

ORIGINAL COMMUNICATION

CO₂ production during acute infection in malnourished Malawian children

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Objective: This study tested the hypotheses that the rate of CO₂ production is less in marasmic children with acute infection when compared to well-nourished children, but greater when compared to uninfected marasmic children.

Design: A descriptive comparison of children aged 12–60 months who had their rates of CO₂ production measured using a stable isotope tracer dilution method while receiving feedings. Body mass index (BMI) was the best measure of lean body mass available in this study.

Setting: Queen Elizabeth Central Hospital, Blantyre, Malawi.

Subjects: A total of 56 children were studied, 28 with marasmus and acute infection, 16 with marasmus, and 12 well nourished with acute infection. Those with acute infection had malaria, pneumonia, or sepsis.

Results: Well-nourished children with acute infection produced more CO₂ than marasmic children (344 ± 60 vs 225 ± 65 mmol CO₂/h, mean \pm s.d., $P < 0.001$; 24.2 ± 4.6 vs 18.4 ± 5.4 mmol CO₂/BMI h, $P = 0.001$). However, the rate of CO₂ production in marasmic children with acute infection was not greater than in uninfected marasmic children (225 ± 65 vs 228 ± 61 mmol CO₂/h). The observed rate of CO₂ production was greater than that which could be produced from the dietary intake alone (29.6 vs. 25.8 mmol CO₂/kg h).

Conclusions: Marasmic children do not increase energy expenditure in response to acute infection, as well-nourished children do. Dietary energy provided to marasmic children should be at least 420 kJ/kg day.

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Introduction

Protein-energy malnutrition (PEM) and acute systemic infection are often coexistent and synergistic in children

(Scrimshaw & SanGiovanni, 1997). In adults, these two physiologic states are important determinants of total energy expenditure; acute infection increases energy expenditure and malnutrition decreases it (Shetty, 1984; Kinney, 1995). Understanding how the coexistence of PEM and acute infection alters energy metabolism in children is important for dietary recommendations and may give insight into the interaction between nutrition and immune status. Unfortunately, very little is known about energy metabolism during acute physiologic stress in young children, primarily because of the complexity and challenges in measuring energy expenditure.

Primary PEM usually occurs in poor populations in the developing world, far removed from the clinical research and intensive care units where studies of energy expenditure are often conducted. We utilized a less cumbersome, stable isotope tracer (¹³C-sodium bicarbonate) dilution method to measure the rate of CO₂ production and

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predict energy expenditure in children with PEM in Malawi (Kien & McClead, 1996). This study tested the hypotheses that the rate of CO₂ production is less in marasmic children with acute infection when compared to well-nourished children, but greater when compared to uninfected marasmic children.

Subjects and methods

Subjects

Children aged 12–60 months with marasmus admitted to Queen Elizabeth Central Hospital in Blantyre, Malawi were eligible. Marasmus was defined as weight-for-age <60% of the reference population (Wellcome Trust Working Party, 1970). Each child was admitted to a special metabolic ward where more intensive nursing care, better parenteral antibiotics, and more frequent feedings were provided. 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 HIV (Vironostika HIV, Organon Teknika, Durham, NC, USA). Every child received parenteral ceftriaxone (Roche Laboratories, Nutley, NJ, USA) for the first 48 h after admission. Body temperature was measured in the axilla, fever was defined as body temperature >38°C. Acute infection was defined as having one of these three clinical conditions: sepsis, clinical signs of sepsis with a positive blood or urine culture; malaria, clinical signs of *Falciparum malaria* with a positive smear for malaria parasites; or pneumonia, cough and tachypnea with a focal infiltrate on chest X-ray. The infections were believed to be acute because each caretaker reported that the child's clinical condition had worsened within the day prior to admission. Children with malaria were treated with parenteral quinine on admission, 19 h prior to the metabolic study. As a comparison group, Malawian children of the same age with weight-for-age indices within the norms of the World Health Organization's reference population (weight-for-age within 2Z-scores of the mean) and acute infection were enrolled. Standard Malawian pre- and post-HIV test counseling was given to all of the caretakers. The study was approved by the Human Studies Committee of Washington University in St Louis, MO and the College of Medicine Research Committee of the University of Malawi.

Diet

The metabolic ward diet provided 350 kJ/kg day (85 kcal) and 1.2 g/kg day of protein to all children. Each feeding contained 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 added to the feeding (Nutraset, Malauney, France). Feedings were administered in equal amounts per kg body weight every 2 h, and children unable to take feedings by mouth were fed through a nasogastric tube.

Measurement of CO₂ production

The rate of CO₂ production was measured using a stable isotope tracer dilution technique, where a known quantity of ¹³C-enriched sodium bicarbonate is infused at a constant rate until a steady state of ¹³C isotopic enrichment is achieved. The rate of CO₂ production can then be calculated assuming the conservation of mass for ¹³C. At 19 h after admission, each child's CO₂ appearance rate was determined using a primed (2.5 μmol/kg) constant (5 μmol/kg h) intravenous infusion of ¹³C-sodium bicarbonate (99% ¹³C; Cambridge Isotopes, Andover, MA, USA) as previously described (Manary *et al*, 2002). The timing of the measurement of the CO₂ production was chosen so that the children were still subject to the acute physiologic stress of infection, but had time to habituate to the feeding regimen. Breath samples were collected using a silicone rubber face mask connected to a nondiffusing gas collection bag, transferred to evacuated glass tubes, and transported to laboratories in St Louis taking care not to expose the samples to low pressure or temperature. Breath samples were collected immediately prior to the initiation of the infusion and after 60, 65, and 70 min.

In 10–20 ml aliquots of breath, ¹³CO₂/¹²CO₂ abundance was measured using an automated gas isotope ratio mass spectrometer (Finnigan MAT Delta + XL, Bremen, Germany; Yarasheski *et al*, 1998).

Calculations

The rate of CO₂ production (Ra_{CO₂}) was calculated from the following equation (Matthews *et al*, 1980): $Ra_{CO_2} = [(Ei/Ep - 1) \times I] / 0.81$, where Ei is the isotopic enrichment of the tracer infused (99%), Ep is the isotopic enrichment of the tracer in breath CO₂, and I is the infusion rate of the tracer. The factor 0.81 accounts for the fact that all CO₂ produced is not expired in breath, but some is incorporated into other metabolic substrates (James *et al*, 1976; Spear *et al*, 1995).

It was expected that the rate of CO₂ production, expressed in mmol/h, would be greater for children of increasing size; so the data were normalized to a measure of metabolically active tissue. Typically, normalization to body weight is most applicable when the body composition of the subjects is similar (Shetty *et al*, 1994). In this study, body weight was not appropriate because groups of children with distinctly different body compositions were compared, marasmic and well-nourished children. Lean body mass correlates with energy expenditure in undernourished children and would have been most suitable for normalizing these data (Salas *et al*, 1990). However, the only surrogate measure of leanness available to us was body mass index (BMI, kg/m²). Fortunately, surface area and weight-for-height (% of reference population) correlate with lean body mass (Hamberger & Lundgren, 1965; Zemel *et al*, 1997). Sedentary daily energy expenditure in infants and children has been noted to be a power function of body weight (Butte *et al*, 2000), and the mathematical relation that best models this is energy expenditure ∝ (weight)^{0.7}, which normalizes for differences

in age and body composition. Predicted basal metabolic rate includes terms for age, sex, weight, and height (Schofield, 1985), and could also be used to normalize the rates of CO₂ production. As a result of the uncertainty over which measure to use to normalize the data, the CO₂ production data were normalized to each of these measures, and the results compared for marasmic children with and without infection and well-nourished children with infection.

In addition, backward regression modeling was done with total CO₂ production for the malnourished children to test whether anthropometric indices, body temperature, heart rate, HIV infection, acute infection, or malaria infection were predictive of the rate of CO₂ production.

Data from CO₂ production for specific foods were used to calculate the expected CO₂ production from the diet, so that it could be compared to the observed rates (Elia, 1991).

Statistical analyses

The estimated sample size was 13 children in each group, assuming that the rate of CO₂ production would vary similarly as energy expenditure (Butte *et al*, 2000), and that differences of 10% in the rate of CO₂ production between groups would be detected with 95% specificity and 90% power. Data are expressed as means \pm s.d. Anthropometric Z-scores were calculated using Epi Info 2000 (WHO/Centers for Disease Control, Atlanta, GA, USA). Comparisons between groups of children were made using ANOVA and Student's *t*-test. Backward linear regression of the rate of CO₂ production with respect to age, sex, anthropometric measurements and indices, HIV infection, heart rate, and body temperature was used to determine which of these parameters significantly influenced energy expenditure for malnourished children (SPSS Professional edition for Windows version 10, Chicago, IL, USA). Statistical differences of $P < 0.05$ were considered to be significant.

Results

A total of 56 children were studied between August 2000 and October 2001: 23 boys and 33 girls (Table 1). The study

population was divided into three groups on the basis of nutritional and infectious status: marasmic with acute infection ($n = 28$); marasmic without acute infection ($n = 16$); and well nourished with acute infection ($n = 12$).

During the ¹³C- sodium bicarbonate infusion, isotopic steady state in ¹³CO₂ was attained after 55 min of constant infusion as previously documented in a subset of these children (Manary *et al*, 2002).

The expected rate of CO₂ production from oxidation of the diet was 25.8 mmol/kg h of CO₂, while the observed rate of CO₂ production was 29.6 mmol/kg h for the 44 marasmic children.

Marasmic children with acute infection produced less CO₂ than well-nourished children; this finding was consistent when the rate of CO₂ production was normalized to any measure related to lean body mass (Table 2). However, these infected marasmic children did not have a greater rate of CO₂ production than uninfected marasmic children. The *post hoc* power of the comparison between marasmic children with and without infection was 93% with respect to the hypothesis that marasmic children with infection had a rate of CO₂ production that was 10% greater than uninfected marasmic children. Body temperature was higher in well-nourished children with infection when compared with the malnourished children with infection (37.4 ± 1.4 vs $36.4 \pm 0.7^\circ\text{C}$, $P = 0.004$) and body temperature correlated with the rate of CO₂ production ($r = 0.4$, $P = 0.003$). None of the malnourished children had fever during the metabolic study, while 4/12 well-nourished infected children did.

For the marasmic children, weight, height, BMI, surface area, weight-for-height Z-score (WHZ), height-for-age Z-score (HAZ), temperature, and heart rate were normally distributed. Backward linear regression modeling of the total rate of CO₂ production in the marasmic children considering these normally distributed variables yielded a model in which age, sex, WHZ, and HAZ were significant predictors ($r = 0.76$, $P < 0.01$ for all variables). Backward linear regression modeling of the rate of CO₂ production normalized to BMI resulted in a model in which age and HAZ were significant predictors ($r = 0.66$, $P < 0.01$ for age and HAZ).

Table 1 Demographic and anthropometric characteristics of study children

	Marasmic with acute infection (M=9, F=19)	Marasmic without acute infection (M=8, F=8)	Well nourished with acute infection (M=5, F=7)
Age (months)	32 \pm 12	27 \pm 13	32 \pm 15
Weight-for-age, Z-score	-4.1 \pm 0.6	-4.0 \pm 0.6	-0.9 \pm 0.6*
Height-for-age, Z-score	-3.4 \pm 1.0	-3.2 \pm 1.1	0.3 \pm 0.9*
Weight-for-height, Z-score	-2.9 \pm 0.6	-2.9 \pm 0.9	-1.2 \pm 0.6*
Hematocrit %	28 \pm 7	29 \pm 4	28 \pm 8
Type of infection, number	11 malaria 14 pneumonia 3 sepsis	None	8 malaria 4 pneumonia
HIV positive, number	14	8	0

*Greater than those of marasmic children, $P < 0.001$.

Table 2 Rate of CO₂ production in marasmic and well-nourished children with acute infection

	Marasmic uninfected (n=16)	Marasmic with acute infection (n=28)	Well nourished with acute infection (n=12)	P-value
CO ₂ production (mmol/h)	228 ± 61	225 ± 65	344 ± 60	<0.001
Weight (kg)	7.5 ± 1.7	7.7 ± 1.4	12.1 ± 2.6	<0.001
Body mass index (kg/m ²)	12.5 ± 1.2	12.2 ± 0.7	14.3 ± 0.8	<0.001
CO ₂ production (mmol/(BMI)/h)	18.1 ± 4.1	18.4 ± 5.4	24.2 ± 4.6	0.001
CO ₂ production (mmol/kg/h)	30.4 ± 4.2	29.0 ± 6.7	28.8 ± 4.7	NS
CO ₂ production (mmol/kg ^{0.7} /h)	55.9 ± 8.4	53.5 ± 12.7	60.4 ± 8.2	0.04
CO ₂ production (mmol WH%/h)	165 ± 60	161 ± 49	302 ± 58	<0.001
CO ₂ production (mmol/(SA m ²)/h)	545 ± 85	524 ± 124	604 ± 86	0.02
CO ₂ production (mmol/(BMR kJ)/h)	103 ± 15	98 ± 24	117 ± 17	0.005

BMI, body mass index; WH%, weight-for-height % of reference population; SA, surface area; BMR, basal metabolic rate.

Consideration was given to whether HIV infection affected the rate of CO₂ production; however, these data do not suggest this to be the case. Among uninfected malnourished children, the rate of CO₂ production was similar between children with and without HIV (218 ± 62 vs 238 ± 63, $P=0.53$) and the HIV term in a simplified regression model including only HAZ, age, sex, and WHZ reached a statistical probability of only $P=0.68$.

Discussion

This approach provided reasonable measurements of the rate of CO₂ production in ill, uncooperative, unintubated, young children who were cared for in a clinical unit with limited resources. While receiving feedings during acute infection, the marasmic children had a lower rate of CO₂ production than well-nourished children, but had a rate of CO₂ production similar to marasmic children without infection.

This study is limited in that the only estimates of body composition were those based on weight and height. An estimate of fat-free mass may have been a useful denominator by which the rate of CO₂ production data could have been normalized. The estimation of the rate of CO₂ production includes a factor to account for the CO₂ which is produced, but not expired; this factor is based on experimental work done in adults and infants (Spear *et al*, 1995; Kien & McClelland, 1996). This factor has been measured in diverse populations, including critically ill children, and varies between 0.70 and 0.85 (Hoerr *et al*, 1989; Bresson *et al*, 1990; Tissot *et al*, 1993) although most reports have identified values between 0.77 and 0.82 (including those from critically ill infants). We did not measure the amount of CO₂ produced that was then incorporated into other metabolic substrates in our population. We used a previously determined value from a population similar to that studied here, and this may have limited the accuracy of our results. We did not account for the potential effect of reduced hemoglobin on our measurements, particularly in malaria, which would reduce CO₂ delivery to the lungs and result in an underestimate in the rate of CO₂ production. Hemo-

globin was not found to be a significant factor in the regression modeling. Despite the uncertainty of the accuracy of the estimations, the relative comparisons made between different clinical groups are likely to be valid, since the same assumptions were applied in all of the calculations.

The rate of CO₂ production in the well-nourished acutely infected children corresponds to energy expenditure of 420 kJ/kg day, which is 19% greater than the predicted total energy expenditure in uninfected healthy children (Butte *et al*, 2000). This suggests that like adults, acute infection in children, especially when associated with fever, increases total energy expenditure.

These data suggest that children with PEM and acute infection expend less energy largely due to lower body temperatures and the absence of fever. Not raising body temperature in response to acute infection conserves scarce nutrients, but also denies the immunologic enhancements of fever. Fever activates cellular immunity, stimulates the acute phase response, enhances iron sequestration, and is associated with better survival (Kluger & Rothenburg, 1979; El-Radhi & Al-Kafaji, 1980; Kreger *et al*, 1980; Ahokas *et al*, 1985). The clearance of malaria parasites is accelerated by fever (Brandts *et al*, 1997). We speculate that part of the immunologic compromise of malnutrition may be related to the inability to respond to infection with fever.

The regression analyses suggest that age, sex, WHZ, and HAZ are associated with lower rates of CO₂ production. The associations between age and sex are not novel, and are recognized as important determinants of basal metabolic rate (Schofield, 1985). Cachexia or wasting represents limited amounts of substrate available for energy production, which understandably could result in less energy expenditure. This association is consistent with evidence from children in the Gambia where the degree of wasting was found to correlate with resting energy expenditure during a malaria infection (Stettler *et al*, 1992).

The association between stunting (HAZ) and energy expenditure has been carefully investigated in older children in Brazil and Jamaica, and it has been found that while stunted children have lower resting metabolic rates, the energy expenditure per lean body mass is similar to well-

nourished children (Soares-Wynter & Walker, 1996; Hoffman *et al*, 2000). Studies in Indian men found that basal metabolic rate is not altered by nutritional status as well (Soares *et al*, 1991). This previous work suggests that the mechanism by which stunting lowers energy expenditure is by altering body composition, rather than decreasing basal active cell metabolism. This same mechanism probably explains why HAZ was found to be an important determinant of the rate of CO₂ production in our study, rather than an effect of stunting on metabolic programming, although we do not have information about the body composition of the children studied here to support this.

Dietary energy intake in the 44 marasmic children studied here was 350 kJ/kg day, the level recommended for malnourished children from experience in treating malnourished children in Jamaica (Waterlow, 1992). The data from the rate of CO₂ production suggest that to match energy expenditure, intake should have been increased by 25% to about 440 kJ/kg day, when the thermic effect of food is considered. Current standard recommendations are that during the initial phase of treatment severely malnourished children should receive 336–420 kJ/kg day (World Health Organization, 1999). Further research is needed to determine whether increased dietary energy improves the response to acute infection, and whether these children might be better served by increasing their dietary intake.

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