Urea Production and Leucine Oxidation in Malnourished Children With and Without Acute Infection

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We compared the kinetics of urea production and leucine oxidation in severely malnourished Malawian children. We tested the hypotheses that the rate of urea production was directly proportional to the rate of leucine oxidation and that the relationship between the two is altered by acute infection. Thirty-six marasmic children, aged 12 to 60 months, were enrolled; 26 had acute infection and 10 did not. The rates of urea and CO_2 production were estimated using primed, constant, intravenous stable isotope-labeled tracer infusions followed by intermittent sampling of breath and blood. The rate of urea production was greater in infected children when compared to uninfected children (169 ± 85 *v* 105 ± 44 μmol urea · kg^{-1} · h^{-1}, $P < .02$). For children with and without infection, the rates of leucine oxidation and urea production were directly correlated ($r = 0.49$ and $r = 0.74$, respectively; $P < .01$), but the slopes of the regression lines were different. In uninfected children the degree of wasting was correlated with the rates of urea production and leucine oxidation ($r = 0.67$ and $r = 0.48$, respectively; $P < .05$). These data suggest that the rates of leucine oxidation and urea production are both measures of nitrogen catabolism, that acute infection alters the relationship between the two, and that less nitrogen is lost as urea in children with more wasting.

TECHNIQUES TO estimate the rate of amino acid oxidation are important research tools in the assessment of the metabolic response to physiologic stress, nutritional status, diet, and pharmacologic interventions. Stable isotope methodologies using ^{13}C-leucine and $^{15}N_2$-urea have been used by a number of investigators to estimate the rate of amino acid oxidation.[1-3] Most methods that use leucine to measure the rate of amino acid oxidation require the cooperation of the research subject for quantitative collections of expired CO_2 and the calculations assume that leucine is oxidized in a similar proportion as other amino acids.[1] Measurement of the rate of urea production requires only a small blood specimen during a steady-state infusion,[2] or a more complete urine collection after an oral dose of urea,[4] but the calculations assume that all urea produced is excreted—an unwarranted assumption if colonic bacteria are recycling nitrogen into amino acids. The challenge of estimating the rate of amino acid oxidation in ill, young children is compounded by the limits of their cooperation and the severity of their clinical condition. Consequently, very little is known about this important metabolic parameter in this population vulnerable to infection and malnutrition. This study used a methodology that obviated the need for a quantitative gas collection to determine the leucine oxidation rate and compared this with the rate of urea production in marasmic children age 1 to 5 years with and without acute infection. The study tested the hypothesis that in this population the rate of urea production is directly correlated with the rate of leucine oxidation, and that this correlation is different for children who do and do not have acute infection.

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MATERIALS AND METHODS

Children aged 12 to 60 months with marasmus admitted to Queen Elizabeth Central Hospital in Blantyre, Malawi were eligible. Each child was admitted to a special metabolic ward, which provided more intensive nursing care, better parenteral antibiotics, more frequent feedings, and more careful clinical monitoring than the hospital ward. The initial evaluation of these children included blood culture, urine culture obtained by sterile catheter, chest x-ray, thick blood smear for malaria parasites, and an enzyme-linked immunoabsorbant assay for human immonodeficiency virus (HIV) infection (Vironostika HIV, Organon Teknika, Durham, NC). The diet provided 418 kJ · kg^{-1} · d^{-1} (78 kcal) and 1.5 g protein · kg^{-1} · d^{-1}. The recipe for the feeding was 40 g full cream milk powder, 40 g of corn oil, and 26 g of sugar mixed in 1 L of water. All children received a mineral multivitamin supplement (Nutriset, Malauney, France).[5] Feedings were administered in equal amounts per kilogram body weight every 2 hours, throughout the day and night, and children unable to take feedings by mouth were fed through a nasogastric tube. Every child received parenteral ceftriaxone for the first 48 hours after admission. Acute infection was defined by a persistent fever, clinical signs of sepsis with a positive blood or urine culture, clinical signs of falciparum malaria with a positive smear for malaria parasites, or cough and tachypnea with a focal infiltrate on chest x-ray. The infections were believed to be acute because by the caretaker's report each child's clinical condition had worsened within the day prior to admission. The study was approved by the Human Studies Committee of Washington University in St Louis, MO and by the College of Medicine Research Committee of the University of Malawi.

Nineteen hours after admission each child's CO_2 appearance rate was determined with a primed (2.5 μmol · kg^{-1}), constant (5 μmol · kg^{-1} · h^{-1}) infusion of ^{13}C-sodium bicarbonate[6] (99% ^{13}C; Cambridge Isotopes, Andover, MA). Before starting the ^{13}C-sodium bicarbonate infusion, 2 breath samples were collected using a silicone rubber face mask connected to a nondiffusing gas collection bag with a 2-way non-rebreathing valve (Hans Rudolph, Kansas City, MO) to determine the baseline $^{13}CO_2$ isotope abundance. Breath samples were collected 60, 65, and 70 minutes after the initiation of the ^{13}C-sodium bicarbonate infusion for the measurement of the CO_2 appearance rate. Immediately following the collection of these breath samples, a primed constant intravenous infusion of $^{15}N_2$-urea (98% $^{15}N_2$; Cambridge

Table 1. Characteristics of the Marasmic Children With and Without Acute Systemic Infection

	With Acute Infection	Without Acute Infection
Male/female	8/18	4/6
Age (mo)	31 ± 12	25 ± 6
Weight-for-age *Z* score	−4.08 ± 0.62	−3.91 ± 0.51
Height-for-age *Z* score	−3.38 ± 1.02	−3.25 ± 1.26
Weight-for-height *Z* score	−2.87 ± 0.60	−2.74 ± 0.86
No. with HIV infection	14	6
No. with		
Pneumonia 14		
Malaria 12		
Urinary tract infection 9		
Sepsis 5		

NOTE. Data are means ± SD. None of the differences between children with and without acute infection were of statistical significance.

Isotopes) and 1-^{13}C-leucine (99% ^{13}C; Cambridge Isotopes) was begun.[7] Each child received $^{15}N_2$-urea (prime 21.5 μmol/kg, infusion 2.15 μmol · kg^{-1} · h^{-1}) and 1-^{13}C-leucine (prime 15.3 μmol/kg, infusion 4 μmol · kg^{-1} · h^{-1}) for 5 hours. During the 5-hour infusion children received feedings in small aliquots every 30 minutes. The amount of each feed was such that it provided the same rate of dietary energy intake (418 kJ · kg^{-1} · d^{-1}) as the 2-hour feedings. This was done so that the metabolic measurements would be representative of the fed state rather than the postabsorptive state. At 4.5 and 5 hours after the start of the urea/leucine infusion, 24 hours after admission and the initiation of feedings, a 1-mL blood sample was drawn to measure isotopic abundance of urea and α-ketoisocaproic acid. Three breath samples were collected 4.5. 4.75, and 5 hours after the start of the urea/leucine infusion for the measurement of $^{13}CO_2$ isotope abundance.

In 20-mL aliquots of breath, $^{13}CO_2/^{12}CO_2$ abundance was measured using an automated gas isotope ratio mass spectrometer (Finnigan MAT Delta+ XL, Bremen, Germany).[8]

Plasma α-ketoisocaproic acid, the intracellular deamination product of leucine, was used to estimate intracellular ^{13}C-leucine enrichment.[1] Serum samples were analyzed by gas chromatography electron impact–quadrupole mass spectrometry after the urea was converted to its *t*-butyldimethylsilyl derivative and α-ketoisocaproic acid was converted to its quinoxalinol trimethylsilyl derivative.[7]

The rates of appearance (Ra) of urea and CO_2 calculated from the following equation, derived from a simple mass balance[1]:

$$\text{Ra} = [(\text{Ei/Ep}) - 1] \times \text{I}$$

where Ei is the isotopic enrichment of the tracer infused (98% to 99%), Ep is the isotopic enrichment of the tracer in serum or breath, and I is the infusion rate of the tracer.

The rate of leucine oxidation was estimated from the following equations[1]:

$$\text{Rate Leu Ox} = (\text{rate of } ^{13}CO_2 \text{ appearance in breath}) \times (\text{fraction of leucine that is labeled with } ^{13}\text{C})/0.81$$

$$\text{Rate Leu Ox} = (Ra_{CO2})(\%\ ^{13}CO_2 \text{ Enrichment in breath}) \times (\text{Ei/Ep} - 1)/81$$

where Ei is the isotopic enrichment of the leucine infused (98% to 99%), and Ep is the isotopic enrichment of the α-ketoisocaproic acid in plasma. The factor 0.81 accounts for the fraction of CO_2 that is produced by leucine oxidation, but not released from the body bicarbonate pool into the breath.

Nutritional status was assessed by anthropometric *Z* scores, a comparison of the child's weight, height, and weight-for-height to the World Health Organization's reference population.[9] Weight-for-height *Z* score is a measure of wasting, with 0 representing no difference between the individual and the reference population, -1 placing the individual 1 SD below the mean, and so forth. Anthropometric *Z* scores were calculated using Epi Info Version 6 (WHO/Centers for Disease Control, Atlanta, GA). Stepwise linear regression analyses of the rates of urea appearance and leucine oxidation with respect to age, sex, anthropometric indices, acute infection, HIV infection, and clinical characteristics such as duration of diarrhea were used to determine which of these parameters significantly influenced nitrogen catabolism (SPSS Professional edition for Windows version 10, Chicago, IL). Linear regression was used to model the relationship between the rates of urea appearance and leucine oxidation. Pearson's correlation was used to test for associations between variables. Comparisons between children with and without acute infection were made using Student's *t* test. Statistical differences of $P < .05$ were considered to be significant.

RESULTS

Thirty-six children were enrolled in August 2000 and from February to April 2001, 26 with acute infection and 10 were uninfected. Pneumonia and malaria were the 2 most common acute infections (Table 1).

To verify that a steady-state isotopic enrichment was achieved during the ^{13}C-bicarbonate infusion, a prolonged infusion with frequent breath sampling to measure the $^{13}CO_2$ isotopic abundance was conducted (Fig 1). Figure 1 also demonstrates that the $^{13}CO_2$ abundance returns to baseline within 2 hours after the infusion was stopped. The variation in ^{13}C abundance in the 2 initial baseline breath samples obtained was 970 ± 1,320 parts per billion (ppb, mean ± SD), and the variation in 2 breath samples obtained at 65 and 70 minutes during the ^{13}C-bicarbonate infusion was 940 ± 1,310 ppb. This similarity suggests that the bicarbonate tracer had equilibrated

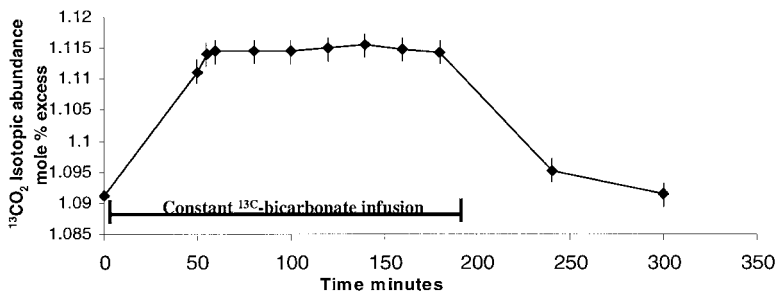

Fig 1. Plot of breath $^{13}CO_2$ isotopic abundance *v* time during a primed constant infusion of ^{13}C-bicarbonate. Isotopic steady-state is reached after 60 minutes. Brackets around each point indicate SEM.

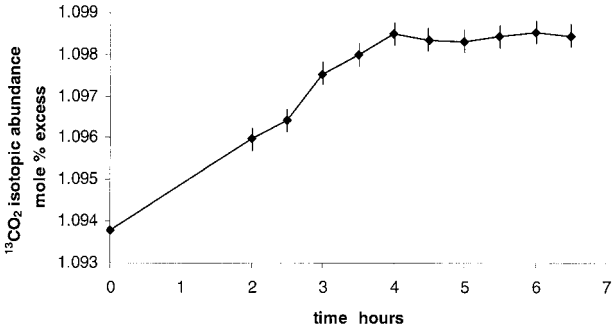

Fig 2. Plot of breath $^{13}CO_2$ isotopic abundance versus time during a primed constant infusion of ^{13}C-leucine. Isotopic steady state is reached after 3.5 hours. Brackets around each point indicate SEM.

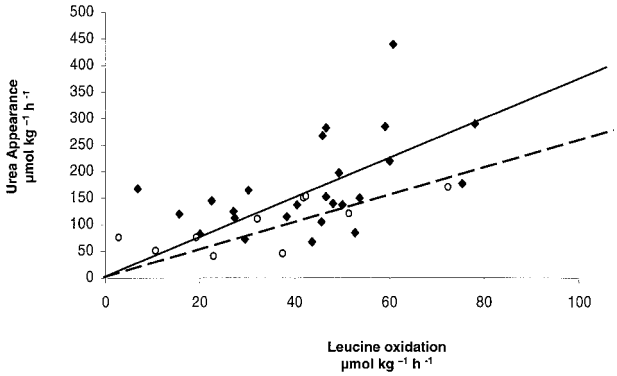

Figure 3. Plot of the rate of urea appearance versus leucine oxidation for 36 children with marasmus with (◆ or without (○) acute infection displaying the direct correlation between the 2 metabolic parameters. Best fit regression lines are shown for children with and without infection are shown; the data are separated for children with and without acute infection because linear regression modeling indicates that infection significantly affects the slope of the regression line ($P < .05$).

in the bicarbonate pool within 60 minutes. Breath samples collected during a ^{13}C-leucine infusion and analyzed for $^{13}CO_2$ isotopic abundance demonstrated that a steady-state had been achieved within 4 hours (Fig 2). The variation in ^{13}C abundance in 2 baseline breath samples was 970 ± 1,320 ppb, and the variation in 2 breath samples obtained during a ^{13}C-leucine infusion at 4.75 and 5 hours was 770 ± 1,080 ppb, corroborating that the infusion has reached a steady-state. Because of ethical and clinical considerations, only 2 blood samples for $^{15}N_2$-urea isotopic abundance could be obtained, at 4.5 hours and at 5 hours. The mean urea enrichment at 4.5 hours was 1.81 mole percent excess and at 5 hours was 1.83 mole percent excess; the mean variation between the 4.5- and 5-hour samples was 2% and was greater than 5% in only 4 pairs of samples. Greater urea enrichment was found at 4.5 hours in 17 of the sample pairs, and greater urea enrichment was found at 5 hours in 17 of the sample pairs; in 2 of the pairs the urea enrichment was the same.

The rate of CO_2 production for the 36 children was 29.0 ± 5.5 mmol · kg^{-1} · h^{-1}. The food quotient of the diet, the ratio of CO_2 produced to O_2 consumed when the food is fully oxidized, was 0.79. Assuming the respiratory quotient was the same as the food quotient, and using the Wier equation[10] for estimating total energy expenditure, the rate of CO_2 production corresponded to 444 kJ · kg^{-1} · d^{-1}. This estimate of total energy expenditure is similar to the dietary energy intake of 418 kJ · kg^{-1} · d^{-1}.

Regression analyses indicated that only the presence or absence of acute infection was a significant predictor of the rates of urea appearance or leucine oxidation. In particular, HIV infection was not associated with increased rates of urea appearance or leucine oxidation (Table 2). The r values in the regression models were improved by less than 1% when HIV status was included in the model. When children with acute infection were compared to those without acute infection, they had higher rates of urea appearance (169 ± 85 *v* 105 ± 44 μmol urea · kg^{-1} · h^{-1}, $P < .02$).

The rates of urea appearance and leucine oxidation were directly correlated in the 26 children with acute infection ($r = 0.49$ $P < .01$) and in the 10 children without acute infection ($r = 0.74$, $P < .01$). A linear regression model best described the relationship between the rates of urea production and leucine oxidation in these children. For children with or without acute infection, the slopes of the lines were 3.7 and 2.6 mol urea/mol leucine, respectively (Fig 3; 95% confidence intervals of slopes, ± 0.5). For the children without acute infection, the rates of urea appearance and leucine oxidation were directly correlated with weight-for-height Z score ($r = 0.67$ and $r = 0.48$, respectively; $P < .05$).

DISCUSSION

This study describes the successful application of a stable isotope tracer method to directly measure the rate of leucine oxidation in critically ill, young children in a modest metabolic unit in southeast Africa. The rate of leucine oxidation was found to directly correlate with the rate of urea appearance, another measure of nitrogen catabolism. The presence or absence of acute infection altered the mathematical relationship between the rate of leucine oxidation and urea appearance, suggesting that nitrogen catabolism differs in these two physiologic states.

Table 2. Rates of Leucine Oxidation and Urea Appearance in Children With and Without HIV Infection

	Children Infected With HIV	Children Without HIV Infection
Urea appearance in children with acute infection (μmol urea · kg^{-1} · h^{-1})	163 ± 68 (n=14)	175 ± 99 (n=12)
Urea appearance in children without acute infection (μmol urea · kg^{-1} · h^{-1})	86 ± 47 (n= 6)	133 ± 21 (n= 4)
Leucine oxidation in children with acute infection (μmol leucine · kg^{-1} · h^{-1})	41.2 ± 17.5 (n=14)	46.3 ± 17.9 (n=12)
Leucine oxidation in children without acute infection (μmol leucine · kg^{-1} · h^{-1})	27.7 ± 24.9 (n= 6)	42.1 ± 7.8 (n= 4)

NOTE. Data are mean ± SD. None of the differences between children with and without HIV was of statistical significance.

One of the primary assumptions upon which the calculations are based is that the stable isotope tracers were infused in such a manner that they had reached a steady-state, the rate of appearance of tracer was equal to the rate of disappearance. Direct evidence for this is presented for the $^{13}CO_2$ and the ^{13}C-leucine infusions. Because of the nature of the clinical subjects, indirect evidence was relied upon to validate this assumption for the $^{15}N_2$-urea infusion. Average ^{15}N-urea enrichment did not increase or decrease when the 4.5-hour and 5-hour measurements were compared. In addition, the same infusion protocol has been used in malnourished, ill Malawian children and no difference in urea enrichment was found over the period of 4.5 hours to 7 hours after the start of the infusion.[11] Jahoor and Wolfe thoroughly investigated issues surrounding the priming dose and constant infusion of $^{15}N_2$-urea in the study of urea production in humans and found that a steady-state isotopic enrichment was achieved after 2 hours and that the model was resilient to a variety of priming doses.[12] This infusion protocol has been used by other investigators investigating ill children and adults and they also have provided evidence to support the steady-state assumption.[2,3] This study was conducted in a clinical scenario where nitrogen recycling is likely to be very small, since all children (with or without acute infection) were receiving broad spectrum parenteral antibiotics, which limits the growth of gastrointestinal bacterial flora. In particular ceftriaxone, the agent used in this study, reduced aerobic and anaerobic fecal bacteria by 2 to 7 log factors dependent on the species within 24 hours, suggesting that greater than 99% of colonic bacteria were eliminated.[13] This assumption may not be valid in other clinical scenarios where the colonic bacteria can contribute amino acids to the nitrogen pool, and the rate of urea appearance may differ from the rate of urea production.[14] Caution should be exercised in applying the specific findings about the rates of urea appearance and leucine oxidation in other clinical populations consuming different diets.

The data demonstrate that the mathematical relationship varies with the clinical condition, relatively more leucine is oxidized when children have acute infection. It is not simply a matter of greater whole-body proteolysis during acute infection. If it were there would be no difference in the slopes of the regression lines for infected and uninfected children. Nor is it that the acute phase proteins associated with acute infection contain less leucine than visceral proteins. In fact, estimates of amino acid composition suggest that the leucine content of acute phase and visceral protein is similar.[15] Instead, the increased leucine oxidation relates to the complete complement of amino acids available from the diet and proteolysis for protein synthesis, and infers that there is a greater excess of leucine in this complement during acute infection. Other investigators have demonstrated that the rate of leucine oxidation is directly proportional to the rate of urea appearance in healthy, well-nourished adults.[16] The slope of their regression line is different than those found here; further evidence that the relationship between the rates of leucine oxidation and urea production varies with physiologic status.

The rate of leucine oxidation has been shown to vary with the leucine content in the diet, whereas the rate of urea appearance varies with the total nitrogen content of the diet.[17] In this study, the fraction of leucine in the diet was substantially greater than the fraction of leucine in body protein (4.77 *v* 3.817 mmol leucine/g N, respectively)[18], and thus it is reasonable to expect that relatively more leucine was oxidized than other dietary amino acids. We have conducted 3 previous studies of Malawian children with severe-protein energy malnutrition and acute infection where urea appearance has been measured using the same infusion protocol; one in the fasted state and the other two while receiving 1.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1}, less dietary protein than the children received in this study.[7,11,19] The mean rate of urea appearance was 56 $\pm$ 35 μmol urea $\cdot$ kg^{-1} $\cdot$ h^{-1} in the fasted state (mean $\pm$ SD), 117 $\pm$ 76 μmol urea $\cdot$ kg^{-1} $\cdot$ h^{-1} for those receiving 1.2 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1}, and 169 $\pm$ 85 μmol urea $\cdot$ kg^{-1} $\cdot$ h^{-1} for those receiving 1.5 g protein $\cdot$ kg^{-1} $\cdot$ d^{-1}. This demonstrates that for children with a similar clinical condition, increased dietary nitrogen intake is associated with increased urea production.

It is interesting to note that HIV infection itself did not accelerate the rate of leucine oxidation or urea production in malnourished children. This observation is consistent with the data of 2 previous studies in which the rate of urea appearance in children with severe protein-energy malnutrition and acute infection in Malawi was determined. Together, these previous studies included 55 children, 19 with HIV infection. The rate of urea appearance in children with and without HIV infection was similar (126 $\pm$ 96 *v* 113 $\pm$ 65 μmol urea $\cdot$ kg^{-1} $\cdot$ h^{-1}, P = .55). These findings are in contrast to studies done among well-nourished adults with asymptomatic HIV infection, which conclude that HIV infection increases the rate of amino acid oxidation and protein turnover.[8] The difference is likely to be due to the differences in nutritional status of the populations studied. The children in the current study were severely malnourished and markedly wasted, while the adults with HIV were treated, well-nourished, and asymptomatic. The direct correlation between either the rate of urea appearance or leucine oxidation and the weight-for-height Z score in the children currently studied suggests that with more severe wasting, there is greater conservation of amino acids (less oxidative disposal). In malnourished children, perhaps the greater amino acid conservation counterbalances the generalized proinflammatory induced protein catabolism seen with HIV infection.

In conclusion, we describe a method for estimating the rate of leucine oxidation which is particularly well suited for use in ill, young children. The rates of leucine oxidation and urea production are interrelated estimates of amino acid oxidation. The relationship between the two is determined by both physiologic state (the presence or absence of acute infection) and dietary protein intake.

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