Glucose production and gluconeogenesis in adults with cerebral malaria

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Summary

Hypoglycaemia is an important complication in severe malaria, ascribed to an inhibition of gluconeogenesis. However, the only data available suggested that in severe malaria, total glucose production is increased. We measured glucose production and gluconeogenesis after an overnight fast in all seven patients with cerebral malaria (CM) consecutively admitted to Bao Loc General hospital over a 2-year period, and in six healthy sex- and age-matched controls. Glucose production was measured by infusion of [6,6–2H2]glucose and the contribution of gluconeogenesis by oral ingestion of 2H2O. Compared to controls, plasma glucose concentration was 42% higher in CM patients (p = 0.004), and glucose production was doubled (p = 0.003). Gluconeogenesis contributed 100% of the total glucose in CM patients but only 58% in controls (p = 0.003). The plasma concentrations of the substrates for gluconeogenesis and the glucoregulatory hormones were not different between patients and controls, except for an increase in lactate and cortisol in the patients. Cerebral malaria is associated with increases rather than decreases in plasma glucose, glucose production and gluconeogenesis, and there is no contribution of glycogenolysis to glucose production. Factors other than malaria per se are involved in the pathogenesis of hypoglycaemia associated with CM.

Introduction

Disturbances in glucose metabolism, especially hypoglycaemia, complicate severe malaria in both adult and paediatric patients, with a prevalence of ~10%.1–6 Hypoglycaemia is associated with a poor prognosis in children with severe malaria, and is considered an independent risk factor for mortality.7,8 Some studies have assumed that hypoglycaemia in severe malaria is caused by impaired glucose production, due to disruption of gluconeogenesis, although gluconeogenesis has not been quantified in severe malaria.9,10 Data on total glucose production in severe malaria in general have so far been provided only by Davis et al.,11 who found that glucose production was increased by 50% compared to convalescence.

Severe malaria includes a spectrum of different manifestations, and is not therefore a homogeneous disease entity. Cerebral malaria is one of the most serious forms, for which the presence of coma is a prerequisite. Of the 18 patients with severe malaria...
studied by Davis, only four had cerebral malaria. As hypoglycaemia is especially found in cerebral malaria, it may be difficult to separate the role of cerebral malaria per se in the induction of hypoglycaemia from the changes in glucose metabolism induced by other forms of severe malaria. We therefore focussed our study on patients with cerebral malaria without additional complications.

The objective of this study was to measure basal glucose production using $\text{d}_6,6\text{-}^2\text{H}_2\text{glucose}$ and the fractional contribution of gluconeogenesis using $^2\text{H}_2\text{O}$ in all patients with cerebral malaria consecutively admitted to Bao Loc General Hospital over a 2-year period.

**Methods**

**Patients**

All adult non-pregnant patients with cerebral malaria, admitted to the ICU of Bao Loc General Hospital between July 1997 and February 1999 were recruited. The inclusion criteria were based on the definition of the WHO for cerebral malaria. Exclusion criteria were: treatment with quinine, severe anaemia (Hct < 15%) and concomitant infectious diseases. Six healthy subjects were enrolled as controls. The study was approved by the local health authorities and by the Medical Ethical Committee, Academic Medical Centre, Amsterdam, The Netherlands.

**Study design**

Patients were recruited on the day of admission after quinine use was excluded by quinine dipstick. All patients were immediately treated with artesunate (Guilin No 2 Pharmaceutical Factory) intravenously according to the standard regimen of Bao Loc hospital, immediately after laboratory confirmation of the diagnosis. After receiving informed consent signed by a first-degree relative, the patient was given a standard meal of ~400–450 ml of soup (rice and pork meat) through a gastric tube (also standard regimen for comatose patients in this hospital) followed by a fast until completion of the study.

The study design is shown in Figure 1. Twelve hours after the end of the last meal, an intravenous cannula was introduced into a forearm vein for blood sampling. The catheter was kept patent by a slow isotonic saline drip, 0.5% enriched with $^3\text{H}_2\text{O}$ (Cambridge Isotope Laboratories).

After obtaining blood samples for determination of various parameters and background enrichment, patients were given (through the gastric tube) 1 g of $^3\text{H}_2\text{O}$ per kg body water at 30-min intervals, for a total of five doses (total dose of 5 g/kg body water). Body water was estimated to be 60% of body weight in male and 50% in female.

The time of the first dose of $^2\text{H}_2\text{O}$ ingestion was set at T = 0. Blood for $^2\text{H}$ enrichment was drawn at T = 6.00; 6.15 and 6.30 h. A blood sample for background enrichment of $[6,6-^2\text{H}_2]\text{glucose}$ was also drawn at 6.30 h. Then (at T = 6.30 h) 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) dissolved in sterile isotonic saline, was administered by a motor-driven, calibrated syringe pump (Perfusor Secura FT, B. Braun) through a Millipore filter (size 0.2 mm; Minisart, Sartorius). Urine for $^3\text{H}$ enrichment was collected between 6.00 and 7.00 h after emptying of the bladder by pressing on it with a urinary catheter installed at 5.00 h.

At T = 8.20 h, three blood samples were collected at intervals of 10 min for determination of plasma glucose concentration and $[6,6-^2\text{H}_2]\text{glucose}$ enrichment. Blood samples for the measurement of plasma concentration of insulin, counter-regulatory hormones, alanine, lactate, FFA and glycerol were also collected at T = 8.30 h. The study ended at T = 8.40 h.

Plasma glucose concentrations were measured regularly to detect hypoglycaemia. Blood samples for measurement of gluconeogenesis were promptly deproteinized by adding an equal amount of 10% perchloric acid. Blood for $[6,6-^2\text{H}_2]\text{glucose}$ enrichment, and that for hormone measurement, was collected in prechilled heparinized tubes, and that for lactate and alanine in fluoride tubes. All samples were kept on ice and centrifuged immediately. Plasma and urine were stored at below $-20\,^\circ\text{C}$, and
were transported on dry ice before assay in The Netherlands.

**Assays**

Plasma samples for glucose enrichments of \( [6,6-{^2}H_2] \) glucose were deproteinized with methanol. 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) DB17 column (30 m x 0.25 mm, d\(_f\) 0.25 \( \mu \)m). Glucose concentrations were determined by gas chromatography using xylose as an internal standard. Glucose was monitored at m/z 187, 188 and 189. The enrichment of glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for natural enrichments.

To measure deuterium enrichment at the C5 position, glucose was converted to hexamethylentetraamine (HMT) as described by Landau et al. HMT was injected into a gas chromatograph mass spectrometer. Separation was achieved on an AT-Amine (Alltech) column (30 m x 0.25 mm, d\(_f\) 0.25 \( \mu \)m). HMT consists of six formaldehyde molecules, originally derived from the C5 of six glucose molecules. The ions monitored for the HMT were m/z 140 and 141. Deuterium enrichment in body water was measured by a method adapted from Previs et al. 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 ionization mode, Hewlett-Packard).

Plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Diagnostic AB): intra-assay coefficient of variation (CV) 3–5%; inter-assay CV 6–9%; detection limit 15 pmol/l. C-peptide was determined by RIA (RIA-coat c-peptide, Byk-Sangtec Diagnostica): intra-assay CV 4–6%, inter-assay CV 6–8%; detection limit 50 pmol/l. Cortisol was measured by enzyme-immunoassay on an Immulite analyser (DPC), intra-assay CV 2–4%; inter-assay CV 3–7%; detection limit 50 nmol/l. Glucagon was determined by RIA (Linco Research): intra-assay CV 3–5%; inter-assay CV 9–13%; detection limit 15 ng/l. Norepinephrine and epinephrine were determined by an in-house HPLC 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 free fatty acids were measured by an enzymatic method (NEFAC; Wako Chemicals): intra-assay CV 2–4%; inter-assay CV 3–6%; detection limit 0.02 nmol/l. TNF-alpha was measured by ELISA (CLB) with a detection limit of 2 pg/ml.

**Calculations and statistics**

Glucose production rate was calculated from the dilution of labelled glucose in plasma. Because the plasma glucose concentration and enrichment percentage of \( [6,6-{^2}H_2] \) glucose varied (albeit little) at T = 8.20, 8.30 and 8.40 h, we applied the Steele equation for non-steady-state conditions with the fraction of total extracellular glucose pool (pV) assumed to be 165 ml/kg.

The rate of gluconeogenesis was calculated by multiplying the total rate of glucose production by fractional gluconeogenesis. The fractional gluconeogenesis = 100% \times \left[ {^[2}H] \right. enrichment on C5 of glucose/\left[ ^{[2}H \right] enrichment in urinary water. The rationale has been discussed in detail by Landau. In brief, carbon 5 of the glucose formed via gluconeogenesis takes its hydrogen from water for both sources, glycerol and phosphoenolpyruvate. The isomerization of hydrogen from water is transferred to that carbon is extensive. There is no exchange of water with the hydrogen bound to C5 of glucose in glycogenolysis. Thus, the ratio of enrichment at C5 of glucose to that at C2 or in water at steady state is a measure of fraction of the glucose formed by gluconeogenesis.

Glucose clearance was calculated by dividing glucose turnover by the plasma concentration.

Data are are means ± SEM, unless otherwise stated. Two sample comparisons were made using the Mann-Whitney test. A p value of <0.05 was taken as significant. The SPSS statistical software program was used for analysis.

**Results**

**Clinical data**

Seven Vietnamese patients with cerebral falciparum malaria, and six healthy controls matched for age and sex were studied. These patients were the only patients admitted for cerebral malaria over the 2-year study period. The main characteristics of both groups are shown in Table 1. All patients presented to the Intensive Care Unit in a comatose state with a Glasgow coma scale <11. None had hypoglycaemia on admission. Their duration of illness was 4 ± 1 days. They were all treated with artesunate intravenously immediately after admission to the hospital. This is the standard treatment for cerebral malaria in Vietnam. Three patients died (patients 1, 3 and 7): patient 3 from refractory pulmonary oedema and patient 7 from respiratory insufficiency, respectively 31 h and 42 h after the
end of the study. Neither had hypoglycaemia at any time. Patient 1 developed liver failure and renal failure one day after the study. He was transferred to tertiary care hospital in Ho Chi Minh City, where he was treated with peritoneal dialysis. The course of his illness was complicated by recurrent hypoglycemia, and he was discharged after 2 weeks of treatment, but later died at home. The other four patients responded well to therapy and recovered completely.

**Glucose metabolism (Figure 2)**

The plasma glucose concentration in the patients was 42% higher than in the healthy controls (6.68 ± 0.31 vs. 4.72 ± 0.20 mmol/l; p = 0.004). The mean glucose production was almost doubled in the patients compared with the controls (27.4 ± 2.1 vs. 13.8 ± 0.8 mmol/kg/min; p = 0.003). Glucose clearance was higher in patients than in controls (4.1 ± 0.3 vs. 2.9 ± 0.2 ml/kg/min; p = 0.01).

The mean $^2$H enrichment in total body water in urine collected from 6.00 to 7.00 h of the experiment was 0.38% (range 0.33–0.44%) in patients and 0.47% (0.43–0.50%) in controls (p = 0.004). Glucose production was 100% from gluconeogenesis in the patients, but only 58% in the controls (p = 0.003). Consequently, gluconeogenesis made no contribution to glucose production in the patients.

**Precursors, free fatty acid, glucoregulatory hormones and TNF-alpha (Table 2)**

The plasma concentrations of alanine and glycerol were not different between patients and controls, while lactate concentrations were higher in the patients. Plasma free fatty acids were not different between the groups. There was no difference in plasma insulin, glucagon, epinephrine and norepinephrine concentrations between the groups except for plasma cortisol, which was significantly higher in patients than in controls. TNF-alpha was also significantly higher in patients than in controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CM patients</th>
<th>Controls</th>
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<tr>
<td>Age (years)</td>
<td>32 ± 5</td>
<td>34 ± 4</td>
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<tr>
<td>Sex (M/F)</td>
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<td>5/1</td>
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<td>BMI</td>
<td>19.3 ± 0.7</td>
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<td>Parasitaemia (per ml)</td>
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<td>Glasgow coma score</td>
<td>6 ± 1</td>
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<td>Haemoglobin (mmol/l)</td>
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<td>Serum AST (U/l)</td>
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<td>NS</td>
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<td>Serum ALT (U/l)</td>
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<td>Creatinine (μmol/l)</td>
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<td>Precursors</td>
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<td>Alanine (μmol/l)</td>
<td>286 ± 29</td>
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<td>Lactate (mmol/l)</td>
<td>2.55 ± 0.82</td>
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<td>Glycerol (μmol/l)</td>
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<td>FFA (mmol/l)</td>
<td>0.8 ± 0.06</td>
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<td>TNF-alpha (pg/ml)</td>
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<td>Hormones</td>
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<td>Insulin (pmol/l)</td>
<td>49 ± 11</td>
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<td>Glucagon (ng/l)</td>
<td>132 ± 33</td>
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<td>Cortisol (nmol/l)</td>
<td>1080 ± 163</td>
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<td>Epinephrine (nmol/l)</td>
<td>0.27 ± 0.13</td>
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<td>Norepinephrine (nmol/l)</td>
<td>4.8 ± 3.5</td>
<td>1 ± 0.1</td>
<td>NS</td>
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</table>

Data are means ± SEM. CM, cerebral malaria; NS, not significant.

**Figure 2.** Total glucose production and gluconeogenesis ratio in patients with cerebral malaria and controls. Data are means ± SEM.
Discussion

Our data clearly show that in our series of consecutively admitted adults with cerebral malaria after 20 h of fasting, plasma glucose concentration was 42% higher than in healthy control subjects, concomitant with a doubling of glucose production. Further, this increased glucose production was entirely dependent on gluconeogenesis.

The data on glucose production in our controls were comparable to those in other studies.21-23 The data on fractional gluconeogenesis were also completely consistent with those in healthy subjects after a comparable time of fasting, measured by the same method17,21,24,25 or by 13C NMR.23 In light of the relationship between disease severity and the degree of stimulation of glucose production found in other diseases, our finding of a doubling of glucose production in cerebral malaria is not unexpected.24 Our group has previously reported that glucose production is 25% higher in adults with uncomplicated falciparum malaria.21 Similarly, Davies et al. showed that in adults with severe malaria, glucose production is 50% higher than in convalescence.11

The finding that the increased glucose production in cerebral malaria was completely dependent on gluconeogenesis was unexpected. Complete dependency on gluconeogenesis has been described earlier, but only during long-term fasting, when glycogen stores are exhausted.17,27 During fasting in healthy subjects, the relative contribution of gluconeogenesis to glucose production is increased but glucose production itself is decreased. Until recently, gluconeogenesis could not be measured reliably in humans due to problems with estimation of enrichment of the precursor for gluconeogenesis (oxaloacetate). Two different stable isotope methods for the quantification of gluconeogenesis have been described, that bypass this problem: mass isotomer distribution analysis (MIDA) based on infusion of [2,13C]glycerol or [U-13C]glucose, and the deuterated water method.27 A third method to estimate gluconeogenesis in humans in vivo is the measurement of changes in glycogen content over time by 13C-NMR, together with the quantification of endogenous glucose production by stable isotopes. By subtraction of changes in glycogen content from total glucose production, gluconeogenesis can be estimated.28 Because the results obtained in healthy subjects by the deuterated water method and 13C-NMR are in close agreement, both methods are presently considered to be the gold standard for measuring gluconeogenesis.

In non-severe malaria, glucose production is increased by ~25%, and gluconeogenesis contributes ~87% to total glucose production.21 When compared to those data, our study supports the notion of a relationship between the severity of malaria on one hand and the rate of glucose production and gluconeogenesis on the other.

The values for gluconeogenesis obtained in the different stages of malaria might be overestimated by the deuterated water method. For instance, triose phosphate cycling, stimulation of the pentose cycle and transaldolase reactions will result in overestimation.24,29 However, in healthy subjects, overestimation by (one of) these possibilities is thought to be only a few percent.24 Doubling or tripling of these pathways will therefore probably not invalidate our conclusion that cerebral malaria is characterized by massive stimulation of gluconeogenesis.

The mechanism of increased glucose production and gluconeogenesis in cerebral malaria and/or malaria in general cannot be determined from this study with any certainty. Cytokines, counter-regulatory hormones and precursor supply are all potentially implicated in the regulation of glucose production in malaria. In this study, plasma concentrations of glucoregulatory hormones were not different between patients and controls, except for cortisol. High plasma cortisol may stimulate gluconeogenesis in humans.22 In our patients, the five-fold increase in plasma concentration supports the idea of a major role for cortisol in the stimulation of gluconeogenesis in patients with malaria. However, the rather limited increase in plasma cortisol (two-fold) associated with a major stimulation of gluconeogenesis in the patients with non-severe malaria in our previous study, makes this explanation less likely.21

Among the gluconeogenic precursors, we found that glycerol was low in patients, whereas alanine was comparable and lactate was higher. Lactate is an important precursor for gluconeogenesis. However, the high plasma concentration of this precursor does not explain the findings of our study. It is well known that precursor supply is important in maintaining basal glucose production, but supply of precursors above a certain minimum does not stimulate glucose production.30 Lastly, it is not likely that our results are influenced by the treatment, as artesunate does not influence glucose metabolism.31

The mechanism inducing hypoglycaemia in cerebral malaria can not be deduced from our study. None of our patients, except patient 1, had hypoglycaemia, but the complications of his illness are in themselves well-known causes for hypoglycaemia. The plasma glucose concentration is the result of glucose production and glucose uptake. In our patients, increases in glucose
production and in glucose clearance were both found. Theoretically, inhibition of production or facilitation of peripheral uptake of glucose are both possible explanations for the induction of hypoglycaemia in cerebral malaria in general. In our series of consecutively admitted patients, the rate of increase in glucose production was much higher than the rate of increase of glucose clearance, resulting in an increase in plasma glucose. Our data therefore indicate that cerebral malaria itself does not induce hypoglycaemia. Additional factors must be involved in the induction of hypoglycaemia in cerebral malaria. Severe anaemia, prolonged starvation, sepsis or malnutrition are provoking possibilities, but in our patients none of these factors were present.

We conclude that cerebral malaria stimulates glucose production to a greater extent than other forms of malaria. In cerebral malaria, glucose production is completely dependent on gluconeogenesis. Remarkably, in our patients with cerebral malaria, hypoglycaemia was absent, suggesting that other factors than malaria per se may be involved in the pathogenesis of malarial hypoglycaemia.

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