Infectious disease-related differences in the adaptation of glucose metabolism to fasting in children and the effect of age
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Very young children with uncomplicated falciparum malaria have higher risk of hypoglycemia: a study from Suriname

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BACKGROUND  Hypoglycemia is considered a major outcome predictor in children with falciparum malaria. Its pathophysiology is unknown, but young age, malnutrition and prolonged fasting with concomitant low endogenous glucose production are considered major risk factors. Measurements of glucose kinetics have changed the insight in the pathophysiology of glucose metabolism in adults with falciparum malaria. However, in young children with malaria these data are scarce.

OBJECTIVE To measure glucose kinetics and the influence of age, nutritional status and fasting duration in children with uncomplicated falciparum malaria under the age of five.

DESIGN Plasma glucose concentration, endogenous glucose production and gluconeogenesis were measured using [6,6-2H2]glucose and 2H2O in seventeen very young (<3 years) and seven older (3-5 years) Surinamese children with uncomplicated falciparum malaria admitted to the Distrikt Hospital Stoelmanseiland and Diakonessen Hospital Paramaribo during a 17 months study period.

RESULTS Plasma glucose concentration was lower in the group of very young children than in the older children (p=0.028). There were no differences in endogenous glucose production and gluconeogenesis between the groups. Overall gluconeogenesis contributed 56% (median, range 17–87%) to endogenous glucose production, with no differences between groups (p=0.240). Glucose clearance was lower in the older children (p=0.026). Glucose concentration did not differ between children with weight for length/height < -1.3 SD and children with weight for length/height > -1.3 SD (p=0.266). Plasma glucose concentration was not predicted by fasting duration (p=0.762).

CONCLUSIONS Our data confirm the higher risk of hypoglycemia in very young children with uncomplicated malaria as plasma glucose concentration was lower in this group. Since this could not be attributed to an impaired endogenous glucose production, and because glucose clearance was lower in the older children, we presume that older children were better capable of reducing glucose utilization during fasting. Studies on glucose kinetics are feasible in very young children with malaria and reveal more insight in the pathophysiology of hypoglycemia.
INTRODUCTION

Hyperglycemia is a frequent finding in sepsis and other acute infections (Sauerwein 1994). In severe falciparum malaria however, hypoglycemia rather than hyperglycemia, is a common and serious complication (White et al. 1983), especially in children (White et al. 1987) and pregnant women (Looareesuwan et al. 1985). Mortality rate increases four- to six fold in children when malaria is complicated with hypoglycemia (Marsh et al. 1995; Schellenberg et al. 1999). Thus hypoglycemia is considered to be one of the major outcome predictors in children with falciparum malaria.

Hypoglycemia is particularly common in young children with falciparum malaria below the age of 3 years (Taylor et al. 1989; WHO 2000). Hypoglycemia could be caused by diminished endogenous glucose production, increased utilization, or both. Limited glucose production capacity could play a role in the pathophysiology of hypoglycemia in children with falciparum malaria, but data on glucose production in these children are scarce. Our previous study in Kenyan children with falciparum malaria showed that glucose production was an important determinant of the plasma glucose concentration (Dekker et al. 1996). This is in contrast with findings in adults with malaria, in whom peripheral uptake seemed to be more important (Davis et al. 1993; Dekker et al. 1997).

So far, glucose production has only been measured in children with malaria at >2 years of age, precluding conclusions about the importance of glucose production in the youngest patients, who have the highest risk for hypoglycemia.

In addition to age, starvation and malnourishment are considered major risk factors for hypoglycemia (Osier et al. 2003; Solomon et al. 1994; van Thien et al. 2004; van Thien et al. 2006). Following a fasting period of 24 hours healthy children are not able to maintain a normal plasma glucose concentration and show a significant steeper decrease in plasma glucose concentrations than adults (Haymond & Sunehag 1999). Data on the influence of malnutrition on glucose production in children are limited and contradictory. In otherwise healthy malnourished children no difference in glucose production was found between the malnourished state and after recovery (Kerr et al. 1978). However, in children with falciparum malaria it was shown that malnutrition did influence glucose kinetics due to diminished precursor supply (Dekker et al. 1997). Hence the combination of malnutrition and another metabolic insult, like infection with Plasmodium falciparum could negatively affect glucose kinetics in children.

Although hypoglycemia is merely a complication of severe malaria, we hypothesize that the risk for hypoglycemia in (very) young children with uncomplicated malaria is increased in the presence of other risk factors that compromise glucose production. Data on glucose kinetics in young children with malaria are scarce and lacking in children less than two years of age. We measured the plasma glucose concentration, total endogenous glucose production (EGP) and gluconeogenesis (GNG) using stable isotope techniques in a group of very young (< 3 years) children compared with a group of older (3-5 years) children with falciparum malaria.
SUBJECTS AND METHODS

Patients
All children admitted to Distrikt Hospital Stoelmanseiland and Diakonessen Hospital Paramaribo with a primary diagnosis of falciparum malaria during the study period of 17 months were considered for inclusion in the study. Inclusion criteria were: acute uncomplicated malaria defined as < 2% of erythrocytes infected, age between 6 months and 5 years and plasma glucose concentration at admission ≥ 3.0 mmol/l. Exclusion criteria were: plasma glucose concentration < 3 mmol/l, complicated malaria according to the WHO-criteria (WHO 2000) (because clinical practice dictates constant glucose infusion in these patients), treatment with quinine or other well-known stimulators of insulin secretion, severe chronic diarrhea (which may induce hypoglycemia in childhood (Bennish et al. 1990)), documented endocrinological disease and other concomitant infectious diseases.

Nutritional status of children can be defined in different ways and in the assessment of child malnutrition a number of measures are available (Ge & Chang 2001). In this study, nutritional status was assessed by weight for length/height on the WHO Child Growth Standards for children under five years of age (WHO 2006). Children with a weight for length/height below -1.3 SD (or 10th percentile) were classified as mildly malnourished. We divided the children in two groups: normal and mildly malnourished (weight for length/height > -1.3 SD and < -1.3 SD). The time the children had their last meal before the start of the study was recorded and considered as the start of the fasting period. Written informed consent was obtained from the accompanying parent or guardian. This study was approved by the Suriname National Ethical Committee and the Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands. Only children with malaria were included, as for ethical reasons studies in healthy young children are not allowed.

Stable isotope techniques
EGP and GNG were measured using stable isotope techniques. Stable isotope techniques are safe and considered highly sensitive, which makes it possible to pick up even small differences between subjects. Measurements of glucose kinetics have changed the insight in the pathophysiology of glucose metabolism in adults with falciparum malaria. These studies also revealed that there are differences in the regulation of glucose metabolism between adults and children (> 2 years of age) with falciparum malaria to various degrees of severity (Dekker et al. 1997, Dekker et al. 1997, Landau 1999, van Thien et al. 2004, van Thien et al. 2006).

The rationale for the measurement of gluconeogenesis by the $^2$H$_2$O technique has been discussed in detail by Landau et al. (1995, 1996). This method is based on the theory that after ingestion of $^2$H$_2$O, the fifth carbon of glucose (C5) is labeled with deuterium at the level of the triose phosphate isomerase reaction during gluconeogenesis and the
second carbon of glucose (C2) is labeled with deuterium during both glycogenolysis and gluconeogenesis (Landau et al. 1996). Therefore the plasma C5/C2 glucose ratio is used to estimate the fraction of glucose derived from gluconeogenesis. Since the third carbon of glucose (C3) and C5 are concurrently labeled during the triose isomerase reaction, the C3/C2 ratio also has been used to assess gluconeogenesis (Jones et al. 2000, Bock et al. 2007).

A major assumption of the deuterated water method is that labeling of the fifth carbon of glucose with deuterium only occurs during gluconeogenesis (Landau et al. 1996). The specific biochemical step in which the C5 hydrogen is exchanged for body water is during the equilibration between glyceraldehyde-3 phosphate and dihydroxyacetone phosphate at the triosephosphate isomerase reaction (Landau et al. 1996). During this exchange the C3 hydrogen is also lost. Therefore, whenever a triose molecule is converted to glucose, enrichment at either C5 or C3 can be used to measure gluconeogenesis. However, enrichment of C5 and also of C3 can be influenced by other processes than gluconeogenesis such as the transaldolase reaction which will result in an overestimate of gluconeogenesis (Bock et al. 2007). On the other hand, exchange of the C3 hydrogen with deuterium during the triosephosphate isomerase reaction may result in a lower than expected enrichment in C3 and therefore an underestimate of gluconeogenesis. Bock et al. (2007) showed that the deuterium on C5 of glucose is lost more rapidly relative to the deuterium on C3. This may result in an overestimation of gluconeogenesis using the C5/C2 ratio and an underestimation using the C3/C2 ratio. The overall influence of these different factors is small in fasting subjects (Landau 1999).

A second assumption is that no 2H label from the [6,6-2H2]glucose is lost in the form of 2H2O for example during exchange that occurs during transamination of pyruvate with alanine (Virakami et al. 1990). The exchange would cause lower 6,62H-glucose enrichment and higher total body water enrichment, resulting in higher measurements of EGP and lower estimates of gluconeogenesis. However, this is opposed by a previous study of our group, in which we compared measurements of gluconeogenesis using the 2H2O and [2-13C]glycerol technique and found higher rates of gluconeogenesis with the 2H2O method (Ackerman et al. 2001).

A third assumption is that simultaneous glycogen synthesis and breakdown does not compromise the results (Landau et al. 1996). The extent of this glycogen cycling has not yet been measured in the fasted state but its influence is estimated to be minimal as estimates of gluconeogenesis by the deuterated water method were in agreement with those reported in studies using the NMR-technique (Landau et al. 1996).

Study design (figure 1)

Patients were recruited immediately after laboratory confirmation of the clinical diagnosis and exclusion of quinine-use by a quinine dipstick (Silamut et al. 1995). Each patient was treated with halofantrine. An electrocardiogram was made to rule out congenital prolonged QT interval (halofantrine would then be contra-indicated). An intravenous
A 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. The catheters were kept patent by a slow isotonic saline drip.

After obtaining a baseline blood sample at \( T = -8.15 \) hr for determination of background isotopic enrichment, plasma glucose, basal hematological and biochemical parameters, the patients were given 1 g of \( ^2\text{H}_2\text{O} \) per kg body water at 30-minute intervals for a total of five times (total dose of 5 g/kg body water). Body water was estimated to be 60% of body weight. The patient was fasted until the end of the study but was allowed to drink water ad libitum, enriched 0.5% with \( ^2\text{H}_2\text{O} \), in order to maintain isotopic steady state.

At \( T = -2.15 \) hr after obtaining a blood sample for plasma glucose concentration, a primed (3.2 mg/kg), continuous (2.4 mg/kg/h) infusion of \([6,6-^2\text{H}_2]\text{glucose} \) (Cambridge Isotope Laboratories, Andover MA, USA) dissolved in sterile isotonic saline and sterilized by passage of the solution through a millipore filter was administered by a motor-driven, calibrated syringe pump (Perfusor® Secura FT, B.Braun, Germany).

At \( T = -0.30, -0.15, \) and 0 hr three blood samples were collected for the measurement of isotopic enrichment and plasma glucose concentration. Blood samples for determination of plasma concentrations of insulin, counterregulatory hormones, alanine and FFA were also collected at \( T = 0 \) hr. Plasma glucose concentrations were measured at least every hour to detect hypoglycemia.

Blood for measurement of gluconeogenesis was promptly deproteinized by adding an equal amount of 10% perchloric acid. Blood for \([6,6-^2\text{H}_2]\text{glucose} \) enrichment as well as hormones and alanine were collected in prechilled heparinized tubes. All samples were centrifuged immediately. Plasma was stored at \(-20^\circ\text{C}\) and was transported on dry ice before assay in the Netherlands.

**Assays**

Plasma samples for glucose enrichments of \([6,6-^2\text{H}_2]\text{glucose} \) were deproteinized with methanol (Reinhauer et al. 1990). 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, FOL, CA, USA) DB17 column (30 m x 0.25 mm, \( d_i = 0.25 \) µm). Glucose concentrations were determined by gas chromatography using xylose as an internal standard. Glucose was monitored at \( m/\text{z} = 187, 188 \) and 189. The enrichment of \([6,6-^2\text{H}_2]\text{glucose} \) was determined by dividing the peak area of \( m/\text{z} = 189 \) by the peak area of 187 and correcting for natural enrichments.
To measure deuterium enrichment at the C5 position, glucose was converted to hexamethylenetetraamine (HMT) as described by Landau et al. (1996) and Ackermans et al. (2001). HMT was injected into a gas chromatograph mass spectrometer. Separation was achieved on an AT-Amine (Alltech, Deerfield, IL, USA) column (30 m x 0.25 mm, df 0.25 µm). HMT consists of six formaldehyde molecules, originally derived from the C5 of six glucose molecules (intra-assay coefficient of variation (c.v.) for deuterium enrichment at the C5 position: 8.5%, inter-assay c.v.: 10%). Deuterium enrichment in body water was measured by a method adapted from Previs et al. (1996) (intra-assay c.v. for deuterium enrichment in body water: 6%, inter-assay c.v.: 6%). All isotopic enrichments were measured on a gas chromatograph mass spectrometer (model 6890 gas chromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionisation mode, Hewlett-Packard, Palo Alto, CA).

Plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden), intra-assay c.v.: 3-5 %, inter-assay c.v.: 6-9 %, detection limit: 15 pmol/l. Cortisol was measured by enzyme-immunoassay on an Immulite analyser (DPC, Los Angeles, CA), intra-assay c.v.: 2-4 %, inter-assay c.v.: 3-7 %, detection limit: 50 nmol/l. Glucagon was determined by RIA (Linco Research, St. Charles, MO, USA), intra-assay c.v.: 3-5 %, inter-assay c.v.: 9-13 %, detection limit: 15 ng/l. Norepinephrine and epinephrine were determined by an in-house HPLC method. Norepinephrine: intra-assay c.v.: 6-8 %, inter-assay c.v.: 7-10 %, detection limit: 0.05 nmol/l. Epinephrine: intra-assay c.v.: 6-8 %, inter-assay c.v. 7-12 %, detection limit: 0.05 nmol/l. Plasma alanine concentration was measured enzymatically on a CobasBio analyzer (Roche, Basel, Switzerland). The enzyme L-alanine dehydrogenase is used to convert alanine into pyruvate and the amount of NADH which is simultaneously formed is measured by the increase of the absorption at 340 nm. Detection limit: 20 µmol/l. Serum free fatty acids were measured by an enzymatic method (NEFAC; Wako chemicals GmbH, Neuss, Germany), intra-assay c.v. 2-4 %, inter-assay c.v.: 3-6 %, detection limit: 0.02 mmol/l.

Calculations

Endogenous glucose production was calculated from the dilution of labelled glucose in plasma. Because plasma glucose concentrations and tracer/tracer ratios for [6,6-2H2] glucose were constant during each sampling phase of the study, calculations for steady state kinetics were applied, adapted for the use of stable isotopes (Wolfe 1992). The rate of gluconeogenesis was calculated by multiplication of the EGP by fractional gluconeogenesis. The fractional gluconeogenesis (%) =

$$100 \times \frac{[\text{2H}] \text{ enrichment on C5 of glucose}}{[\text{2H}] \text{ enrichment in total body water}}.$$  
Glucose clearance was calculated as the glucose production rate divided by the plasma glucose concentration.
Statistics

SPSS statistical software program version 12.0 was used for analysis. The subjects were divided in a group of very young (<3 years) and a group of older children (3-5 years). The Mann-Whitney non-parametric test for two independent groups was used to analyse the data. Since some data were not normally distributed, ranks of valuables were used. Data are represented as the median and range.

Univariate linear regression analysis was performed to identify a correlation between the plasma glucose concentration and the fasting duration as well as other factors that could influence plasma glucose concentration: parasite load, haemoglobin, CRP, the plasma concentrations of the glucoregulatory hormones and FFA. The (unstandardized) residuals were normally distributed (Wilk-Shapiro’s W >0.95) and showed constant variance. A p-value of < 0.05 was assumed to reflect statistical significance.

RESULTS

Clinical data

Clinical and laboratory details are given in table 1. Twenty-four Surinamese children under the age of five years with acute uncomplicated falciparum malaria were studied: 17 children were younger than 3 years and 7 children were between 3 and 5 years of age. Plasma creatinine values were higher in the older children but all values were within the reference range for age. There were no differences in any of the other clinical

<table>
<thead>
<tr>
<th>Table 1. Clinical and biochemical characteristics.</th>
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<tr>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Weight for length/height (SD)</td>
</tr>
<tr>
<td>Fasting duration (hours)</td>
</tr>
<tr>
<td>Duration of illness (days)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>Parasitemia (/µl)</td>
</tr>
<tr>
<td>Haemoglobin (mmol/l)</td>
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<tr>
<td>CRP (mg/l)</td>
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<tr>
<td>Serum AST (U/l)</td>
</tr>
<tr>
<td>Serum ALT (U/l)</td>
</tr>
<tr>
<td>Bilirubin (µmol/l)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
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</table>

Data are medians and ranges. † = p < 0.001 for comparison with < 3 years.
and biochemical characteristics between the groups and all values of biochemical characteristics were within the reference range for age. All patients responded well to therapy and made uneventful recoveries.

Glucose kinetics
The data on enrichment and glucose kinetics are shown in tables 2 and 3. Plasma glucose concentration was lower in the very young children \((p=0.028)\). There was no difference in endogenous glucose production and gluconeogenesis between the very young and the older children. Glucose clearance was higher in the very young children \((p=0.026)\). Glucose kinetics did not differ between children with a weight for length/height \(< -1.3 SD\) \((n=10)\) compared with children with a weight for length/height \(> -1.3 SD\) \((n=14)\): plasma glucose concentration \(p=0.266\), endogenous glucose production \(p=0.079\), gluconeogenesis \(p=0.143\) and glycogenolysis \(p=0.412\).

Univariate linear regression analysis revealed that plasma glucose concentration was not predicted by fasting duration \((p=0.762)\), or by any of the other factors that could influence plasma glucose concentration.

Hormones, precursors and free fatty acids
The results are shown in table 4. Plasma insulin was higher in the group of older children \((p=0.022)\). There was no difference in the plasma concentration of the other glucoregulatory hormones, the gluconeogenic precursor alanine and the free fatty acids.

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**Table 2.** Plasma glucose concentration, \([6,6-2H_2]\)enrichment of glucose, \([2H]\) enrichment on C5 of glucose and in total body water.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-30</th>
<th>-15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose concentration (mmol/l)</td>
<td>5.1 ± 0.5</td>
<td>5.0 ± 0.5</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>([6,6-2H_2])glucose</td>
<td>0.0087 ± 0.0012</td>
<td>0.0088 ± 0.0011</td>
<td>0.0087 ± 0.0012</td>
</tr>
<tr>
<td>([2H]) enrichment on C5 of glucose</td>
<td>0.2150 ± 0.0756</td>
<td>0.2150 ± 0.0756</td>
<td>0.2150 ± 0.0756</td>
</tr>
<tr>
<td>([2H]) enrichment in total body water</td>
<td>0.4225 ± 0.0349</td>
<td>0.4225 ± 0.0349</td>
<td>0.4225 ± 0.0349</td>
</tr>
</tbody>
</table>

Data are means ± SD. Steady state in plasma glucose concentration and \([6,6-2H_2]\)glucose enrichment was reached in all children between \(t = -30\) and \(t = 0\) min.

**Table 3.** Glucose kinetics in groups at \(t = 0\).

<table>
<thead>
<tr>
<th></th>
<th>&lt; 3 years</th>
<th>3 – 5 years</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration (mmol/l)</td>
<td>5.0 (4.0 - 5.5)</td>
<td>5.4 (4.8 - 6.1)</td>
<td>0.028</td>
</tr>
<tr>
<td>Glucose production (µmol/kg•min)</td>
<td>38.5 (31.5 - 49.6)</td>
<td>36.8 (29.0 - 47.0)</td>
<td>0.505</td>
</tr>
<tr>
<td>Gluconeogenesis (µmol/kg•min)</td>
<td>18.7 (8.3 - 32.8)</td>
<td>21.5 (8.0 – 26.0)</td>
<td>0.295</td>
</tr>
<tr>
<td>Glycogenolysis (µmol/kg•min)</td>
<td>18.0 (4.8 – 38.4)</td>
<td>14.7 (8.4 – 39.0)</td>
<td>0.240</td>
</tr>
<tr>
<td>Glucose clearance (µmol/kg•min)</td>
<td>1.39 (1.16-1.87)</td>
<td>1.30 (1.0-1.59)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Data are medians and ranges.
Discussion

Hypoglycemia is a frequent and serious complication in falciparum malaria in children. Our data confirm the higher risk of hypoglycemia in very young children with malaria, as the plasma glucose concentration was lower in the very young children. However, this could not be attributed to a difference in glucose production, indicating that factors influencing peripheral uptake are important for maintenance of the plasma glucose concentration.

The effect of age on glucose concentration is not specific for children with malaria. It is well-known that young children are more vulnerable to low plasma glucose concentrations than adults (Chaussain et al. 1977; Haymond et al. 1982). We did not find differences in endogenous glucose production between children < 3 years and children 3-5 years of age. Age is generally considered an important determinant of endogenous glucose production: glucose requirements in preterm infants are approximately 44 µmol/kg•min (van Kempen et al. 2003), glucose production in term infants is approximately 33 µmol/kg•min and ranges between 27 and 43 µmol/kg•min in 1 month to 6 year old children. Thereafter EGP decreases with age to 20 µmol/kg•min at the age of 8 to 10 years and 13 µmol/kg•min in adolescents (Haymond & Sunehag 1999; Cowett et al. 1983; Kalhan et al. 1986; Bier et al. 1977; Sunehag et al. 2001). Glucose production in children between 2.5 and 3.9 years with idiopathic ketotic hypoglycemia ranged between 31.2 and 39.6 µmol/kg•min after an overnight fast (Huidekoper et al. 2007). Glucose production rates of the 3-5 year old children with uncomplicated falciparum malaria in this study were comparable with the production rates in Kenyan children between 3-5 years of age in our previous study (29.6 µmol/kg•min, p = 0.072) (Dekker et al. 1996). EGP in both age-groups is within the range of EGP in healthy children between 1 month and 6 years (Haymond & Sunehag 1999; Bier et al. 1977). However, there are insufficient data in the literature to differentiate

Table 4. Hormones, the precursor alanine and free fatty acids at t=0.

<table>
<thead>
<tr>
<th></th>
<th>&lt; 3 years</th>
<th>3 – 5 years</th>
<th>Reference postabsorptive range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/l)</td>
<td>20 (15-35)</td>
<td>30 (18-109)</td>
<td>43-172</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>87 (43-109)</td>
<td>65 (48-76)</td>
<td>40-140</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>330 (90-940)</td>
<td>500 (220-1610)</td>
<td>220-650</td>
</tr>
<tr>
<td>Epinephrine (nmol/l)</td>
<td>0.10 (0.05-1.38)</td>
<td>0.22 (0.10-1.42)</td>
<td>&lt;0.55</td>
</tr>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td>0.57 (0.07-1.34)</td>
<td>0.74 (0.26-3.02)</td>
<td>&lt;3.25</td>
</tr>
<tr>
<td>Alanine (µmol/l)</td>
<td>123 (49-523)</td>
<td>111 (36-160)</td>
<td>158-314</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.76 (0.08-1.08)</td>
<td>0.71 (0.5-0.88)</td>
<td>0.6-1.5</td>
</tr>
</tbody>
</table>

† p = 0.022 for comparison with < 3 years.
between children less than 3 years and above 3 years of age. If we assume that glucose production decreases with age, EGP should have been higher in the younger age-group compared with the older children in our study. As we found no differences between the age-groups, this indicates either that EGP was ‘suppressed’ in the younger infants or that it was ‘increased’ in the older age-group. In other words: either young children are not capable of increasing EGP or older children are insulin resistant. In the absence of more detailed reference values for the younger children and with normoglycemia in both study-groups it is impossible to draw definite conclusions.

The lack of glucose production as a predictor of the plasma glucose concentration indicates that factors influencing peripheral uptake must play a more important role, because the plasma glucose concentration is the resultant of the balance between glucose supply and glucose utilization. This is supported by the higher plasma concentration combined with a comparable EGP resulting in a lower glucose clearance in the older children. In addition, the plasma insulin concentration was higher in the group of older children. Because insulin regulates glucose metabolism by stimulation of peripheral glucose uptake and inhibition of hepatic glucose production (Rizza et al. 1981), the higher insulin concentration indicates that peripheral insulin sensitivity was lower in the older age-group compared with the younger children, resulting in reduced peripheral glucose uptake. Therefore, we presume that older children were better capable of reducing glucose utilization in order to maintain the plasma glucose concentration.

Overall gluconeogenesis contributed 56% (median, range 17–87%) to glucose production and there were no differences between the very young and the older children (p=0.240). This is in the same order of magnitude as gluconeogenesis in children between 2.5 and 3.9 years with idiopathic ketotic hypoglycemia gluconeogenesis (45%), healthy pre-pubertal children and adolescents (50%) and healthy adults after an overnight fast (Huidekoper et al. 2007, Sunehag et al. 2001, Landau et al. 1996). In Kenyan children between 2 and 6.5 years with uncomplicated malaria glucose production was largely derived from gluconeogenesis (73%) after an eight hour fast (Dekker et al. 1997), whereas in Vietnamese adults with uncomplicated malaria gluconeogenesis contributed 87% to glucose production after a seven hour fast (Dekker et al. 1997. We conclude that the contribution of gluconeogenesis to glucose production differs between populations depending on the age-group and underlying disease.

Our data do not support an influence of nutritional status since there was no difference in any of the parameters of glucose kinetics between children with a weight for length/height > -1.3 SD and children with a weight for length/height < -1.3 SD. This is in line with our previous findings in older Kenyan children with uncomplicated malaria (Dekker et al. 1997; Dekker et al. 1997). However, these data need prudent interpretation, as most children in our study were only mildly malnourished. Only two children fulfilled the criteria of moderate malnutrition (weight for length/height < -2 SD) (WHO 2006). Therefore, at this moment there are insufficient data to draw definite conclusions about the influence of malnutrition on glucose kinetics in young children with falciparum malaria.
The fasting duration did not emerge as a predictor for the plasma glucose concentration in our study. This was against our expectations, as we and others have shown a relationship between fasting and the occurrence of hypoglycemia (Osier et al. 2003; van Thien et al. 2006; Haymond et al. 1999; Bennish et al. 1990; Kawo et al. 1990). The maximum duration of fasting in our study was 18 hours, which is probably too short to be of influence on the plasma glucose concentration. Healthy children are able to maintain a normal plasma glucose concentration during a fasting period up to 24 hours (Haymond & Sunehag 1999). Furthermore the assessment of the fasting duration may play a role: in the present study the fasting duration prior to admission was obtained from the patient history, and could not be confirmed objectively. It is well-known that diet histories in general are unreliable, and this could be true for the fasting duration in our study as well. Therefore further studies on glucose kinetics during an objectively recorded fasting period in children with falciparum malaria are necessary.

A minor point in our study is the relatively small number of patients. For ethical reasons children with hypoglycemia cannot be included in studies like this, because hypoglycemia requires prompt treatment and therefore glucose kinetics cannot properly be studied. Also for ethical reasons it is unacceptable to study an appropriate control group consisting of healthy children of similar age. We found no data in the literature on glucose kinetics in children under the age of two years with or without disease to compare our results. In that aspect this study is unique since it is the first to provide these data in this age group.

In conclusion: our data confirm the higher risk of hypoglycemia in very young children with uncomplicated falciparum malaria. This could not be attributed to an impaired glucose production, but is presumably due to a lack in reduction of glucose utilization. The influence of nutritional status and the influence of fasting remain unclear, and must be subject for further studies. Studies on glucose kinetics are feasible in very young children with UFM and reveal more insight in the pathophysiology of hypoglycemia.

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