13[2]	141 ± 13.5	160 ± 10
Marasmic Kwashiorkor ($n = 7$)[3,4]	54 ± 8[2,5]	128 ± 3.7	147 ± 11
Kwashiorkor ($n = 7$)[3,4]	55 ± 7.5[2,5]	99 ± 9.7[2,5]	120 ± 11.6[5]
All edematous ($n = 14$)[3,4]	58 ± 5[2,5]	113 ± 6.2[2,5]	135 ± 8

[1] All values are $\bar{x}$ ± SEM. FFM, fat-free mass; clinical phase 1, ≈3 d after admission when the subjects were infected and malnourished; clinical phase 2, ≈15 d after admission when the subjects were still severely malnourished but no longer infectedand edematous; clinical phase 3, ≈55 d after admission when the subjects were recovered. Within clinical phase 1 (baseline), values were compared by unpaired *t* test; to determine the effect of clinical phase (nutritional status) and diagnosis on outcome, a 2-factor repeated-measures ANOVA was performed with clinical phase as the repeat factor and diagnosis as the between factor.

[2] Significantly different from corresponding clinical phase 2 and 3 values, $P < 0.001$.

[3] Main effect of clinical state, $P < 0.0002$.

[4] Diagnosis × clinical state interaction, $P < 0.0001$.

[5] Significantly different from nonedematous group in the same clinical state, $P < 0.001$.

[6] Significantly different from clinical phase 3 value, $P < 0.001$.

TABLE 4
Plasma nitrate plus nitrite (NOx) concentration and fractional and absolute synthesis rates in children with edematous and nonedematous severe undernutrition[1]

	Clinical phase 1	Clinical phase 2	Clinical phase 3
NOx concentration (μmol/L)			
All subjects ($n = 21$)	78 ± 13.5	70 ± 12.1	77 ± 16
Nonedematous ($n = 7$)	100 ± 28	84 ± 29	95 ± 37
All edematous ($n = 14$)	64 ± 13	64 ± 13	66 ± 14
Marasmic Kwashiorkor ($n = 7$)	82 ± 22	75 ± 16	68 ± 23
Kwashiorkor ($n = 7$)	48 ± 6	50 ± 5	64 ± 12
NOx fractional synthesis rate (% plasma pool/h)			
All subjects ($n = 21$)	1.51 ± 0.25	1.87 ± 0.29	1.75 ± 0.28
Nonedematous ($n = 7$)	1.72 ± 0.36	1.64 ± 0.41	1.29 ± 0.35
All edematous ($n = 14$)	1.50 ± 0.3	1.96 ± 0.4	1.88 ± 0.4
Marasmic Kwashiorkor ($n = 7$)	1.12 ± 0.31	2.09 ± 0.51	1.99 ± 0.53
Kwashiorkor ($n = 7$)	1.83 ± 0.45	1.69 ± 0.44	1.78 ± 0.44
NOx absolute synthesis rate (μmol · L plasma^{-1} · h^{-1})			
All subjects ($n = 21$)	1.29 ± 0.40	1.59 ± 0.47	1.71 ± 0.52
Nonedematous ($n = 7$)	2.29 ± 1.0	2.06 ± 1.18	1.99 ± 1.23
All edematous ($n = 14$)	0.81 ± 0.17	1.26 ± 0.25	1.45 ± 0.42
Marasmic Kwashiorkor ($n = 7$)	0.78 ± 0.21	1.65 ± 0.36	1.38 ± 0.47
Kwashiorkor ($n = 7$)	0.84 ± 0.21	0.92 ± 0.23	1.52 ± 0.618

[1] All values are $\bar{x}$ ± SEM. Clinical phase 1, ≈3 d after admission when the subjects were infected and malnourished; clinical phase 2, ≈15 d after admission when the subjects were still severely malnourished but no longer infected and edematous; clinical phase 3, ≈55 d after admission when the subjects were recovered. Within clinical phase 1 (baseline), values were compared by unpaired *t* test; to determine the effect of clinical phase (nutritional status) and diagnosis on outcome, a 2-factor repeated-measures ANOVA was performed with clinical phase as the repeat factor and diagnosis as the between factor. There were no between-group or within-group significant differences.

groups but not in the nonedematous group. Between groups, at clinical phase 1, flux was significantly slower ($P < 0.001$) in the marasmic kwashiorkor and kwashiorkor groups than in the nonedematous group. These slower fluxes in the marasmic kwashiorkor and kwashiorkor groups at clinical phase 1 were associated with lower plasma arginine concentrations than at clinical phases 2 and 3. In the nonedematous group, although there was no significant difference in arginine flux among clinical phases, the plasma concentration was lower ($P < 0.01$) at clinical phase 1 than at clinical phases 2 and 3. When the marasmic kwashiorkor and kwashiorkor subjects were grouped together, arginine flux was significantly slower ($P < 0.001$) in the edematous group at clinical phase 1 than at clinical phases 2 and 3 and slower than at the corresponding clinical phase 1 value of the nonedematous group. In both groups, arginine flux did not change significantly from clinical phase 2 to 3.

There was no statistically significant effect of clinical phase or diagnosis on nitric oxide outcome measures. When all subjects were grouped together, there was no significant difference in plasma NOx concentration or fractional or absolute synthesis rates among clinical phases (**Table 4**). Similarly, when the subjects were separated into different groups according to diagnosis, there were no significant differences in plasma NOx concentrations or fractional or absolute synthesis rates between the different groups. None of these parameters changed significantly in response to treatment.

DISCUSSION

In this study, we aimed to test the hypothesis that at the time they are admitted to the hospital for treatment, children with edematous SCU will have a slower arginine flux but faster NO synthesis rate than do children with nonedematous SCU, and that arginine flux will be slower in the malnourished state than in the recovered state in the edematous group but not in the nonedematous group. Our results show that arginine flux was slower in children with edematous SCU than in children with nonedematous SCU at clinical phase 1, when the children were both severely undernourished and infected. At clinical phase 1, arginine flux was slower in the children with edematous SCU than at clinical phase 2, when the children were still anthropometrically undernourished but their infections were cured, and at clinical phase 3, when they were recovered. This difference in arginine flux was not present, however, in children with nonedematous SCU. In both the edematous and the nonedematous SCU groups, there were no significant differences in plasma NOx concentration or fractional or absolute synthesis rates among clinical phases. These results suggest that children with edematous SCU cannot maintain arginine production in the undernourished and infected state but those with nonedematous SCU can. Finally, all children with SCU can maintain NO synthesis rates despite the slower arginine production of those with edematous SCU when they are infected and undernourished.

In humans, arginine is regarded as a semi-essential amino acid, meaning that it is produced in adequate quantities to meet the requirements of healthy adults but not of growing children or persons stressed by severe trauma or sepsis (6). Because its total flux depends on dietary intake, release from protein breakdown, and de novo synthesis, it is highly likely that arginine will be in short supply in children with SCU. Our data, however, show that this is only true for children with edematous SCU. In children with nonedematous SCU, arginine flux was not slower in the undernourished state than in the recovered state. However, despite maintaining arginine flux at a rate not significantly different from the rate at recovery, the plasma arginine concentration was lower than the value at recovery, which suggests that arginine

utilization was greater than production in the infected undernourished state. In past studies, we reported that whole-body protein breakdown, the major contributor to overall amino acid flux, was not diminished in children with marasmus (2, 5), which indicates that they may still be able to maintain arginine flux in the infected and undernourished state. There is evidence, however, that arginine utilization and catabolism are increased in patients with systemic infections and that this is associated with lower plasma concentrations, which suggests that flux cannot keep up with utilization in this condition (6, 13). Because 6 of the 7 marasmic children had clinical evidence of infections at clinical phase 1, our finding of a lower plasma arginine concentration suggests that these children could not increase de novo arginine synthesis to compensate for inadequate dietary intake and also to meet any increased demands due to their infections.

Whereas the children with marasmus did not have a slower arginine flux when undernourished and infected, those with edematous SCU did. In the latter group, arginine flux was ≈50% slower than at clinical phases 2 and 3. On the basis of our finding that the whole-body protein breakdown rate was markedly slower in children with edematous SCU when they were undernourished than when they were recovered (2, 5), it is expected that these children will have slower fluxes of most amino acids at clinical phase 1. In the case of the dietary nonessential amino acids, however, it is possible for de novo synthesis to fill the gap created by any reduction in the amount released from protein breakdown. We have found this to be true for glycine and serine, but not for cysteine, a nonessential amino acid that depends on the availability of the essential amino acid methionine for de novo synthesis (3–5). Our present findings indicate that in the case of the semi-essential amino acid arginine, de novo synthesis was not sufficient to fill the shortage created by inadequate dietary intake plus decreased release from protein breakdown when the children with edematous SCU were severely undernourished and infected. Furthermore, the lower plasma arginine concentration at clinical phase 1 suggests that flux of arginine was not sufficient to meet requirements in the infected undernourished state. Similarly, at clinical phase 2, although arginine flux doubled to a rate almost identical to the rate at recovery, the plasma pool was still not fully replenished. That is, despite a concurrent increase of 107% in the plasma arginine concentration from clinical phase 1 to 2, it was still lower than the value at recovery. Hence, flux was still not enough to meet requirements and to replenish the plasma pool. In the case of subjects with kwashiorkor, at recovery, plasma concentrations were still lower than the corresponding values of the subjects with marasmus, which suggests that the plasma pool was still not fully replenished or that a systemic difference exists between the 2 types of SCU. Finally, it is very likely that increased de novo synthesis contributed to the increased arginine flux at clinical phase 2, because in a previous study we reported that the protein breakdown rate is still slower at clinical phase 2 than at recovery in children with edematous SCU. If true, this would suggest that in the children with edematous SCU, de novo arginine synthesis was impaired in the infected undernourished state.

Besides its importance for protein synthesis, ammonia detoxification, wound healing, and immune function, arginine as a precursor for NO synthesis serves important roles in the regulation of blood pressure plus the numerous other regulatory roles attributed to NO (6). Low plasma arginine and increased NO production have been reported in septic patients (6), which suggests an increased conversion of arginine to NO despite reduced arginine availability. The findings of Fechner et al (7) of a 100% higher plasma NOx concentration in children with kwashiorkor than in healthy controls suggest that the same phenomenon may exist in children with edematous SCU and infections. The findings of the present study do not corroborate those of Fechner et al (7), because we found no differences in plasma NOx concentration or fractional or absolute synthesis rates from the severely undernourished and infected state to the recovered state in the children with edematous SCU. The same was true for the children with nonedematous SCU. Similarly, although arginine flux was ≈50% slower in the children with edematous SCU than in the children with nonedematous SCU in the severely undernourished and infected state, there were no significant differences in plasma NOx concentration or fractional or absolute synthesis rates, which suggests that there was still sufficient arginine to support NO synthesis in the children with edematous SCU. For example, NOx synthesis in the kwashiorkor group at clinical phase 1 was 0.84 μmol · L plasma^{-1} · h^{-1}, which, assuming 70 mL plasma/kg at this stage, translates to 0.059 μmol · kg^{-1} · h^{-1}. This amount of NO will require the metabolism of just 0.16% of the 35 μmol · kg^{-1} · h^{-1}of arginine being produced by these subjects. If anything, these results in the kwashiorkor group are opposite those reported by Fechner et al (7), which suggests that increased NO production may not be contributing to the oxidative stress of children with kwashiorkor.

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