The relationship between glucose production and plasma glucose concentration in children with falciparum malaria

Evelien Dekker, Johannes A. Romijn, Catherine Waruiru, Mariette T. Ackermans, Gerrit J. Weverling, Robert W. Sauerwein, Erik Endert, Norbert Peshu, Kevin Marsh and Hans P. Sauerwein

Department of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; KEKIRI Clinical Research Centre, Kilifi Unit, Kilifi, Kenya; NATEC, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; Department of Medical Microbiology, University Hospital, University of Nijmegen, Nijmegen, The Netherlands

Abstract
The pathophysiology of hypoglycaemia in children with acute falciparum malaria, a frequent and serious complication, is unknown due to absence of data on glucose kinetics. We investigated the correlation between basal glucose production and plasma glucose concentration in 20 children (8 girls) with acute, uncomplicated falciparum malaria by infusion of [6,6-2H]glucose. Median plasma glucose concentration was 4.5 (range 2.1–6.5) mmol/L and median glucose production 5.0 (range 4.1–8.4) mg/kg/min. There was a positive correlation between basal glucose production and plasma glucose concentration (r=0.53, P<0.016). There was no correlation between the rate of glucose production and the plasma concentrations of alanine, lactate, counter-regulatory hormones or cytokines. It was concluded that, in children with acute uncomplicated falciparum malaria, endogenous glucose production is an important determinant of plasma glucose concentration, contrary to previous findings in adults with malaria, in whom peripheral uptake seems to be more important than glucose production in determining plasma glucose concentration.

Keywords: malaria, Plasmodium falciparum, glucose production, children, hypoglycaemia

Introduction
Hyperglycaemia is a frequent finding in acute infections. Acute falciparum malaria is a peculiar infectious disease with respect to glucose metabolism as hyperglycaemia instead of hyperglycaemia is a common complication, especially in children and pregnant women (Looareesuwan et al., 1985; White et al., 1987). Its incidence in children with acute falciparum malaria is 13.2% in Kilifi, Kenya, and it is associated with a much increased risk of death (Marsh et al., 1995).

Hypoglycaemia is caused by inhibition of glucose production and/or stimulation of glucose disposal. Basal glucose production in falciparum malaria has been measured only in adults, with plasma glucose concentrations above 3 mmol/L. In adults with complicated malaria, and in pregnant patients with uncomplicated malaria, average glucose production was increased during acute falciparum malaria compared to convalescence (Davis et al., 1993, 1994). In non-pregnant patients there was an inverse correlation between glucose production and plasma glucose concentration (Davis et al., 1993). The authors concluded that facilitated peripheral uptake (by the host and/or the parasite) was the factor most decisive in determining plasma glucose concentration in these adults.

There are no data available on glucose production during falciparum malaria in children, although globally children are by far the largest group at risk for the development of hypoglycaemia (White et al., 1987; Marsh et al., 1995). The first objective of this study was therefore the measurement of basal glucose production in children with acute falciparum malaria by primed, continuous infusions of [6,6-2H]glucose. We studied only those children with plasma glucose concentrations ≥2.2 mmol/L, because lower blood glucose levels would have necessitated intravenous glucose infusion. Theoretically, low levels of glucose uptake, counter-regulatory hormones and high levels of cytokines may be implicated in the induction of hypoglycaemia. The second objective was therefore the evaluation of the relation between the rate of basal glucose production and these factors.

Methods
Subjects
All children admitted to Kilifi District Hospital in Kenya with a primary diagnosis of malaria during the study period of 6 weeks were considered for inclusion in the study. Inclusion criteria were acute falciparum malaria, age between 2 and 10 years, and a fasting period of at least 4 h. Exclusion criteria were admission plasma glucose concentration <2.2 mmol/L, complicated malaria according to the World Health Organization criteria (WHO, 1986) (because clinical practice dictates constant glucose infusion in all such patients), treatment with quinine (quinine stimulates insulin secretion by the pancreas; Henquin et al., 1975), concomitant infectious disease, severe malnutrition, and severe chronic diarrhoea (which may induce hypoglycaemia in childhood; Bennish et al., 1990). Witnessed informed consent was obtained from the accompanying parent or guardian. The study protocol was approved by the Kenya National Ethical Committee.

Study design
Patients were recruited immediately after laboratory confirmation of the clinical diagnosis and exclusion of quinine use by a quinine screening test (Sturm et al., 1995). Each patient was weighed and treatment with Fansidar®, in some cases combined with chloroquine, was given. An intravenous cannula was introduced in a forear arm vein for isotope infusion. A second cannula for blood sampling was introduced into a suitable vein of the contralateral arm. The catheters were kept patent by a slow saline drip.

After obtaining a baseline blood sample for determination of background isotope enrichment, plasma glucose, plasma cytokine concentrations and basal haematological and biochemical tests, a primed (5.4 mg/kg), continuous (60 µg/kg/min) infusion of [6,6-2H]glucose (99%, Isotec Inc., Miamisburg, Ohio, USA), dissolved in sterile isotonic saline and sterilized by passage through a Millipore™ filter (<0.2 µm; Minisart, Sartorius AG, Göttingen, Germany), was administered by a motor driven, calibrated syringe pump (Perfusor Secura FT™, Braun AG, Melsungen, Germany). The rate of [6,6-2H]glucose infusion was calculated from the measured concentration of glucose in the infusate. The time at the start of the infusion was set at t=0.

After 90 min of [6,6-2H]glucose infusion for equilibration, 3 blood samples were collected at intervals of 15
min for determination of plasma glucose concentration and [6,6-\textsuperscript{2}H\textsubscript{4}]glucose enrichment. Blood samples for the measurement of concentrations of insulin, counter-regulatory hormones, alanine and lactate were collected at the end of the study (t=120 min).

Blood samples for determination of plasma glucose, [6,6-\textsuperscript{2}H\textsubscript{4}]glucose enrichment, insulin, counter-regulatory hormones and cytokines were collected in pre-chilled heparinized tubes and plasma samples for alanine and lactate into fluoride tubes. All samples were kept on ice and centrifuged promptly. Aliquots of separated plasma were stored below -20°C and transported on dry ice before assay.

**Assays**

All measurements were performed in duplicate and all samples of each individual subject were analysed in the same run. Glucose concentration and [6,6-\textsuperscript{2}H\textsubscript{4}]glucose enrichment in plasma were measured by gas chromatography/mass spectrometry using selected ion monitoring. The method was adapted from that of REINAUER et al. (1990), using phenyl-\(\beta\)-D-glucose as internal standard.

Plasma insulin concentration was measured by commercial radioimmunoassay (RIA) (Pharmacia Diagnostics, Uppsala, Sweden), glucagon by RIA (Daichi Radioisotope Laboratories, Tokyo, Japan), using glucagon antiserum elicited in guinea-pigs against pancreatic specific glucagon; cross-reactivity with glucagon-like substances of intestinal origin less than 1%, catecholamines by high-performance liquid chromatography and electrochemical detection, after purification on Biorex 70\textsuperscript{TM} and concentration by solvent extraction (SMEDES et al., 1982), and cortisol by fluorescence polarization immunoassay on TDx\textsuperscript{TM} (Abbott Laboratories, Chicago, Illinois, USA). Plasma alanine concentration was determined by amino acid analyser (Chromocon 500\textsuperscript{TM}, Kontron, Italy) and plasma lactate by an enzymatic method (Boehringer Mannheim, Almere, The Netherlands) on a Cobas Bio-Centrifugal\textsuperscript{TM} analyser.

Turnour necrosis factor \(\alpha\) (TNF-\(\alpha\)) concentrations were measured by enzyme amplified sensitivity assay (EASIA) (Medigenix, Amersfoort, The Netherlands) with a detection limit of 5 pg/mL. Stable TNF-receptors types I and II (sTNF-RI and sTNF-RII) were measured by EASIA, with detection limits of 0.1 and 0.5 ng/mL, respectively. Plasma concentrations of interleukin (IL) 1 were measured by immunoradiometric assay (Medigenix, Amersfoort, The Netherlands), detection limit 10 pg/mL, and IL-6 was determined by an enzyme-linked immunosorbent assay (ELISA) (CLB, Amsterdam, The Netherlands), detection level 2 pg/mL. Plasma concentrations of IL-10 were measured by ELISA (kindly provided by Schering–Plough Research Institute, Kenilworth, New Jersey, USA), detection limit 20 pg/mL.

**Calculations and statistics**

Glucose production rate was calculated from the dilution of the infused tracer in plasma. Because plasma glucose concentrations and tracer/tracer ratios for [6,6-\textsuperscript{2}H\textsubscript{4}]glucose remained constant during the study, calculations for steady state kinetics were applied, adapted for the use of stable isotopes (WOLF, 1992).

Data are reported as medians and ranges. A correlation coefficient was calculated for plasma glucose concentrations and glucose production, using linear regression analysis to investigate the relationship. Statistical significance was set at \(P<0.05\).

**Results**

**Clinical data**

Twenty children (including 8 girls) with uncomplicated acute falciparum malaria were studied (Table 1). Their illness had a median duration of 3 d (range 1–6 d) and they had not fed for a median period of 12 h (range 4–24 h) before the study. Median axillary temperature was 39.3°C (range 37.0–40.8°C). Albumin concentrations were normal except in one child, who did not differ from the other subjects in terms of weight-for-height or height-for-age by the US National Center of Health Statistics standards (HAMIL et al., 1979). Using these standards 10 children were below the tenth percentile of weight-for-height and 12 were below the tenth percentile of height-for-age. All patients responded quickly to therapy and made uneventful recoveries.

**Glucose kinetics**

Plasma glucose concentrations at times \(t=90\) min, \(t=105\) min and \(t=120\) min were similar. (Table 1). Although one of the inclusion criteria was plasma glucose concentration >2.2 mmol/L, one patient developed a plasma glucose level of 2.1 mmol/L during the study. The individual plasma glucose concentrations, including the low values, were also constant during the time of the study.

Isotopic steady state was obtained because there was no difference in tracer/tracer ratios between \(t=90\) min, \(t=105\) min and \(t=120\) min (data not shown). There was a positive correlation between plasma glucose concentration and basal glucose production (\(r=0.53, P=0.016\) (Figure).

No correlation was found between age, parasite count or body temperature, duration of fasting or duration of illness at the start of the study and plasma glucose concentration or the rate of glucose production.

**Hormones, precursors and cytokines**

Plasma concentrations of insulin and glucose counter-
Table 2. Plasma concentrations of insulin, counter-regulatory hormones, gluconeogenic precursors and cytokines in 20 Kenyan children with uncomplicated falciparum malaria

<table>
<thead>
<tr>
<th>Patients</th>
<th>Normal fasting range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (milliunits/L)</td>
<td>4 (1-25)</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>0-62 (0-14-2-27)</td>
</tr>
<tr>
<td>Glucagon (ng/mL)</td>
<td>165 (45-496)</td>
</tr>
<tr>
<td>Noradrenaline (nmol/L)</td>
<td>1-18 (0-6-3-90)</td>
</tr>
<tr>
<td>Adrenaline (nmol/L)</td>
<td>0-60 (0-15-1-98)</td>
</tr>
<tr>
<td>Alanine (µmol/L)</td>
<td>132 (74-290)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1-3 (0-8-27)</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>135 (18-468)</td>
</tr>
<tr>
<td>sTNF-R1 (ng/mL)</td>
<td>6-4 (2-1-14-7)</td>
</tr>
<tr>
<td>sTNF-R2 (ng/mL)</td>
<td>44-4 (12-6-121-0)</td>
</tr>
<tr>
<td>IL-1 (pg/mL)</td>
<td>&lt;10 (&lt;10)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>47 (6-342)</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>605 (185-3215)</td>
</tr>
</tbody>
</table>

*Median (range in parentheses). Abbreviations: TNF = tumour necrosis factor, sTNF-RI and sTNF-R2 = soluble TNF receptors, IL = interleukin.

regulatory hormones, alanine and lactate are shown in Table 2. Plasma insulin concentrations were appropriately low and plasma concentrations of the counter-regulatory hormones were appropriately high in all children, with 2 exceptions. One child had a plasma insulin concentration of 25 milliunits/L; glucose production in this child was 5-4 mg/kg/min. Another child had a low plasma cortisol concentration (0-14 µmoll/L), and a glucose production rate of 4-5 mg/kg/min. These values for glucose production were both intermediate in our study and had no other underlying disease; their plasma lactate concentrations were normal.

Discussion

This was the first study of glucose production in children with falciparum malaria. The children we studied required hospital admission, but had no defining feature of severe malaria (WHO, 1986). They were thus intermediate between mild outpatient malaria cases and those with the highest risk of hypoglycaemia. In these children there was a positive correlation between plasma glucose concentration and basal glucose production, indicating that children with the lowest plasma glucose concentration had the lowest glucose production rates. Our data suggested that plasma glucose concentrations are primarily determined by glucose production in children with uncomplicated falciparum malaria.

The clinical data indicated that the Kenyan children evaluated in this study had a weight-for-height and height-for-age around the tenth percentile of the US standards (HAMILL et al., 1979). None of the, physical examination indicated that the children were overweight and had no other underlying disease; their plasma albumin concentrations supported this finding. Hence, stunted growth in the past and not malnourishment at the time of the study was the most probable explanation for the low anthropometric values.

The declaration of Helsinki, serving as a guideline for clinical research, does not allow invasive studies in healthy children. We were therefore unable to obtain data from healthy Kenyan children for comparison with our patients. Studies in convalescence were inappropriate, for the same reason. There are only 2 published studies on basal glucose production measured by means of a non-recycling isotope in healthy children of the same age group elsewhere in the world (BIER et al., 1977; HAYMOND et al., 1978). Basal glucose production was, on average, approximately 30% lower in the Kenyan children in our study than in healthy American children with similar body weight (BIER et al., 1977). The present study suggested that, contrary to the findings in adults, glucose production in children with uncomplicated falciparum malaria is not increased. Although the comparison with healthy American children may not be optimal, this does not invalidate our observation of a positive correlation between basal glucose production and glucose production in children with malaria.

It is unlikely that the duration of fasting was a decisive factor in our study, as the mean duration was only 12 h and literature data suggest that only prolonged fasting (>1 d) is a factor contributing to hypoglycaemia in children with falciparum malaria (TAYLOR et al., 1988; KAWO et al., 1990). Moreover, there was no correlation between the duration of fasting and plasma glucose concentration or glucose production.

The only antimalarial drug known to influence glucose metabolism is quinine, which stimulates insulin release (HENQUIN et al., 1975; WHITE et al., 1983; DAVIS et al., 1990). This was not a confounding variable in our study, as none of the children had detectable plasma quinine concentrations and plasma insulin concentrations were appropriately low.

Theoretically, cytokines and/or counter-regulatory hormones could be implicated in the regulation of glucose production in our children with malaria as it is well recognized that catecholamines, glucagon and relatively low levels of the cytokines TNF-α and IL-6 can stimulate glucose production in humans (SHAMOON et al., 1981; GELFAND et al., 1984; VAN DER POL et al., 1991; STOUTARD et al., 1995), whereas IL-1 induces hypoglycaemia in animal models (FISCHER et al., 1991). As TNF-α is secreted episodically and the concentrations of soluble TNF receptors in plasma are considered to be better markers of TNF activity (GODFRIED, 1994), we also included the plasma concentrations of soluble TNF receptors in our analysis. A significant stimulation of counter-regulatory hormone and cytokine production was found in our patients, in accordance with other reports in malaria (KERN et al., 1989, 1992; KWIATKOWSKI et al., 1990; PHILLIPS et al., 1993; PEYRON et al., 1994).

However, our correlation analyses between the rate of glucose production and the plasma concentrations of these substances, separately or combined, suggest that neither of them could be implicated as a major determinant for glucose production in these children with uncomplicated malaria.

In untreated malaria, glucose metabolism is determined by factors related to host and parasite, and it has been suggested that a large glucose requirement by the parasite may contribute to the induction of hypoglycaemia (WHITTE et al., 1983). Although some contribution seems plausible, the direct role of the parasite appears to be limited. It can be calculated from data obtained from growth studies in vivo on Plasmodium falciparum parasites (ZOLG et al., 1984) that, in severe infections, the parasite consumes at the most 10% of total glucose production, suggesting that changes in the host are the most important precipitating factors for hypoglycaemia. Moreover, if increased peripheral use of glucose were a decisive factor in determining glucose concentration in malaria, a negative correlation would be expected between the rate of glucose production and the plasma glucose concentration. In contrast, we found a positive cor-
relation between glucose production and glucose concentration. Therefore, a major effect on glucose concentration by the parasite seems improbable in our patients.

In adults with severe falciparum malaria there is an inverse correlation between plasma glucose concentration and glucose production (Davis et al., 1993), suggesting that facilitated peripheral glucose uptake rather than decreased production was the most important determinant for glucose concentration. In contrast, in children with uncomplicated falciparum malaria there was a positive correlation between glucose production and glucose concentration, suggesting that the rate of production of glucose rather than glucose uptake was the most important determinant of plasma glucose concentration. These data suggest that glucose metabolism in falciparum malaria is differently regulated in children and adults.

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References


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