FFAs are not involved in regulation of gluconeogenesis and glycogenolysis in adults with uncomplicated *P. falciparum* malaria

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Van Thien, Huynh, G. J. Weverling, M. T. Ackermans, Nguyen canh Hung, E. Endert, P. A. Kager, and H. P. Sauerwein. FFAs are not involved in regulation of gluconeogenesis and glycogenolysis in adults with uncomplicated *P. falciparum* malaria. *Am J Physiol Endocrinol Metab* 287: E609–E615, 2004. First published May 27, 2004; 10.1152/ajpendo.00026.2004.—In normal subjects, elevation of plasma free fatty acid (FFA) levels stimulates gluconeogenesis (GNG) (11) and inhibits glycogenolysis (GLY) (11). We hypothesized that FFAs are regulators of GNG and GLY in uncomplicated *falciparum* malaria, we investigated the effect of inhibition of lipolysis by acipimox in 12 patients with uncomplicated *falciparum* malaria. Six of them were given acipimox, and six served as controls. Also as controls, six matched healthy subjects were studied on two occasions with and without acipimox. After 16 h of fasting, glucose production and GNG were significantly higher in the malaria patients compared with the healthy controls (*P* = 0.003 and <0.0001, respectively), whereas GLY was significantly lowered (*P* < 0.001), together with elevated plasma concentrations of cortisol and glucagon. During the study, glucose production in patients declined over time (*P* < 0.0001), without a statistically significant difference between the acipimox-treated and untreated patients. In controls, however, with acipimox the decline was less outspoken compared with nontreated controls (*P* = 0.005). GNG was unchanged over time in patients as well as in healthy controls, and no influence of acipimox was found. In patients, GLY declined over time (*P* < 0.001), without a difference between acipimox-treated and untreated patients. In contrast, in controls treated with acipimox, no change over time was found, which was statistically different from the decline in untreated controls (*P* = 0.002). In conclusion, in *falciparum* malaria, FFAs are not involved in regulation of glucose production, nor of GNG or GLY.

*Plasmodium falciparum*; free fatty acids

**Subjects and Methods**

**Subjects.** Twelve adult, nonpregnant patients with uncomplicated *falciparum* malaria consecutively admitted to Bao Loc General Hospital were recruited. Entrance criteria were demonstration of asexual *P. falciparum* parasites in a blood smear and clinical appearance of malaria. Exclusion criteria were severe malaria based on the definition by the World Health Organization (40), treatment with quinine (10), concomitant infectious diseases, and prior history of peptic ulcer disease. Six healthy volunteers matched in sex and age were enrolled as controls. They were studied twice, one time with and one time without acipimox. These studies were done in balanced assignment and separated by a 4-wk washout period.

The study was approved by the local health authorities and by the Medical Ethics Committee, Academic Medical Center, Amsterdam, The Netherlands.

**Study design.** Patients were recruited on the day of admission after quinine use was excluded by quinine dipstick (32). All patients were treated orally with artesunate. Six patients also received acipimox (see below), a known inhibitor of lipolysis; six did not receive acipimox. After signing informed consent, patients were asked to have dinner at 6 PM, followed by a fast until completion of the study. During the study, they could drink ad libitum water 0.5% enriched by deuterium water. The study design is shown in Fig. 1.

Ten hours after the end of last meal (*t* = −6 h), after a urine sample was obtained for determination of background 2H2O as well as drawing of blood for measurement of background (natural) isotope abundance, the patients/controls ingested 1 g 2H2O/kg body weight (Cambridge Isotope Laboratories, Andover, MA) at 30-min intervals.

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for a total of five times (total dose of 5 g/kg body water). Body water was estimated to be 60% of body weight in males and 50% in females.

Four hours later (t = −2 h), an intravenous cannula was introduced into each forearm, one for blood sampling, which was kept patent with a slow saline drip, 0.5% enriched with 2H2O, and the other for stable isotope enrichment. After blood was drawn for background isotope abundance and basal hematological and biochemical tests, a primed (17.6 µmol/kg), continuous (13.2 µmol·kg−1·h−1) infusion of [6,6-2H2]glucose (Cambridge Isotope Laboratories) dissolved in sterile isotonic saline, tested for pyrogenicity, and sterilized by passage of the solution through a Millipore filter (size 0.2 µm; Minisart, Sartorius, Germany) was administered by a motor-driven, calibrated syringe pump (Perfusor Secura FT, B. Braun, Melsungen, Germany).

At t = −0.30 h, after 90 min of [6,6-2H2]glucose infusion for equilibration, three blood samples were collected at intervals of 15 min (t = −0.30, −0.15, and 0 h) for determination of plasma glucose concentration and [6,6-2H2]glucose enrichment.

At the start of the study, (t = 0 h), blood samples were taken for measurement of basal plasma concentrations of alanine, lactate, glycero1, FFAs, insulin, and counterregulatory hormones (glucagon, cortisol, epinephrine, and norepinephrine). Then, the patients received either acipimox (AF group) administered as one capsule of 250 mg at t = 0 h, one at t = 2 h, and two capsules at t = 4 h or placebo (F group). The healthy controls served as their own controls and were studied twice in the same way with a 4-wk interval, once with group). The healthy controls served as their own controls and were studied twice in the same way with a 4-wk interval, once with

Blood samples for glucose concentration and [6,6-2H2]glucose enrichment were collected every 30 min from the beginning until the end of the study (t = 6 h). Blood samples for FFA concentrations were collected hourly from t = 1 h until the end of the study. Blood for 2H enrichment at C-5 in glucose for measurement of GNG was also drawn at t = 2, 4, and, at the end, t = 6 h. Urine for 2H enrichment in body water was collected at 0, 2, 4, and 6 h after emptying of bladder at t = −1, 0, 2, and 4 h. Plasma insulin, cortisol, glucagon, and catecholamines were measured at t = 4 and 6 h.

At the end of the study (t = 6 h), blood samples for measurement of the plasma concentrations of alanine, lactate, and cytokines were taken.

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

Assays. Glucose concentration, [6,6-2H2]glucose enrichment, deuterium enrichment on the C-5 position in glucose, and body water enrichment were determined as described previously (1). In summary, [6,6-2H2]glucose enrichment and glucose concentration (using xylose as internal standard) were measured as aldonitril pentacetae derivative in deproteinized plasma (28). Separation was achieved on a J&W Scientific, Palo Alto, CA) DB17 column (30 m × 0.25 mm, df 0.25 µm). Glucose was monitored at mass-to-charge ratios (m/z) 187, 188, and 189. The enrichment of [6,6-2H2]glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for background enrichments. To measure deuterium enrichment at the C-5 position, glucose was converted to hexamethylenetetramine (HMT), as described by Landau et al. (22). HMT was injected into a gas chromatograph-mass spectrometer. Separation was achieved on an AT-Amine (Alltech, Deerfield, IL) column (30 m × 0.25 mm, df 0.25 µm). Deuterium enrichment in body water was measured by a method adapted from Previs et al. (26). 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, Palo Alto, CA).

Plasma insulin concentration was determined by RIA (Insulin RIA 100. Pharmacia Diagnostic, Upsala, Sweden): intra-assay coefficient of variation (CV) 3–5%, interassay CV 6–9%, detection limit 15 pmol/l. Cortisol was measured by enzyme-immunoassay on an Immulite analyzer (DPC, Los Angeles, CA): intra-assay CV 2–4%, interassay CV 3–7%, detection limit 50 nmol/l. Glucagon was determined by RIA (Linco Research, St. Charles, MO): intra-assay CV 3–5%, interassay 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%, interassay CV 7–10%, detection limit 0.05 nmol/l; epinephrine intra-assay CV 6–8%, interassay CV 7–12%, detection limit 0.05 nmol/l. Serum FFAs were measured by an enzymatic method (NEFA-C; Wako chemicals, Neuss, Germany): intra-assay CV 2–4%, interassay CV 3–6%, detection limit 0.02 nmol/l. TNF-α was measured by ELISA (CLB, Amsterdam, The Netherlands) with a detection limit of 2 pg/ml. Plasma concentrations of IL-10 were measured by ELISA (Schering-Plough Research Institute, Kenilworth, NJ): detection limit 20 pg/ml.

Calculations and statistics. The glucose production rate was calculated from the dilution of labeled glucose in plasma. Because the plasma glucose concentrations and enrichments for [6,6-2H2]glucose remained constant during each sampling phase of the study, calculations for steady-state kinetics were applied, adapted for the use of stable isotopes (39).

The rate of GNG was calculated by multiplication of the total rate of glucose production by fractional GNG. The fractional GNG = 100 × (3H enrichment on C-5 of glucose/2H enrichment in urinary water%). The rationale for these calculations has been discussed in detail by Landau et al. (22). In brief, during GNG, but not during
Table 1. Clinical and biochemical characteristics of 12 patients and 6 controls at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P. falcip. Patients</th>
<th>P. falcip./ Acipimox Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26±4</td>
<td>23±2</td>
<td>26±4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>20.4±0.7</td>
<td>19.6±0.8</td>
<td>20.9±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>5/1</td>
<td>5/1</td>
<td>5/1</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>37.8±0.2</td>
<td>38.3±0.4</td>
<td>36.8±0.1</td>
<td>0.016*</td>
</tr>
<tr>
<td>Parasitemia, per µl</td>
<td>5.91±2.185</td>
<td>4.875±3.595</td>
<td>ND</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin, mmol/l</td>
<td>7.8±0.7</td>
<td>7.6±0.2</td>
<td>8.7±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum AST, U/l</td>
<td>63±16</td>
<td>61±20</td>
<td>24±2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ALT, U/l</td>
<td>60±16</td>
<td>87±50</td>
<td>22±3</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, µmol/l</td>
<td>115.7±6.6</td>
<td>106.7±5.7</td>
<td>111.5±2.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. P. falcip., Plasmodium falciparum malaria; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ND, not detectable; NS, not significant. *P = 0.016 and 0.018, P. falcip. (either group) patients vs. controls.

RESULTS

Clinical data. Twelve Vietnamese patients with uncomplicated P. falciparum malaria and six healthy controls were studied. The characteristics of the three groups are shown in Table 1. The different groups were well matched for age, sex, body mass index, kidney function, and plasma liver enzyme values. The two groups of patients (one with and one without acipimox) were completely comparable. The duration of illness of the patients with malaria-acipimox was 3 ± 1 days and in the patient group without acipimox 4 ± 1 days. Two patients, both in the without-acipimox group, had taken an anti-malarial agent (chloroquine and sulfadoxine-pyrimethamine, respectively) before admission. All (including these two) were treated orally with artesunate, to which they responded with complete recovery.

FFAs. There was no difference in FFAs among groups in the basal state. In the control subjects, without acipimox, FFAs increased during fasting (P = 0.008). In the malaria patients (control group), FFAs did not increase over time. This difference in FFA pattern between patients and controls was statistically significant (P = 0.0003). Acipimox suppressed FFAs significantly both in patients and controls (P < 0.001 and 0.0003 respectively; Fig. 2).

Glucose kinetics between malaria patients and healthy controls in the basal state. In the basal state, after 16 h of fasting, plasma glucose concentration, glucose production, and GNG, in percentage or in absolute value, were all significantly higher in malaria patients compared with healthy controls (P = 0.043, 0.003, and <0.0001, respectively), whereas GLY was significantly lower (P < 0.001) (Table 2).

Glucose metabolism. Although glucose production declined over time in the patients (P < 0.0001), there was no statistically significant difference in rate of decline between the acipimox-treated and untreated patients, nor was there a time-dependent influence observed from acipimox (Fig. 3). In the controls, acipimox inhibited this decline compared with the nontreated controls (P = 0.005).

No changes in the absolute rate of GNG were found in patients and in controls with or without acipimox. However, in the healthy controls 4 h after the acipimox treatment, GNG tended to be lower in acipimox-treated controls compared with untreated controls at that time point (P = 0.06, t-test).

In the patients, the absolute rate of GLY declined over time (P < 0.0001) without an effect by acipimox. In contrast, in the healthy controls, acipimox inhibited the decline in GLY found over time in the untreated subjects (P = 0.002).

Precursors, glucoregulatory hormones, and cytokines. After an overnight fast, the plasma concentrations of alanine were significantly lower (227 ± 19 vs. 383 ± 34 µmol/l, P = 0.001), and the concentrations of glucagon, cortisol, and IL-10 were significantly higher in the malaria patients compared with controls.
the healthy controls (92 ± 10 vs. 55 ± 3 ng/l, \( P = 0.001 \); 377 ± 46 vs. 185 ± 19 nmol/l, \( P = 0.001 \); and 288 ± 72 vs. 14 ± 3 pg/l, \( P = 0.007 \), respectively; Table 3); the other parameters were not different. These values were not different between both malaria groups (AF vs. F); neither was a difference found in the basal values in the control group on the two occasions.

Acipimox had no statistically significant influence on any of these parameters in either the patients groups or the control group.

### DISCUSSION

Uncomplicated \( P. falciparum \) malaria infection results in increased gluconeogenesis and decreased glycogenolysis with a mean glucose production 20% higher and a doubling of plasma cortisol and glucagon concentrations compared with healthy controls after 16 h of fasting. Although inhibition of lipolysis by acipimox stimulated glycogenolysis and (tended to) decrease gluconeogenesis in healthy humans, no such effect on gluconeogenesis and glycogenolysis was seen in the patients with \( P. falciparum \) malaria, suggesting that FFAs have no role in the regulation of intrahepatic glucose fluxes in uncomplicated malaria, contrary to the findings in type 2 diabetics, who have changes in gluconeogenesis and glycogenolysis comparable to those found in our malaria patients.

Plasma cortisol and glucagon were significantly higher in our patients and therefore were potential confounders. Although plasma cortisol was higher in our patients than in our control subjects, these levels were comparable to those in patients reported by Boden et al. \( [362 ± 54 \text{ nmol (Ref. 2)}] \), who showed a clear-cut regulatory role for FFAs in type 2 diabetics. This makes cortisol a less likely confounder. However, we cannot exclude the possibility that, with suppression of plasma cortisol, a regulatory role of FFAs would become visible. However, this does not invalidate our conclusion about the absence of a glucoregulatory role by FFAs in malaria, as
stimulation of cortisol secretion is an integral part of this disease. The same way of reasoning can be followed for the slightly elevated plasma glucagon levels in our malaria patients.

**Glucose production.** Our data in healthy subjects without acipimox were completely in agreement with literature showing a slight fall in glucose production over time with a decline only in glycogenolysis, whereas gluconeogenesis remained constant (4, 5, 8, 19, 31). Also in accord with others is the fact that those studies (2, 5) did not report in malaria (12, 25), but IL-10 has no known effect on glucose production. Elevated IL-10 levels has been less likely the cause as discussed previously (36, 37). Lower plasma glucagon levels comparable to those in healthy controls (36). An increase in plasma cortisol levels was also lower the cause as discussed previously (36, 37). Lower plasma alanine concentrations can certainly not explain an increase in glucose production. Elevated IL-10 levels has been reported in malaria (12, 25), but IL-10 has no known effect on glucose metabolism.

The present study rules out an important role for FFAs. These findings point to intrahepatic factors as an explanation. In fact, in past years, evidence has accumulated on the importance of an extensive paracrine network within the liver that exerts a potent glucoregulatory role. The prominent paracrine mediators are adenosine, prostaglandins, thromboxane A2, and cytokines (9). These substances have many interactions with the classical hormones and with each other in exerting their effects on glucose metabolism. This complex paracrine signaling system seems to operate between Kupffer cells, hepatic endothelial cells, and hepatocytes (9). Kupffer cells are the major producer of prostaglandins and cytokines (9, 14). Malaria induces changes in Kupffer cells by loading them with malarial pigment and inducing hyperplasia (40). This raises the possibility that these pathological changes in malaria could influence the intrahepatic pathways of glucose metabolism. At least in nonsevere malaria it has been shown that prostaglandins could be involved in this regulation (13).

**Table 3. Changes in plasma glucose regulatory hormones, alanine, lactate, TNF-α and IL-10 at 22 h of fasting compared with values at basal state (16 h of fasting)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of fasting, h</th>
<th>Control</th>
<th>Acipimox-Control</th>
<th>Malaria</th>
<th>Acipimox-Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>22</td>
<td>16</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>38 ± 3</td>
<td>27 ± 3</td>
<td>44 ± 9</td>
<td>18 ± 4</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>Glucagon*, ng/l</td>
<td>54 ± 5</td>
<td>66 ± 8</td>
<td>56 ± 3</td>
<td>80 ± 13</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>Cortisol*, nmol/l</td>
<td>190 ± 23</td>
<td>157 ± 23</td>
<td>180 ± 33</td>
<td>398 ± 85</td>
<td>343 ± 62</td>
</tr>
<tr>
<td>Epinephrine, nmol/l</td>
<td>0.23 ± 0.06</td>
<td>0.43 ± 0.11</td>
<td>0.38 ± 0.08</td>
<td>0.83 ± 0.15</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Norepinephrine, nmol/l</td>
<td>0.97 ± 0.17</td>
<td>0.95 ± 0.21</td>
<td>1.55 ± 0.48</td>
<td>1.42 ± 0.30</td>
<td>0.91 ± 0.26</td>
</tr>
<tr>
<td>Alanine*, μmol/l</td>
<td>381 ± 54</td>
<td>281 ± 36</td>
<td>384 ± 48</td>
<td>353 ± 27</td>
<td>213 ± 35</td>
</tr>
<tr>
<td>Lactate*, mmol/l</td>
<td>1.10 ± 0.13</td>
<td>1.01 ± 0.11</td>
<td>1.16 ± 0.11</td>
<td>1.82 ± 0.25</td>
<td>1.13 ± 0.16</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>&lt;2</td>
<td>&lt;2 ± 0.6</td>
<td>2.3 ± 0.9</td>
<td>3.2 ± 1.3</td>
<td>6.7 ± 5.7</td>
</tr>
<tr>
<td>IL-10*, pg/ml</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>22 ± 12</td>
<td>295 ± 136</td>
</tr>
</tbody>
</table>

None of these parameters revealed statistical difference by ANOVA adjusted for multiple testing. *Statistical significant difference between malaria patients and healthy subjects at basal state.

rate of glycogenolysis. These data also suggest that in malaria there is an unknown mechanism dominating glycogen kinetics, escaping the suppressive effect of FFAs. There is a link between glycogen content and the rate glycogen breakdown (23), and it could be argued that the absence of an effect of acipimox in our malaria patients originates from a partially depleted glycogen store. However, in the malaria patients with acipimox, glycogenolysis was still 1.6 μmol·kg⁻¹·min⁻¹, equal to 9% of total glucose production at the end of their study, making less likely the impossibility for acute changes in the rate of glycogenolysis.

Malaria results in increased gluconeogenesis and decreased glycogenolysis, with the mean glucose production ~20% higher than in controls after 16 h of fasting (12). The pathophysiological mechanism behind this is still not elucidated. Glucagon can stimulate glucose production and is increased in cerebral malaria a doubling of glucose production is found despite plasma glucagon levels comparable to those in healthy controls (36). An increase in plasma cortisol levels was also less likely the cause as discussed previously (36, 37). Lower plasma alanine concentrations can certainly not explain an increase in glucose production. Elevated IL-10 levels has been reported in malaria (12, 25), but IL-10 has no known effect on glucose metabolism.
During short-term starvation, the rate of lipolysis increases over time with an increase in plasma FFA concentration (5, 21, 30). The plasma FFA levels in the healthy Vietnamese subjects showed this expected pattern. A remarkable finding was the absence of an increase in the FFA levels over time in the malaria patients. It seems to be a characteristic finding in malaria, as similar observations are made in adults with cerebral malaria (38), pregnant patients with malaria (37), and children with malaria (20). The mechanism responsible for this finding is unknown. Lipolysis is stimulated by catecholamines and inhibited by insulin. Insulin and catecholamine levels were not different between the patients and controls and are therefore no explanation for this observation. However, in vitro data point to a unique metabolic effect of malaria toxin. These data show that, in rat adipocytes, malaria toxin acts synergistically with insulin to inhibit lipolysis (35).

In conclusion, in P. falciparum malaria, FFAs are not involved in the regulation of glucose production, gluconeogenesis, or glycogenolysis.

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