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Published in:
QJM

Citation for published version (APA):

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The influence of alanine infusion on glucose production in ‘malnourished’ African children with falciparum malaria

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Received 17 February 1997 and in revised form 20 April 1997

Summary

By US standards, about half of African children are malnourished, although most appear clinically normal. It is possible that precursor supply for gluconeogenesis is limited to a greater extent in these seemingly malnourished African children than in healthy children, consequently limiting glucose production. Since in malaria peripheral glucose utilization is increased, precursor supply could play an even more critical role in maintaining glucose production in African children suffering from falciparum malaria. We studied the effect of alanine infusion (1.5 mg/kg/min) on glucose production (measured by infusion of [6,6–¹³C₂]glucose) and plasma glucose concentration in 10 consecutive children with acute, uncomplicated falciparum malaria. By US standards, six children were below the 10th percentile of weight for height and seven were below the 10th percentile of height for age. Plasma concentrations of alanine increased during alanine infusion from 153 ± 21 to 468 ± 39 μmol/l, whereas plasma lactate concentrations did not change (1.4 ± 0.2 vs. 1.3 ± 0.2 mmol/l). Plasma glucose concentration and glucose production did not change during alanine infusion: 4.6 ± 0.3 vs. 4.5 ± 0.3 mmol/l and 5.8 ± 0.4 vs. 5.7 ± 0.3 mg/kg/min, respectively. Gluconeogenic precursor supply is sufficient for maintainance of glucose production in African children with uncomplicated malaria who are malnourished by US standards.

Introduction

In healthy adults, gluconeogenic substrate supply does not play a major role in the regulation of total glucose production during fasting for 16 h.¹² Since young children have limited glycogen stores, gluconeogenesis becomes the predominant source of glucose production during fasting earlier in children than in adults, and the high glucose tissue utilization rate in children allows only a precarious balance to be maintained.³ This is reflected by the inability of children aged 1.5–9 years to maintain a normal plasma glucose concentration during a 24-hour fast.⁴ Of the American weight-for-age standards, 80% or 75% are cut-off points widely used to define malnutrition. If these were applied to African children, approximately half of the children would have to be considered as malnourished, although most of them appear clinically normal.⁵ It is possible that gluconeogenic precursor supply is limited to a greater extent in these seemingly malnourished African children, consequently limiting glucose production.

Since in malaria peripheral glucose utilization is increased,⁶ precursor supply could play an even more critical role in maintaining glucose production in these African children suffering from falciparum malaria.
malaria. This is an interesting issue, as hypoglycaemia, not due to hyperinsulinism caused by the administration of quinine, is a frequent complication in African children with severe falciparum malaria, associated with a high morbidity and mortality.

The objective of this study was to evaluate the effect of extra precursor supply by infusion of alanine (1.5 mg/kg/min) on glucose production (measured by primed, continuous infusion of $[6,6^{-2}\text{H}_2]\text{glucose}$) and plasma glucose concentration in African children with uncomplicated falciparum malaria, maltrenished according to American standards.

Methods

Patients

All children admitted to Kilifi District Hospital 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 4–24 h. Exclusion criteria were: admission plasma glucose concentration <2.2 mmol/l, complicated malaria according to the WHO-criteria (because clinical practice dictates constant glucose infusion in all these patients), treatment with quinine (quinine stimulates insulin secretion by the pancreas), concomitant infectious disease, severe malnutrition (defined as kwashiorkor or marasmus) and severe chronic diarrhoea (which may induce hypoglycaemia in childhood). Fully informed consent was obtained from the accompanying parent or guardian. This study 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 dipstick. Each patient was weighed and treatment with fansidar, in some cases combined with chloroquine, was given. An intravenous cannula was introduced in a forearm 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.

The study design is shown in Figure 1. After obtaining a baseline blood sample for determination of background isotope enrichment, plasma glucose, plasma cytokine concentrations and basal hematological and biochemical tests, a primed (5.4 mg/kg), continuous (60 µg/kg/min) infusion of $[6,6^{-2}\text{H}_2]\text{glucose}$ (99%, Isotec), dissolved in sterile isotonic saline and sterilized by passage of the solution through a sterile membrane filter (pore size 0.2 µm; Minisart, Sartorius) was administered by a motor-driven, calibrated syringe pump (Perfusor Secura FT, Braun, Melsungen). The rate of $[6,6^{-2}\text{H}_2]\text{glucose}$ 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 $[6,6^{-2}\text{H}_2]\text{glucose}$ infusion for equilibration, three blood samples were collected at intervals of 15 min for determination of plasma glucose concentration and $[6,6^{-2}\text{H}_2]\text{glucose}$ enrichment. Blood samples for the measurement of concentrations of alanine and lactate were also collected at this time (t=120 min).

At t=120 min, alanine infusion (1.5 mg/kg/min) was started by a motor-driven, calibrated syringe pump. After another 90 min of $[6,6^{-2}\text{H}_2]\text{glucose}$ and alanine infusion for equilibration, three blood samples were collected at intervals of 15 min for determination of plasma glucose concentration and $[6,6^{-2}\text{H}_2]\text{glucose}$ enrichment. Blood samples for the measurement of concentrations of insulin, counter-regulatory hormones, alanine and lactate were also collected at the end of the study (t=240 min).

Figure 1. Study design.
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 were 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 [6,6-2H2]glucose enrichment in plasma were measured by gas chromatography/mass spectrometry using selected ion monitoring. The method was adapted from Reinauer et al., using phenyl-β-D-glucose as internal standard.13

Plasma insulin concentration was measured by commercial RIA (Pharmacia Diagnostics), glucagon by RIA (Daiichi Radioisotope Laboratories; glucagon-antiserum elicited in guinea pigs against pancreatic-specific glucagon; cross-reactivity with glucagon-like substances of intestinal origin, <1%), catecholamines by HPLC and electrochemical detection, after purification on Biorex 70 and concentration by solvent extraction,14 and cortisol by fluorescence polarization immunoassay on TDx (Abbott Laboratories). Plasma alanine concentration was determined by aminoacid analyzer (Chromcon 500, Kontron), plasma lactate by an enzymic method (Boehringer Mannheim) on a Cobas Bio Centrifugal analyzer.

Cytokine assays: TNF-α concentrations were measured by EASIA (Medgenix) with a detection limit of 5 pg/ml. Soluble TNF-receptors type I and II were measured by EASIA (Medgenix) with detection limits of 0.1 and 0.5 ng/ml, respectively. Plasma concentrations of IL-1 were measured by IRMA (Medgenix), detection limit 10 pg/ml, and IL-6 was determined by an ELISA (CLB), detection level 2 pg/ml. Plasma concentrations of IL-10 were measured by ELISA (kindly provided by Schering-Plough Research Institute, Kenilworth, NJ, 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/tracee ratios for [6,6-2H2]glucose remained constant during the study, calculations for steady-state kinetics were applied, adapted for the use of stable isotopes.15

Data are reported as means ± SEM, unless otherwise stated. Statistical analysis was by paired t-test. Statistical significance was set at p < 0.05.

Results

Clinical data

Ten children (five girls) with uncomplicated acute falciparum malaria were studied. Clinical and laboratory details are given in Tables 1 and 2. Their illness had a median duration of 3 (range 2–6) days, and they had not eaten for a median period of 12 (range 4–24) h before the study. Median axillary temper-

Table 1  Plasma glucose concentrations and hepatic glucose production in 10 Kenyan children with uncomplicated falciparum malaria

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>[Glucose] (mmol/l) Basal–during alanine</th>
<th>Ra glucose mg/kg/min Basal–during alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.0</td>
<td>M</td>
<td>130</td>
<td>21.1</td>
<td>5.7–5.7</td>
<td>5.9–5.4</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>F</td>
<td>108</td>
<td>14.2</td>
<td>4.5–4.6</td>
<td>4.7–5.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>M</td>
<td>103</td>
<td>12.4</td>
<td>4.4–3.6</td>
<td>7.5–7.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>M</td>
<td>81</td>
<td>11.8</td>
<td>4.1–4.1</td>
<td>4.4–4.5</td>
</tr>
<tr>
<td>5</td>
<td>5.5</td>
<td>F</td>
<td>104</td>
<td>16.1</td>
<td>3.8–3.0</td>
<td>6.4–5.6</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>M</td>
<td>82</td>
<td>9.4</td>
<td>3.5–3.8</td>
<td>6.6–6.7</td>
</tr>
<tr>
<td>7</td>
<td>4.5</td>
<td>F</td>
<td>102</td>
<td>14.1</td>
<td>5.1–4.9</td>
<td>5.6–6.2</td>
</tr>
<tr>
<td>8</td>
<td>4.5</td>
<td>F</td>
<td>102</td>
<td>14.2</td>
<td>6.5–5.9</td>
<td>7.4–6.5</td>
</tr>
<tr>
<td>9</td>
<td>7.0</td>
<td>M</td>
<td>113</td>
<td>17.5</td>
<td>4.1–4.0</td>
<td>4.8–5.3</td>
</tr>
<tr>
<td>10</td>
<td>7.0</td>
<td>F</td>
<td>109</td>
<td>15.1</td>
<td>4.3–4.9</td>
<td>4.9–4.7</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>4.9 ± 0.7</td>
<td>103 ± 5</td>
<td>14.6 ± 1.0</td>
<td>4.6 ± 0.3–4.5 ± 0.3</td>
<td>5.8 ± 0.4–5.7 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Clinical and biochemical data for 10 Kenyan children with uncomplicated falciparum malaria

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitaemia (/µl)</td>
<td>225630</td>
<td>77–699200</td>
</tr>
<tr>
<td>Haemoglobin (g%)</td>
<td>9.1</td>
<td>6.6–11.2</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>36</td>
<td>30–47</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>29</td>
<td>24–41</td>
</tr>
<tr>
<td>SGPT (U/l)</td>
<td>10</td>
<td>6–18</td>
</tr>
<tr>
<td>Plasma bilirubin (µmol/l)</td>
<td>14</td>
<td>3–28</td>
</tr>
</tbody>
</table>
ature was 38.9 (range 37.0–40.5) °C. By US standards, six were below the 10th percentile of weight for height and seven were below the 10th percentile of height for age (National Center of Health Statistics, USA\(^{19}\)). All patients responded quickly to therapy and made uneventful recoveries.

**Glucose metabolism**

During alanine infusion, plasma alanine concentration increased significantly from 153 ± 21 to 468 ± 39 μmol/l (\(p < 0.05\)); plasma lactate concentration did not change (1.4 ± 0.2 vs. 1.3 ± 0.2 mmol/l).

Plasma glucose concentrations did not change during alanine infusion: 4.6 ± 0.3 vs. 4.5 ± 0.3 mmol/l (Table 1, Figure 2a). There were no differences in tracer/tracce ratios between \(t = 90\), \(t = 105\) and \(t = 120\) min, or between \(t = 210\), \(t = 225\) and \(t = 240\) min (data not shown). During alanine infusion, glucose production did not change: 5.8 ± 0.4 vs. 5.7 ± 0.3 mg/kg/min (Figure 2b).

**Hormones and cytokines**

Plasma concentrations of insulin and glucocounter-regulatory hormones are presented in Table 3. Plasma insulin concentrations were low and plasma concentrations of all counter-regulatory hormones (except noradrenaline) were high in all patients.

Plasma concentrations of the cytokines TNF-\(\alpha\), soluble TNF receptors types I and II (sTNF-RI and RII), IL-1, IL-6 and IL-10 are presented in Table 4. The concentrations of all cytokines and soluble receptors, except for IL-1, were elevated in the majority of the patients, as found previously in patients with falciparum malaria.\(^{17–20}\)

**Discussion**

In these Kenyan children with uncomplicated falciparum malaria, alanine infusion did not increase glucose production. These results suggest that despite the rather low plasma alanine concentrations, gluconeogenic precursor supply was not a factor limiting glucose production. These children had weight-for-
heights and height-for-ages around the 10th percentile of the US standards of weight for height and height for age. Furthermore, physical examination indicated that these children were well-proportioned and had no other underlying disease. Moreover, plasma albumin concentrations were within the normal range.

The absence of an increase in glucose production during alanine infusion is not due to inadequate alanine infusion, since (i) plasma alanine concentrations increased significantly, by approximately 200%, and (ii) if all alanine had been transformed into glucose, an increase of glucose production by approximately 25% could be expected. Moreover, the same dose of alanine infusion was given to adults and prevented a decrease in glucose production during dichloroacetate (DCA) administration.

The children we studied required hospital admission, but had no defining features of severe malaria. They are thus intermediate between mild out-patient malaria and those with the highest risk of hypoglycaemia. The clinical impression that they were indeed sick, is supported by the high plasma cytokine concentrations in these children.

In this study, in children with uncomplicated malaria considered malnourished, plasma concentrations of alanine were in the low-normal range: those of lactate were normal. However, White et al. found raised concentrations of alanine and lactate in children with severe falciparum malaria, especially in those with hypoglycaemia. The cause of this discrepancy in plasma concentrations could be due to diminished liver perfusion that has been documented in severe malaria but not in uncomplicated malaria, as indicated by indocyanine-green-clearance studies by Molyneux et al. Consequently, decreased clearance rates of gluconeogenic precursors may be present in severe malaria, but not in uncomplicated malaria.

Basal glucose production in these Kenyan ‘malnourished’ children with uncomplicated falciparum malaria was approximately 25% lower compared to healthy American children with similar body weight, and equal to or approximately 20% lower compared to healthy American children with similar age. The rate of glucose production in normal Kenyan children is unknown. Unfortunately, we are unable to obtain reference values in healthy children for ethical reasons dictated by the Declaration of Helsinki.

Compared to the abovementioned data, glucose production was not increased in these Kenyan children despite increases in the counter-regulatory hormones and cytokines. Relative impairment of glucose production is therefore a possibility in normoglycaemic children and in the pathophysiology of hypoglycaemia in malaria. One of the possible causes of a relative impairment of glucose production is shortage of precursor supply for gluconeogenesis. In adults, in the postabsorptive state, gluconeogenic substrate supply does not play a major role in the regulation of total glucose output, unless its supply diminishes below a certain level. Several studies in postabsorptive adults tested the effect of extra gluconeogenic precursor supply on total glucose production by infusing glycerol or lactate, and reported no increase in glucose production. However, after prolonged starvation (3–4 weeks) in adults, extra precursor supply did influence glucose kinetics. These adults had low levels of gluconeogenic precursors associated with hypoglycaemia and a reduced glucose production rate, and only in this circumstance did alanine infusion cause an increase in plasma glucose concentrations and an increased incorporation of [14C]label from alanine into glucose. Comparable results were obtained in another study in adults, where fluxes of the major gluconeogenic substrates were decreased by DCA. Replenishing gluconeogenic precursor supply (by exogenous alanine infusion) during DCA administration prevented the decrease in glucose production. These data suggest that adequate amounts of gluconeogenic precursors are always necessary to maintain glucose production. Only one study on the relation between gluconeogenic precursor supply and glucose production in children has been published in Anglo-American literature. Kerr et al. tested the effect of infusion of alanine in malnourished and recovered children after an overnight fast, and found an increase in plasma glucose concentration (≈ 4 mg/100 ml or 1.7 mmol/l), but not in glucose production, in both groups. These data suggest that in malnourished children, precursor supply is not critical.

Our data suggest that the amount of gluconeogenic precursors required to maintain an adequate glucose production does not exceed the supply of these precursors by the body in Kenyan children with uncomplicated malaria who are malnourished by US standards.

Acknowledgements

This paper was published with the permission of the Director of KEMRI. We are indebted to doctors, nursing and laboratory staff at the KEMRI unit, Kilifi and at the Kilifi District Hospital for their pleasant collaboration and helpful advice, to the technicians of the Endocrinology Laboratory for their assistance and to Theunis Eggelte for providing the quinine dipsticks. This work was supported by the Metabolic Research Fund AMC, KEMRI and the Wellcome Trust. JAR is a clinical investigator supported by the Netherlands Organisation for Scientific Research (NWO) and the Dutch Diabetes Foundation. KM is a Wellcome Trust Senior Fellow in Clinical Science.
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