Glucose Kinetics during Fasting in Young Children with Severe and Non-severe Malaria in Suriname

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Abstract. Fasting could be an important factor in the induction of hypoglycemia in children with malaria because fasting results in a decrease in endogenous glucose production. The influence of extended fasting on plasma glucose concentration, glucose production, and gluconeogenesis were measured using [6,6-2H2]glucose and 2H2O in 12 Surinamese children with severe malaria and compared with 16 children with non-severe malaria during a 16-hour controlled fast. Glucose concentration and glucose production were comparable after 8 hours of fasting and decreased in both groups (P < 0.001) with an extension of the fast up to 16 hours. Glucose concentration decreased faster in the non-severe group than in the severe group (P = 0.029). The decrease in glucose production was not different between groups (P = 0.954). Thus, fasting predisposes for hypoglycemia in young children with Plasmodium falciparum malaria. Hypoglycemia caused by fasting develops later in young children with severe malaria than in children with non-severe malaria.

INTRODUCTION

Hypoglycemia is a common and serious complication in children with severe malaria, and it also is indicative of mortality caused by this disease.1,2 Hypoglycemia occurs more frequently in children (up to 25%)3–6 than in adults (8%).7 The pathogenesis of hypoglycemia in malaria is still incompletely understood. There are several generally accepted risk factors, such as prolonged fasting, severity of the infection, young age, and malnutrition.8–16

Fasting is considered a risk factor because it leads to glycogen depletion, which can result in decreased glucose production and hypoglycemia.8,17,18 Healthy adults are able to maintain normal plasma glucose levels up to 86 hours of fasting.19 However, healthy children are not able to maintain a normal plasma glucose concentration during a fasting period of 24 hours and show a significant steeper decrease in plasma glucose concentration than adults.10–12

In earlier studies, we found that infection with Plasmodium falciparum resulted in an increase in glucose production in different patient groups, both in non-severe P. falciparum malaria and in severe P. falciparum malaria.8,13,14,19 In adults with non-severe malaria, glucose production increased 20% compared with that in healthy controls.8,19 In adults with severe malaria and adults with cerebral malaria, glucose production doubled.14,20 In pregnant women, infection with P. falciparum resulted in higher glucose production and higher glucose concentrations during 24 hours of fasting, thereby delaying the occurrence of hypoglycemia.8

There are few studies of glucose production in young children with malaria. In older children with non-severe malaria, endogenous glucose production (EGP) was a significant determinant of plasma glucose concentration.21 Other studies showed that glucose production was higher in children with severe malaria than in children with non-severe malaria.22,23 There are no studies that compared glucose kinetics in children with severe malaria and those with non-severe malaria during a controlled and objectively observed fasting period.

Hypoglycemia is particularly common in young children (less than three years of age) with P. falciparum malaria.3,15 Because glycogen stores in the young child are limited, a period of prolonged fasting could be a major risk factor for hypoglycemia. Nutritional status has an effect on glucose production but data in children are limited and contradictory.16,23,24

We hypothesized that prolonged fasting is an important determinant in the development of hypoglycemia in children with P. falciparum malaria and that children with severe malaria are more at risk than children with non-severe malaria. The primary objective of this study was to measure the influence of a prolonged period of controlled fasting on the plasma glucose concentration, EGP, and the role of gluconeogenesis in children with severe malaria and non-severe malaria in Suriname.

MATERIALS AND METHODS

Patients. All children admitted during the 2.5-year study period to Diakonessen Hospital in Paramaribo, Suriname, which is the main referral hospital for the interior of Suriname, with a primary diagnosis of P. falciparum malaria and a plasma glucose concentrations at admission ≥ 3 mmol/L were eligible for inclusion in the study. Six children with non-severe malaria were studied at Distrikt Hospital Stoelmansiland in interior Suriname.

Exclusion criteria were a plasma glucose concentration < 3 mmol/L, treatment with quinine or other well-known stimulators of insulin secretion, severe chronic diarrhea, documented endocrinologic disease, and concomitant infectious diseases. Children were considered to have severe malaria according to the World Health Organization (WHO) criteria if > 2% of their erythrocytes were infected, if they had severe anemia (hemoglobin level < 5 g/dL or 3.1 mmol/L), hypoglycemia (plasma glucose concentration < 3 mmol/L, in that case the patient would be excluded), if they were prostrated or in respiratory distress (in case of impaired consciousness, multiple convulsions within the past 24 hours), or if they had (other) signs of cerebral malaria (Blantyre coma score ≤ 2),15 which was not attributable to any other cause. All patients with suspected cerebral malaria had a lumbar puncture to exclude other causes of coma. Patients with non-severe ma-
laria were defined as having < 2% of their erythrocytes infected and none of the above mentioned criteria.

All severely ill children admitted to Diakonessenhuis Hospital were treated according to the guidelines of proper pediatric intensive care medicine. For respiratory or circulatory insufficiency and metabolic dysregulation, this was promptly corrected. Patients with hypoglycemia were immediately treated and were not eligible for the study.

Patients with a hemoglobin concentration < 3.5 mmol/L were given a blood transfusion of at least 20 mL/kg. If their hemoglobin concentration after the blood transfusion was > 5 mmol/L, they were eligible for the study.

Nutritional status was assessed by weight-for-length/height on the WHO Child Growth Standards for children less than five years of age. Children with a weight-for-length/height below −2 SD were considered malnourished. The time the children had their last meal or drink either at home or in the hospital was recorded and considered the start of the fasting period prior to the study.

Written informed consent was obtained from the accompanying parent or guardian. This study was reviewed and approved by the Suriname National Ethical Committee and the Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands.

**Study design.** After admittance, patients were stabilized and recruited immediately after laboratory confirmation of the clinical diagnosis and exclusion of quinine use by a quinine dipstick test. Basal hematologic and biochemical parameters were measured for clinical purposes.

Patients with non-severe malaria were treated with halofantrine. An electrocardiogram was conducted to rule out congenital prolonged QT interval (halofantrine would then be contraindicated). Patients with severe malaria were treated with intramuscular artemotil (β-arteether).

An intravenous cannula was introduced in a peripheral vein for stable isotope infusion. A second cannula for blood sampling was introduced into a suitable vein in the contralateral arm or foot. Both cannulas were introduced after application of Emla cream (2.5% lidocaine, 2.5% prilocaine) for local anesthesia. Blood sampling from the venous catheter proved to be technically possible and was well tolerated by all children. Whenever possible, blood samples for study purposes were also used for clinical analysis. The catheters were kept patent by a slow isotonic saline drip.

The study design is shown in Figure 1. After obtaining a baseline blood sample at t = −8.15 hours for determination of background isotopic abundance and plasma glucose, the patients were given 1 gram of 2H2O per kg of body water at 30-minute intervals a total of five times (total dose = 5 g/kg of body water). Body water was estimated to be 60% of body weight for boys and girls. The patient fasted until the end of the study but was allowed to drink water enriched 0.5% 2H2O ad libitum to maintain isotopic steady state.

At t = −2.15 hours, a blood sample was drawn for measurement of plasma glucose concentration and enrichment of [6,6-2H2]glucose. Immediately thereafter, a primed (3.2 mg/kg), continuous (2.4 mg/kg/hour or 0.33 μmol/kg/minute) infusion of [6,6-2H2]glucose (Cambridge Isotope Laboratories, Andover, MA) dissolved in sterile isotonic saline and sterilized by passage of the solution through a Millipore (Billerica, MA) filter was administered by a motor-driven, calibrated syringe pump (Perfusor; Secura FT, B. Braun, Germany). At t = −0.30 hours (7.45 hours of fasting), three blood samples were collected at intervals of 15 minutes for the measurement of isotopic enrichment and plasma glucose concentration. Between t = 0 hours and t = 8 hours (16 hours of fasting; end of the study), blood samples were obtained every hour for measurement of plasma glucose concentration and enrichment of [6,6-2H2]glucose. Blood samples for 2H-enrichment in glucose (at the C5 position) were drawn at t = −8, t = 0, t = 4, and t = 8 hours. Blood samples for determination of plasma concentration of insulin, counterregulatory hormones, and free fatty acids (FFAs) were collected at t = 0 hr and t = 8 hr.

![Study design diagram](image-url)
Safety measures. Two major concerns had to be addressed in this study: the risk of hypoglycemia and amount of blood to be sampled. To detect hypoglycemia without delay, glucose concentrations were checked hourly during the entire study using a bedside point of care device (Precision Q·I·D: MediSense Inc., Abbott Park, IL), in addition to the glucose measurements on admission and samples taken for the study. If hypoglycemia occurred (blood glucose < 3 mmol/L), the patient was promptly treated and excluded from further study. However, none of the patients developed hypoglycemia during the study.

The maximum amount of blood to be taken for study purposes was set at 5 mL/kg of body weight with a maximum absolute amount of 36.8 mL for the entire study. For that reason, in one child with a body weight of 7.1 kg, only blood samples for glucose concentrations and enrichment were taken (total amount 24.8 mL); samples for measurement of hormones, alanine, and FFAs were omitted. Hemoglobin concentration was checked after a blood transfusion (if applicable) and after the study.

Assays. Blood for measurement of gluconeogenesis was promptly deproteinized by adding an equal amount of 10% perchloric acid. Blood for [6,6-2H2]glucose enrichment and measurement of hormones was collected in prechilled heparinized tubes. All samples were kept on ice and centrifuged immediately. Plasma was stored at −20°C and was transported on dry ice before assay in The Netherlands.

Plasma samples for glucose enrichments of [6,6-2H2]glucose were deproteinized with methanol.27 The aldonitril penta-acetate derivative of glucose was injected into a gas chromatograph/mass spectrometer system. Separation was achieved on a J&W (J&W Scientific, Folsom, CA) DB17 column (30 m × 0.25 mm, df = 0.25 μm). Glucose concentrations were determined by gas chromatography using xylose as an internal standard. Glucose was monitored at 187, 188, and 189 nmol/L.

For deuterium enrichment in body water was measured by a gas chromatograph mass spectrometer (Model 6890 gas chromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionization mode; Hewlett-Packard, Palo Alto, CA).

Cortisol was measured with a chemiluminiscent immunometric assay (intra-assay variation at 47 pmol/L = 6%; at 609 pmol/L = 3%; inter-assay variation at 91 pmol/L = 4%, at 120 pmol/L = 6%, detection limit = 15 pmol/L). Glucagon was measured by radioimmunoassay (Linco Research, St. Charles, MO) (intra-assay cv = 3–5%, inter-assay cv = 9–13%, detection limit = 15 ng/L). Norepinephrine and epinephrine were measured by an in-house high performance liquid chromatography method (norepinephrine: intra-assay cv = 6–8%, inter-assay cv = 7–10%, detection limit = 0.05 nmol/L; epinephrine: intra-assay cv = 6–8%, inter-assay cv = 7–12%, detection limit = 0.05 nmol/L. Serum FFAs were measured by an enzymatic method (NEFAC; Wako Chemicals GmbH, Neuss, Germany) (intra-assay cv = 2–4%, inter-assay cv = 3–6%, detection limit = 0.02 mmol/L).

Calculations. The glucose rate of appearance (Ra) was calculated by the isotope dilution from the [6,6-2H2] enrichment of glucose in plasma using non–steady state equations as described by Steele and others.31,32

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Ra = \frac{F - pV \times [(C2 + C1)/2] + [(E2 - E1)/(E2 - E1)]}{E + E1/2}
\]

where Ra = rate of appearance of glucose (in μmol/kg-minute), F = [6,6-2H2]glucose infusion rate (in μmol/kg-minute), E = percent of glucose molecules enriched with 2H (in absolute values), C = plasma glucose concentration (in nmol/L), t = time at the sampling points (in minutes), and pV = effective distribution volume of glucose, assumed to be 75% of the extracellular water volume. The volume of extracellular water was calculated by nomogram from body weight and height.33 To calculate the endogenous glucose production rate exogenously, infused glucose was subtracted from glucose Ra. The fractional gluconeogenesis (%) = 100 × ([3H] enrichment on C5 of glucose/[3H] enrichment in total body water). The rationale for these calculations has been discussed in detail by Landau and others.28

Statistical analysis. To investigate the influence of fasting duration, severity of infection, age, and weight-for-length/height percentile on plasma glucose concentration and EGP, mixed models analysis with repeated measurements analysis of variance (SPSS version 12.0.1; SPSS Inc., Chicago, IL) was used with time as a linear variable. Because some data of glucose kinetics were not normally distributed, ranks of values were used. To investigate after how often children with severe malaria and non-severe malaria would become hypoglycemic, extrapolation of the plasma glucose concentration was performed using parametric linear mixed model analysis. For each of the models, the residuals were normally distributed (Wilk-Shapiro’s W > 0.95) and showed constant variance. Differences between the severe and the non-severe group in clinical and laboratory data and glucose kinetics at t = 0 were analyzed by the t-test for independent variables. Paired data of hormones and FFAs were analyzed by the paired samples t-test. Data are represented as the median and range unless otherwise stated. Statistical significance was set at P < 0.05.

RESULTS

Clinical data. Clinical and laboratory details are shown in Table 1. Twenty-eight children in Suriname less than five years of age with acute P. falciparum malaria were studied: 12
children had severe malaria and 16 children had non-severe malaria. Three children with severe disease had cerebral malaria. The severe malaria children had a longer duration of illness, were more severely anemic, and had higher concentrations of C-reactive protein, aminotransferases, bilirubin, and creatinine, all consistent with severe disease.

All children with severe anemia had hemoglobin values > 5 mmol/L after blood transfusion before entering the study. None of the children had a decrease in hemoglobin level > 0.5 mmol/L after the study.

All patients, including the children with cerebral malaria, responded well to therapy and had uneventful recoveries. None of the children had any side effects (nausea or vertigo) from the deuterated water.

**Basal glucose kinetics.** After eight hours of controlled fasting, all parameters of glucose kinetics were comparable in the children with severe malaria and those with non-severe malaria (Table 2).

**Glucose kinetics during extended fasting.** Figure 2 shows the plasma glucose concentration between 8 and 16 hours of fasting. Plasma glucose concentration decreased significantly over time in both groups: from 5.1 (3.5–7.6) to 4.5 (3–6.6) mmol/L in the severe group, and from 5 (4–6.1) to 4.4 (2.6–5.5) mmol/L (P < 0.001) in the non-severe group. The decrease was faster in the non-severe group (18%) than in the severe group (13%) (P = 0.029).

Figure 3 shows EGP between 8 and 16 hours of fasting. The EGP decreased significantly over time in both groups: from 35.9 (27.1–49.9) to 32.9 (22.6–41.6) μmol/kg-minutes in the severe group, and from 37.4 (29.3–47) to 30.9 (20.9–44.3) μmol/kg-minutes (P < 0.001) in the non-severe group. There were no statistically significant differences between the groups (P = 0.954). Gluconeogenesis did not change over time in either group: from 21.3 (10.7–34.8) to 19.1 (10.5–35.1) μmol/kg-minutes in the severe group, and from 20.6 (8–33) to 20.4 (8.7–35.5) μmol/kg-minute in the non-severe group (P = 0.151 for change over time, P = 0.841 for difference between groups).

The contribution of gluconeogenesis to endogenous glucose production increased in both groups between 8 and 16 hours of fasting; from 59% to 61% in the severe group and from 59% to 65% in the non-severe group (P < 0.001 for change over time, no difference between groups, P = 0.963). Age and nutritional status did not influence glucose kinetics over time: P = 0.244 and P = 0.987 respectively, for plasma glucose concentration; P = 0.271 and P = 0.954, respectively for EGP; and P = 0.454 and P = 0.841, respectively, for gluconeogenesis.

**Measurement of hormones, gluconeogenic precursor alanine, and FFAs.** Data are shown in Table 3. During the period of extended fasting, plasma insulin concentrations decreased and plasma FFA concentrations increased in both groups. After 8 hours of fasting, plasma cortisol concentrations were higher in children with severe malaria (870 [310–1,670] nmol/L) than in children with non-severe malaria children (360 [170–940] nmol/L) (P < 0.001). Between 8 and 16 hours of fasting, cortisol concentrations increased only in the children with non-severe malaria by 254 nmol/L (95% confidence interval = 112–396 nmol/L). At the end of the study, there were no differences in plasma concentrations of glucoregulatory hormones, precursors, or FFAs between the groups.

**DISCUSSION**

We studied glucose kinetics in children less than five years of age with *P. falciparum* malaria during a 16-hour period of controlled fasting. Fasting was an important risk factor for hypoglycemia in young children with severe and non-severe malaria. Contrary to the general opinion,15 severe malaria did not induce hypoglycemia, but the decrease in glucose concentration was slower in children with severe malaria than in those with non-severe malaria. This finding indicates that hypoglycemia caused by prolonged fasting would develop later in those with severe malaria than in those with non-severe malaria and those with non-severe malaria.
malaria. Age and nutritional status were not major determinants of glucose kinetics in the children during this study. Plasma glucose concentrations were comparable in the children with severe malaria and those with non-severe malaria children after eight hours of controlled fasting. During the following 8 hours after controlled fasting, plasma glucose concentrations decreased in both groups, but the decrease was faster in the group with non-severe malaria. With the assump-
tion that the decrease in plasma glucose concentration would be linear in time in both groups, we calculated the period after which hypoglycemia (mean plasma glucose < 3 mmol/L) would occur. Linear regression analysis using a mixed linear model showed that the children with non-severe malaria would develop hypoglycemia after 26 hours of controlled fasting and the children with severe malaria would develop hypoglycemia after 33 hours of controlled fasting ($P = 0.036$). The fasting duration prior to the study must also be taken into account. However, this information can only be obtained from the patient history, and can not be confirmed objectively. In this study, the fasting duration prior to the study was approximately 12 hours in both groups. Adding this fasting duration, children with non-severe malaria would become hypoglycemic after 38 hours and children with severe malaria would become hypoglycemic after 45 hours.

The influence of severity of infection is consistent with the observation that hyperglycemia rather than hypoglycemia is frequently found in sepsis and other acute infections, as well as in earlier studies of subjects with *P. falciparum* malaria. Because the plasma glucose concentration decreased less rapidly in the more severely infected children, it apparently is not malaria in itself that causes hypoglycemia. There are no studies on glucose kinetics in children less than five years of age during controlled extended fasting with which we can compare our results. Because healthy prepubertal children are not able to maintain a normal plasma glucose concentration after a fasting period of 24 hours, younger healthy children may develop hypoglycemia after even a shorter period of fasting. We extrapolated that children with malaria can maintain a normal plasma glucose concentration for at least 26 hours of fasting.

There were some differences in clinical and biochemical parameters in the two groups of children studied that could affect the decrease in plasma glucose concentration. The children with severe malaria had a longer duration of illness, although this did not result in a faster decrease in plasma glucose concentration or endogenous glucose production than in the children with non-severe malaria. Other differences between the two groups were all consistent with severe disease. These differences also include nutritional status. Although there were no differences between the groups, three of the children with severe malaria and one of the children with non-severe malaria had a weight-for-height $< -2$ SD and could be considered malnourished. This finding may have been caused by alteration of the initial nutritional status because of longer duration of illness and more severe disease, which led to acute malnutrition in some of the children with severe malaria. Nevertheless, nutritional status did not influence plasma glucose concentration during the extended fasting period. The one determining factor for the decrease in plasma glucose concentration in both groups of children was time of controlled fasting during the study. We conclude that the most important determinant for hypoglycemia in young children with severe and non-severe malaria is the duration of fasting.

The plasma glucose concentration is the result of the balance between glucose supply and glucose use. Hypoglycemia can be caused by decreased glucose production, an increase in glucose clearance, or a combination of both. Endogenous glucose production decreased in both groups during fasting, but there was no difference between the non-severe and the severe groups. Because plasma glucose concentration decreased faster in the children with non-severe malaria, glucose clearance was lower in the children with severe malaria. This finding indicates that malaria, like other inflammatory diseases, results in decreased glucose clearance in these children, a phenomenon that is well known during acute infections in humans.

Absolute gluconeogenesis did not change over time in either group. However, as endogenous glucose production decreased, the fractional gluconeogenesis (contribution of gluconeogenesis to glucose production) increased in both groups. When compared with other groups of patients with malaria, fractional gluconeogenesis in these children was low: fractional gluconeogenesis was 100% after 20 hours of fasting in adults with cerebral malaria and 90% in adults with non-severe malaria, 74% in pregnant women with *P. falciparum*, and 74% in children in Kenya 2–6.5 years of age with non-severe malaria after 8 hours of fasting. However, this finding also implies that glycogenolysis still contributed 35–40% to glucose production after 16 hours of fasting in the children we studied. This finding is remarkable because glyco- gen stores in infants and young child are supposed to be only adequate for a fasting period of 12 hours. There are no other studies measuring liver glycogen content in children less than five years of age. There are also no data on glucose production and gluconeogenesis in healthy children or in children with malaria or other comparable illnesses during prolonged fasting less than five years of age with which to compare our results.

In conclusion, fasting predisposes for hypoglycemia in young children with severe and non-severe *P. falciparum* malaria. Hypoglycemia caused by fasting develops later in young children with severe malaria than in children with non-severe malaria. This finding is most likely caused by a difference in

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**Table 3**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>8-hour fast†</th>
<th>16-hour fast†</th>
<th>Change (95% CI) 8-hour fast†</th>
<th>16-hour fast†</th>
<th>Change (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/L</td>
<td>22 (15–109)</td>
<td>15 (15–97)</td>
<td>-6 (-11–1)</td>
<td>22 (15–35)</td>
<td>15 (15–30)</td>
</tr>
<tr>
<td>Glucagon, ng/L</td>
<td>87 (48–281)</td>
<td>90 (47–239)</td>
<td>+12 (-3–21)</td>
<td>74 (43–109)</td>
<td>83 (39–144)</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>870 (310–1,670)†</td>
<td>980 (145–1,180)</td>
<td>-92 (-448–264)</td>
<td>360 (170–940)</td>
<td>660 (250–1,220)</td>
</tr>
<tr>
<td>Epinephrine, nmol/L</td>
<td>0.21 (0.08–0.64)</td>
<td>0.35 (0.10–0.87)</td>
<td>+0.12 (-0.10–0.34)</td>
<td>0.10 (0.05–1.42)</td>
<td>0.28 (0.05–1.60)</td>
</tr>
<tr>
<td>Norepinephrine, nmol/L</td>
<td>0.72 (0.10–3.90)</td>
<td>0.54 (0.10–5.54)</td>
<td>+0.16 (-0.25–0.56)</td>
<td>0.70 (0.19–3.62)</td>
<td>0.63 (0.26–2.34)</td>
</tr>
<tr>
<td>Free fatty acids, mmol/L</td>
<td>0.89 (0.42–1.21)</td>
<td>1.04 (0.57–1.73)</td>
<td>+0.21 (0.03–0.39)</td>
<td>0.75 (0.12–1.08)</td>
<td>1.07 (0.62–2.04)</td>
</tr>
</tbody>
</table>

* CI = confidence interval.
† Values are medians (ranges).
‡ $P < 0.001$ for comparison with non-severe malaria group.
peripheral uptake of glucose, indicating that children with severe malaria are more insulin resistant than children with non-severe malaria.

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