Lipid kinetic differences between children with kwashiorkor and those with marasmus

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ABSTRACT

Background: It has been hypothesized that one factor associated with poor prognosis in kwashiorkor, but not in marasmus, is impaired lipid catabolism, which limits the supply of energy that is essential for survival when dietary intake is inadequate. However, this hypothesis has not been tested.

Objective: The objective was to measure lipid kinetics in malnourished children with kwashiorkor or marasmus.

Design: Glycerol concentration and flux (index of total lipolysis), palmitate concentration and flux (index of net lipolysis), and palmitate oxidation rate (index of fatty acid oxidation) were measured in 8 children (5 boys and 3 girls) with kwashiorkor and 7 (4 boys and 3 girls) with marasmus, aged 4–20 mo, in the postabsorptive state. The measurements were made ≈3 d after admission, when the children were malnourished, and after the children attained normal weight-for-length, ie, at recovery.

Results: The glycerol concentration was higher in the malnourished stage than at recovery for the marasmus and kwashiorkor groups combined. Glycerol flux tended to be lower (P = 0.067) and palmitate flux significantly lower (P < 0.05) in the kwashiorkor group than in the marasmus group. Palmitate oxidation was significantly lower in the malnourished stage than at recovery in the kwashiorkor group but not in the marasmus group. In the malnourished stage, palmitate oxidation was slower in the kwashiorkor group than in the marasmus group, but no significant differences between groups were observed at recovery.

Conclusions: Children with kwashiorkor break down fat and oxidize fatty acids less efficiently than do children with marasmus; this factor may explain the better survival rate in marasmus.

KEY WORDS Malnutrition, children, lipolysis, palmitate, lipid oxidation, marasmus, kwashiorkor

INTRODUCTION

Although children with kwashiorkor have a smaller weight deficit for age than do children with marasmus (1), they usually are sicker, are more difficult to treat, and have a higher mortality rate (2) because kwashiorkor is associated with more complex physiologic and pathologic changes (3, 4). Despite extensive research, the pathogenic factors that cause a child to develop a particular type of malnutrition in response to reduced food intake with or without the synergistic effect of infection are still not clear. A hypothesis put forward since 1968, first by Gopalan (5) and later by Whitehead and Alleyne (6), is that some children adapt appropriately to food deprivation and become marasmic, whereas others do not and develop kwashiorkor.

Whitehead and Alleyne (6) reasoned that the adaptation to food deprivation, as seen in marasmus, involves the gradual breakdown of fat and muscle to provide energy for survival and amino acids to protect various metabolic processes, such as the synthesis of proteins essential for homeostasis. In contrast, in kwashiorkor, the breakdown of muscle protein and adipose tissue does not occur to the same extent, which limits the supply of amino acids and fatty acids necessary to fill the shortage created by inadequate dietary intakes. More recently, this hypothesis was partially supported by protein kinetic data from our work (7) and that of Manary et (8), which showed slower rates of whole-body protein breakdown in children with kwashiorkor than in those with marasmus. It is not known, however, whether there are similar differences in lipid kinetics between kwashiorkor and marasmus.

Early observations by Lewis et al (9) that plasma free fatty acids and glycerol concentrations were elevated in children with kwashiorkor and that palmitate flux, an index of net lipolysis, was markedly elevated in 6 malnourished children relative to 4 healthy control subjects led to the proposal that lipolysis is stimulated in malnourished children (10). The data showed that lipolysis was much higher in 4 children with kwashiorkor than in 2 with marasmus, which suggests that lipid breakdown is faster in kwashiorkor than in marasmus, contrary to the hypothesis of Whitehead and Alleyne (6). However, the sample size was too small to make such a conclusion. Furthermore, the tracer method used by Lewis et al (10) to measure palmitate flux may have been flawed because the infusion time (30 min) was too short to achieve an isotopic steady state without prior priming of the plasma palmitate pool with tracer (11). With respect to lipid oxidation, some researchers (12, 13) have proposed that it is


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impaired in kwashiorkor. Iputo et al (13) found lower rates of oxidation of an oral dose of [13C]cholesterol in children with kwashiorkor than in healthy children. A limitation of this study is that malabsorption of the tracer might have played a role in the lower lipid oxidation (14). Hence, it is not known whether there is a difference in lipid oxidation rate between the malnourished and the well-nourished state in the same child or between children with kwashiorkor and marasmus.

The present study aimed to measure lipid kinetics in severely malnourished children to uncover possible differences that may exist between those with kwashiorkor and marasmus. Isotopically labeled glycerol and palmitate were used to measure glycerol flux (an index of total lipolysis), palmitate flux (an index of net release of fatty acids into the circulation), and palmitate oxidation (an index of plasma fatty acid oxidation). We also calculated nonoxidative palmitate disposal—an index of fatty acids available for hepatic reesterification to triacylglycerol.

SUBJECTS AND METHODS

Subjects

Fifteen children were recruited from among patients admitted to the Tropical Metabolism Research Unit of the University of the West Indies, Jamaica, for treatment of primary severe malnutrition. The subject group consisted of 9 boys and 6 girls aged 4–20 mo with a diagnosis of kwashiorkor and marasmus according to the Wellcome Classification (1). Treatment followed a standard protocol as described previously (15). The University Hospital, University of the West Indies Faculty of Medical Sciences Ethics Committee, and the Baylor Affiliates Review Board for Human Subject Research, Baylor College of Medicine, approved the study. Written informed consent was obtained from a parent or guardian of each child enrolled.

Rehabilitation diet

During hospitalization, the children were managed according to a standard treatment protocol that is based on an understanding of the metabolic state at different stages of treatment (3) with a milk-based diet (Nan; Nestlé SA, Vevey, Switzerland) diet. The initial phase of treatment lasted from admission until the children were treated for infection, edema resolved, and electrolyte disturbances, affect, and appetite improved. During this period, the children were fed a resuscitative diet (4.8% of energy as protein, 74.2% of energy as carbohydrate, and 20.9% of energy as fat) that was restricted to provide ≈417 kJ·kg⁻¹·d⁻¹ and ≈1.2 g protein·kg⁻¹·d⁻¹ adequate for maintenance of body weight. After this period, feeds supplemented with glucose (9.3% of energy as protein, 50.5% of energy as carbohydrate, and 40.2% of energy as fat) were offered to promote catch-up growth. The diets were supplemented with vitamins (Tropivite; Federated Pharmaceuticals, Kingston, Jamaica) and a mineral mix of potassium, magnesium, and zinc salts (37.28 g KCl + 50.84 g MgCl₂ · 6H₂O + 3.36 g Zn (CH₃COO)₂ · 2H₂O/L; BDH Chemicals, Poole, United Kingdom). In addition, 60 mg iron sulfate/d was given during the catch-up period only.

Study design

The study was conducted in 2 groups of severely malnourished children, one group with kwashiorkor and the other with marasmus. Measurements were carried out in each child in the postabsorptive state at 2 stages: 4 ± 1 (x ± SEM) d after admission, in the severely malnourished state, and at the recovery stage when the subjects had attained ≥90% of the appropriate weight in relation to their length. The measurements were done by using stable-isotope-tracer methodology with the infusion of tracers over 6 h. Glycerol flux, a direct index of the total rate of lipolysis was measured with the use of [2H₅]glycerol. Palmitate flux, an index of the net release of fatty acids into the circulation, and carbon dioxide production (necessary to calculate palmitate oxidation) were measured with infusions of [1-13C]palmitic acid and NaH[13CO₃]₂. Each isotope infusion started 3 h after the subject’s last bolus of feed. Blood and breath samples for lipid kinetic measurements were collected 3 h after the start of the infusion until the end of the infusion. Hence, all subjects had been without food for 6–9 h at the time that the kinetic measurements were made. During each study, the children received 3 mg glucose · kg⁻¹ · min⁻¹ to avoid the possibility of hypoglycemia.

Tracer-infusion protocol

Sterile solutions of [2H₅]glycerol, [1-13C]potassium palmitate, and NaH[13CO₃]₂ (Cambridge Isotope Laboratories, Andover, MA) were prepared. After baseline breath and blood (3 mL) samples were collected, the NaH[13CO₃]₂ solution (prime: = 4.5 μmol/kg; infusion rate: 6 μmol·kg⁻¹·h⁻¹) was administered intravenously for 2 h. At the end of the NaH[13CO₃]₂ infusion, prime solutions of 5 μmol [2H₅]glycerol/kg and of 12 μmol [13C]palmitate/kg were injected followed by constant infusions of the tracers at 10 and 12 μmol·kg⁻¹·h⁻¹, respectively for 4 h. More breath samples were taken at 15-min intervals during the second and final hours of the infusions. Additional 1-mL blood samples were obtained hourly during the 4-h infusions.

Sample analyses

The blood samples were centrifuged at 1000 × g for 15 min at 4 °C, and the plasma was removed and stored immediately at −70 °C for later analyses. Plasma palmitate and glycerol concentrations were measured by in vitro isotope dilution with the use of 2,2-[2H₂]palmitate (98% 2H) and 2-[13C]glycerol (99% 13C; Cambridge Isotope Laboratories) as internal standards as previously described by us (15). The ratio of plasma palmitate tracer to tracer was determined on its pentfluorobenzyl derivative by negative chemical ionization gas chromatography–mass spectrometry (GC-MS) by selective ion monitoring at mass-to-charge ratios of 255 and 256 (257 for the in vitro isotope dilution sample) on a Hewlett-Packard (Fullerton, CA) GC-MS system. The ratio of plasma glycerol tracer to tracer was measured by positive chemical ionization GC-MS on the glycerol triacetate derivative, with selective monitoring of ions at mass-to-charge ratios of 159 to 164. The abundance of 13C in carbon dioxide in expired air was measured by gas isotope ratio mass spectrometry (Europa Scientific, Crewe, United Kingdom), with monitoring ions at mass-to-charge ratios of 44 and 45.

The tracer-tracee ratio (mol/100 mol) of each sample was calculated by the method of Brauman (16). The net tracer-tracee ratio of a sample was obtained by subtracting the background tracer-tracee ratio of the sample taken before the isotope infusion started.

Calculations

The standard steady state equation was used to calculate the flux, or rate of appearance (Ra), of glycerol, palmitate, and carbon dioxide:
normalized to a tracer infusion rate of 12 μmol·kg⁻¹·h⁻¹.

Ra (μmol·kg⁻¹·h⁻¹) = \left(\frac{\text{tr/tr}_{\text{inf}}/\text{tr/tr}_{\text{plat}}}{D}\right)

(1)

where tr/tr_{inf} and tr/tr_{plat} are the tracer-tracer ratios of palmitate or glycerol or of carbon dioxide in the infusion and plasma (or expired air) at plateau, and D is the rate of infusion of the tracer in μmol·kg⁻¹·h⁻¹.

The palmitate oxidation rate was calculated according to the following equation:

Palmitate oxidation (μmol·kg⁻¹·h⁻¹) = \frac{E_{C02} \times Ra_{CO2}/\text{tr/tr palm} \text{tr}}{1}

(2)

where E_{CO2} and Ra_{CO2} are the isotopic enrichment and the production rate of carbon dioxide, respectively, and tr/tr-palmitate is the steady state tracer-tracer ratio of plasma palmitate.

The kinetic data in the malnourished stage were expressed per edema free body weight. Edema weight was estimated as the difference between body weight on the day of the malnourished stage experiment and the lowest postexperiment weight observed. During this period, all patients were fed a diet that has been estimated to maintain weight in healthy children and to not promote growth. The actual intake (x ± SE) was 373 ± 14 kJ·kg⁻¹·d⁻¹ and ≈ 1.07 ± 0.04 g·kg⁻¹·d⁻¹ of protein.

**Statistical analysis**

The data are expressed as means ± SEMs. The data were analyzed by a 2-factor repeated-measures analysis of variance (RMANOVA) with the between-group factor being the diagnosis group and the measurement over time (malnourished stage to recovery stage) being the repeated factor. If the interaction term from the RMANOVA was significant, then post hoc pairwise comparisons were performed with Tukey’s test. When necessary, transformation to ensure normality was done. STATA Statistical Software Version 8 for WINDOWS (Stata Corporation, College Station, TX) was used for the analysis. The results were considered to be statistically significant if P < 0.05.

**RESULTS**

The physical characteristics of the study subjects are presented in Table 1. All subjects were severely malnourished, with markedly lower than expected weights-for-age and weights-for-length. Weight-for-age was significantly lower in the children with marasmus than in the children with kwashiorkor. Age, weight, and length were not significantly different between the kwashiorkor and marasmus groups, and all 3 variables increased significantly from the malnourished to the recovered stage.

Data for glycerol and palmitate concentrations and kinetics are shown in Table 2 and Figure 1. There was a significant main effect of stage of study on the glycerol concentration, which indicated a higher concentration in the malnourished than in the recovered stage. The main effect of diagnosis was of borderline significance (P = 0.054), which indicated a tendency for a lower concentration in the kwashiorkor group. The main effect of stage of study on glycerol flux was not statistically significant, but the main effect of diagnosis showed a tendency for a slower flux in the kwashiorkor group than in the marasmus group (P = 0.067). The main effect of diagnosis was not statistically significant for

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Malnourished stage</th>
<th>Recovery stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kwashiorkor (n = 8)</td>
<td>Marasmus (n = 7)</td>
</tr>
<tr>
<td>Age (mo)²</td>
<td>11.8 ± 1.7 *</td>
<td>11.9 ± 1.5 *</td>
</tr>
<tr>
<td>Weight (kg)²</td>
<td>6.7 ± 0.5 *</td>
<td>5.4 ± 0.3 *</td>
</tr>
<tr>
<td>Length (cm)²</td>
<td>66.6 ± 2.6 *</td>
<td>65.6 ± 2.0 *</td>
</tr>
<tr>
<td>Weight-for-age (%)³,⁴</td>
<td>70.3 ± 2.1</td>
<td>57.1 ± 1.6</td>
</tr>
<tr>
<td>Weight-for-length (%)⁴</td>
<td>85.3 ± 2.7</td>
<td>78.4 ± 2.9</td>
</tr>
<tr>
<td>Length-for-age (%)⁴</td>
<td>91.9 ± 1.4</td>
<td>88.2 ± 1.6</td>
</tr>
</tbody>
</table>

¹ All values are x ± SEM.
² Stage-by-diagnosis interaction, P < 0.05 (repeated-measures ANOVA).
³ Significantly different from the corresponding condition in the recovery stage, P < 0.05 (repeated-measures ANOVA).
⁴ Percentage of the median for a child of the same age or the same length per the EPI INFO 2002 database and statistical software, Centers for Disease Control and Prevention, Atlanta, GA.
⁵ Main effect of diagnosis term (kwashiorkor vs marasmus), P < 0.01 (repeated-measures ANOVA).
⁶ Main effect of stage term (malnourished vs recovery), P < 0.02 (repeated-measures ANOVA).
TABLE 2
Glycerol flux and palmitate kinetics in children with kwashiorkor or marasmus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kwashiorkor (n = 8)</th>
<th>Marasmus (n = 7)</th>
<th>All subjects (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol concentration (μmol/L)</td>
<td>182 ± 43</td>
<td>244 ± 36</td>
<td>211 ± 29(^1)</td>
</tr>
<tr>
<td>Glycerol flux (μmol · kg(^{-1}) · h(^{-1}))</td>
<td>423 ± 56</td>
<td>568 ± 50</td>
<td>499 ± 41</td>
</tr>
<tr>
<td>Palmitate concentration (μmol/L)</td>
<td>126 ± 48</td>
<td>278 ± 64</td>
<td>197 ± 43</td>
</tr>
<tr>
<td>Palmitate flux (μmol · kg(^{-1}) · h(^{-1}))</td>
<td>226 ± 32</td>
<td>394 ± 43</td>
<td>304 ± 34</td>
</tr>
<tr>
<td>Palmitate flux oxidized (%)</td>
<td>25 ± 3</td>
<td>26 ± 3</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Palmitate nonoxidative disposal (μmol · kg(^{-1}) · h(^{-1}))</td>
<td>169 ± 26</td>
<td>298 ± 41</td>
<td>229 ± 28</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm \text{SEM}.

DISCUSSION

The primary aim of this study was to determine whether there are differences in lipid kinetics between children with kwashiorkor and those with marasmus. The rates of lipolysis and palmitate oxidation were slower in the children with kwashiorkor than in those with marasmus. In addition, children with kwashiorkor had palmitate oxidation rates that were slower in the malnourished stage than in the recovered and well-nourished stages. These results suggest inefficient mobilization and utilization of lipid for energy in kwashiorkor than in marasmus and may be one factor contributing to the poorer survival of children with kwashiorkor.

Our finding that both total and net lipolysis (the rate of entry of fatty acids into the circulation) were slower in the kwashiorkor group than in the marasmus group suggests the impaired breakdown and release of stored lipids in response to chronic food deprivation in children with kwashiorkor compared with children with marasmus. This finding does not agree with data published 40 y ago by Lewis et al (10), who reported that palmitate flux in 4 children with kwashiorkor was 2.4 times that of 2 marasmus children. Furthermore, the average palmitate flux (51.7 μEq · min\(^{-1}\) · kg\(^{-1}\), or 3102 μmol · kg\(^{-1}\) · h\(^{-1}\)) of the 6 malnourished patients studied by Lewis et al (10) is \(\approx 10\) times the mean value of 304 μmol · kg\(^{-1}\) · h\(^{-1}\) for the 15 patients in the present study. In all likelihood, the discrepant results of these 2 studies are due to methodologic differences. Lewis et al (10) used a 30-min constant infusion of \(^{[14]}\)Cpalmitate and calculated flux with the steady state equation using the specific activities of samples taken from 10 to 30 min after the infusion started. It is highly unlikely to achieve an isotopic steady state in such a short time without prior priming of the plasma palmitate pool with tracer (11). This will result in an average specific activity that is much lower than the value that would have been reached at actual isotopic steady state; hence, flux will be grossly underestimated.

In contrast, in the present study we used a primed constant 4-h infusion of tracer and the ratio of plasma palmitate tracer to tracee reached a plateau (Figure 1).

Our data suggest that differences in the rates of 2 pathways, triacylglycerol hydrolysis and fatty acid reesterification within adipocytes, may contribute to the slower release of fatty acids...
into the circulation of children with kwashiorkor. The 26% slower glycerol flux in the children with kwashiorkor suggests that hormone-sensitive lipase activity was slower in the children with kwashiorkor than in the children with marasmus. Although we are not aware of any in vitro measurement of hormone-sensitive lipase activity in adipose tissue taken from severely malnourished children, a study by Agbedana et al (17) reported lower postheparin serum total and hepatic lipase activity in children with kwashiorkor than in children with marasmus, which suggests that lipoprotein-triacylglycerol hydrolysis may be slower in kwashiorkor than in marasmus. This finding, together with slower glycerol flux in the present study, suggests that the activities of both enzymes responsible for triacylglycerol hydrolysis (i.e., lipoprotein and hormone-sensitive lipase) are slower in kwashiorkor than in marasmus.

The slower fatty acid oxidation in children with kwashiorkor than in children with marasmus may be a direct result of the decreased availability of fatty acids, because the portions of palmitate flux oxidized by both groups were almost identical (≈25%). One cannot rule out the possibility that this may represent an impairment in fatty acid oxidation in the kwashiorkor group, however, because in the recovered stage these children were oxidizing significantly more palmitate (35% of flux) than in the malnourished stage (25%). Decreased fatty acid transport by carnitine across the inner mitochondrial membrane for β-oxidation could be a factor contributing to slower oxidation in children with kwashiorkor. It has been shown that the concentration of plasma carnitine is lower in children with kwashiorkor compared with when they had recovered from malnutrition (18, 19), with marasmus children (20) and with normal children (21, 22). It is a distinct possibility that carnitine synthesis is decreased in children with kwashiorkor, hence the lower concentrations, because impaired protein breakdown (Jahoor et al 2005) results in a shortage of its precursors lysine and methionine. Another possibility is that the proposed defect in the peroxisomal oxidation of lipids (12) may be greater in kwashiorkor than in marasmus.

Compared with the recovered stage, glycerol concentration and flux trended higher in both the kwashiorkor and marasmus groups, which suggests that there was an overall stimulation of total lipolysis. Palmitate oxidation, however, did not follow a similar trend—it was slower in the kwashiorkor group when they were malnourished than when they had recovered, and there was no difference in palmitate oxidation within the marasmus group. Our finding of slower fatty acid oxidation in the kwashiorkor group is in agreement with previous data that suggests reduced lipid oxidation in kwashiorkor (12, 13, 21).

Recent studies by us and others have shown that protein breakdown is slower in children with kwashiorkor than in children with marasmus (8, 23), which partially supports the previously proposed hypothesis that the usual adaptation to food deprivation occurs to a lesser extent in kwashiorkor than in marasmus (5, 6). Central to this hypothesis is that, in kwashiorkor, the gradual breakdown of muscle and fat to provide fatty acids for energy and amino acids for the synthesis of proteins, peptides, and other biomolecules necessary for survival is inadequate. Indeed, we have shown that the pattern of lipid breakdown is similar to that of protein breakdown, being lower in children with kwashiorkor than in those with marasmus. In addition, our present finding that the amount of energy derived from lipid oxidation is lower in kwashiorkor than in marasmus suggests that utilization of fat as a source of energy is impaired in children with kwashiorkor. Together, our findings that both lipid breakdown and oxidation and protein breakdown are slower in children with kwashiorkor than in children with marasmus provide experimental support for the hypothesis of Gopalan (5) and Whitehead and Alleyne (6) that impaired muscle and adipose tissue breakdown may be involved in the pathogenesis of kwashiorkor.

Finally, the established approach for the dietary management of severely malnourished children constitutes 2 types of diet (24). The initial diet is restricted to maintain body weight during acute resuscitation within the functional metabolic capacity of the child while infections are treated, fluid and electrolyte balance is reestablished, and specific micronutrient deficiencies are corrected before the second high-energy catch-up growth diet is given (3). These diets are usually prepared from regular milk powder or milk formula with added oil. Although the additional lipid may be appropriate for marasmic children, it may not be for children with kwashiorkor, who seem to have impaired mobilization and oxidation of lipids, particularly palmitic acid. This is of greater concern with the initial treatment diet, but might also be an important consideration for the catch-up growth diet because it is not known whether lipid oxidation is restored to normal by the time this diet is started, usually as early as 11 d after admission.

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