

Adiponectin and glucose production in patients infected with *Plasmodium falciparum*

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Abstract

Infections are often complicated by an increase in glucose production due to stimulation of the secretion of glucose counter-regulatory hormones and cytokines. Adiponectin, a fat-derived hormone with insulin-sensitizing properties, could play a regulatory role in the degree of stimulation of glucose production by the infectious agent. Therefore, we investigated the possible correlation between glucose production and plasma adiponectin levels in 25 subjects: 7 patients with cerebral malaria, 6 with uncomplicated malaria, and 12 matched controls.

Glucose production was significantly higher in patients with malaria compared to healthy controls ($P < .001$). Adiponectin levels were not different between the patients with malaria and the control group. However, patients with cerebral malaria had significantly higher values for adiponectin than the patients with uncomplicated malaria ($P < .005$).

Glucose production and gluconeogenesis were positively correlated to plasma adiponectin in the patients ($r = 0.835$, $P < .001$ and $r = 0.846$, $P < .001$, respectively), whereas these correlations were absent in the controls ($r = -0.329$, NS and $r = -0.028$, NS, respectively).

In conclusion, adiponectin levels were not different between patients with malaria and their matched controls. However, patients infected with *Plasmodium falciparum* who have higher glucose production also have higher adiponectin levels. In healthy subjects such a correlation was not found.

As adiponectin is known to inhibit glucose production, stimulation of adiponectin secretion during infection could be intended to restrain the glucose production stimulating properties of hormones and cytokines secreted during infection.

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1. Introduction

Adipose tissue is an active endocrine organ, producing and secreting several hormone-like peptides [1,2]. One of these so-called adipocytokines is adiponectin, a hormone with insulin-like properties. In mice it decreases glucose plasma levels by stimulating glucose uptake in muscles and by increasing the ability of insulin to suppress hepatic glucose production. Adiponectin also increases insulin

sensitivity by stimulation of skeletal muscle free fatty acid (FFA) uptake and oxidation [3–5]. These properties are in harmony with the results of euglycemic-hyperinsulinemic clamp studies in human beings, in which adiponectin plasma levels were positively correlated with insulin sensitivity [6] and negatively correlated with endogenous glucose production [7]. Adiponectin plasma concentrations were found to be decreased in insulin-resistant patients with obesity, type 2 diabetes, and HIV lipodystrophy [8–10].

Infections are often complicated by disturbances in glucose and lipid metabolism. Catabolic hormone production is enhanced while insulin sensitivity decreases. The consequences are increases in glucose production, gluconeogenesis, and lipolysis. The mechanisms, via which

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these changes in metabolism during infection are induced, are uncertain. The major modulators of these changes are considered to be the pro-inflammatory cytokines, like interleukin-1, interleukin-6, tumor necrosis factor α (TNF- α), and the counter-regulatory hormones, like catecholamines, cortisol, and glucagon [11–17].

A role for adiponectin in disturbed glucose metabolism due to infection has not yet been described. Two theories can be put forward. (1) As an infection induces insulin resistance and low plasma adiponectin levels are found in insulin-resistant states, it can be hypothesized that in infected patients, plasma adiponectin levels are lower than those in healthy controls. (2) Adiponectin increases the ability of insulin to suppress glucose production. It can therefore also be hypothesized that in patients with infection, a negative correlation between plasma adiponectin and glucose production will be found.

These 2 hypotheses were tested in a population of patients with the same kind of infection, that is, malaria, but different degrees of disease severity. Malaria is frequently complicated by disturbances in glucose metabolism. Although hypoglycemia has been described often, most studies on glucose kinetics in patients with uncomplicated and cerebral malaria have found high glucose plasma levels together with an increased rate of glucose production and gluconeogenesis [18–22]. In addition, patients with uncomplicated and severe malaria were reported to have reduced tissue insulin sensitivity [23,24]. We measured plasma adiponectin, glucose production, and the fractional contribution of gluconeogenesis in patients with uncomplicated *Plasmodium falciparum* malaria, in patients with cerebral malaria as well as in matched controls.

2. Material and methods

2.1. Subjects

Thirteen Vietnamese patients (7 with cerebral malaria and 6 with uncomplicated malaria) and 12 healthy subjects matched for age, sex, and body mass index were included in the study. Exclusion criteria were treatment with quinine and concomitant infectious diseases. The study was approved by the local health authorities and by the Medical Ethical Committee, Academic Medical Center, Amsterdam, The Netherlands. The data on glucose production and gluconeogenesis in the patients with malaria have been published before [21,25].

2.2. Study design

To measure glucose production in our subjects, we used [6,6- $^2\text{H}_2$]glucose. The fractional contribution of gluconeogenesis was measured using $^2\text{H}_2\text{O}$, which is presently considered to be the gold standard [26–28].

Patients were recruited on the day of admission and were treated with artesunate, immediately after laboratory confirmation of the diagnosis. After receiving informed

consent, the patient was given a standard meal followed by a fast until completion of the study. During the study, 2 intravenous catheters were introduced into both forearm veins: one for blood sampling, the other for stable isotope infusion. The catheters were kept patent by a slow isotonic saline drip, 0.5% enriched with $^2\text{H}_2\text{O}$. Twelve hours after the last meal, after having a urine sample for determination of background $^2\text{H}_2\text{O}$ enrichment in urine, as well as drawing blood for measurement of background enrichment, patients were given 1 g of $^2\text{H}_2\text{O}$ per kg body water at 30-minute intervals for a total of 5 times. Body water was estimated to be 60% of body weight in males and 50% in females. Four hours after the $^2\text{H}_2\text{O}$ gift, after drawing blood for background isotope abundances, a primed (3.2 mg/kg), continuous (2.4 mg/kg per hour) infusion of [6,6- $^2\text{H}_2$]glucose, dissolved in sterile isotonic saline, was administered by a motor-driven, calibrated syringe pump (Perfusorâ Secura FT, B. Braun, Melsungen, Germany) through a millipore filter (0.2 mm; Minisart, Sartorius, Gottingen, Germany). Six hours after the $^2\text{H}_2\text{O}$ gift, 3 blood samples for ^2H enrichment were taken at intervals of 15 min. Urine for ^2H enrichment was collected between the sixth and seventh hour after the $^2\text{H}_2\text{O}$ gift, after emptying of the bladder 1 hour before. At the end of the study, 3 blood samples were collected at intervals of 15 min for determination of plasma glucose and [6,6- $^2\text{H}_2$]glucose enrichment. Plasma levels of insulin, cortisol, glucagon, catecholamines, TNF- α , FFAs, and adiponectin were also collected at this time point. During the study, patients could drink water ad libitum, 0.5% enriched with $^2\text{H}_2\text{O}$.

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

2.3. Blood variables

Plasma samples for glucose enrichments of [6,6- $^2\text{H}_2$]glucose were deproteinized with methanol [29]. 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, Folsom, CA) DB17 column (30 m \times 0.25 mm, d_f 0.25 μm). 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 hexamethylene tetra-amine as described by Ackermans et al [30] and Landau et al [31]. Hexamethylene tetra-amine was injected into a gas chromatograph mass spectrometer. Separation was achieved on an AT-Amine (Alltech) column (30 m \times 0.25 mm, d_f 0.25 μm). The ions

Table 1

Characteristics of the study subjects

Characteristics	Patients with malaria		Controls
	UM	CM	
Number	6	7	12
Age (y)	26 ± 4	32 ± 5	31 ± 3
Sex (M/F)	5:1	6:1	10:2
Body mass index (kg/m ²)	20.4 ± 0.7	19.3 ± 0.7	21.2 ± 0.5

Values are given as mean ± SEM.

UM indicates uncomplicated malaria; CM, cerebral malaria.

No significant differences were found between UM vs CM, nor between all patients with malaria (UM + CM) vs controls.

monitored for the hexamethylene tetra-amine were m/z 140 and 141. Deuterium enrichment in body water was measured by a method adapted from Previs et al [32]. All isotopic enrichments were measured on a 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 radioimmunoassay (RIA) (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden): intra-assay coefficient of variation (CV) 3% to 5%, inter-assay CV 6% to 9%, detection limit 15 pmol/L. C-peptide was measured by RIA (RIA-coat C-peptide, Byk-Sangtec Diagnostica, Dietzenbach, Germany): intra-assay CV 4% to 6%, inter-assay CV 6% to 8%, detection limit 50 pmol/L. Cortisol was measured by enzyme immunoassay on an Immulite analyzer (DPC, Los Angeles, CA): intra-assay CV 2% to 4%, inter-assay CV 3% to 7%, detection limit 50 nmol/L. Glucagon was determined by RIA (Linco Research, St Charles, MO): intra-assay CV 3% to 5%, inter-assay CV 9% to 13%, detection limit 15 ng/L. Catecholamines were measured by an in-house high-performance liquid chromatography method, norepinephrine: inter-assay CV 6% to 8%, intra-assay CV 7% to 10%, detection limit 0.05 nmol/L; and epinephrine: inter-assay CV 6% to 8%, intra-assay CV 7% to 12%, detection limit 0.05 nmol/L. Plasma FFAs were measured by an enzymatic method (NEFAC, Wako Chemicals, Neuss, Germany): intra-assay CV 2% to 4%, inter-assay CV 3% to 6%, detection limit 0.02 mmol/L. Tumor necrosis factor α was measured by enzyme-linked immunosorbent assay (CLB) with a detection limit of 2 pg/mL. Plasma adiponectin concentrations were measured in duplicate by a RIA kit (Linco Research): intra-assay CV 4% to 6%, inter-assay CV 6% to 9%, detection limit 0.5 μ g/mL.

2.4. Calculations and statistics

Glucose production was calculated from the dilution of labeled glucose in plasma. Because the plasma glucose concentration and enrichment percentage of (6,6-²H₂) glucose varied (albeit little), we applied the Steele equation for non-steady-state conditions [33] with the fraction of total extracellular glucose pool 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% × ([²H₂]

enrichment on C5 of glucose) / ([²H₂] enrichment in urinary water). The rationale has been discussed in detail by Landau et al [31]. In brief, carbon 5 of the glucose formed by gluconeogenesis takes its hydrogen from water for both sources, glycerol and phosphoenolpyruvate. The isomerization of hydrogen from water that is transferred to carbon is extensive. There is no exchange with water of 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.

Data are presented as mean ± SEM. Comparisons between the groups were made by the Fisher exact test (sex) or the Mann-Whitney *U* test (other parameters). Correlations between adiponectin and other parameters were analyzed by Spearman correlation coefficient. All tests were 2-tailed and a *P* value of less than .05 was considered to be statistically significant. The SPSS statistical software program version 11.5 (SPSS Inc, Chicago, IL) was used for statistical analysis.

3. Results

3.1. Baseline characteristics

The main characteristics of the control and the malaria groups are shown in Table 1.

3.2. Glucose metabolism

Glucose production was much higher in patients than in controls (24.5 ± 1.5 vs 16.5 ± 0.5 μ mol/kg per minute; *P* < .001). Glucose production was almost entirely dependent on gluconeogenesis in patients (93.2%) but not in controls (54.6%). Consequently, glycogenolysis contributed only a little to glucose production in the patients.

Glucose production was significantly higher in patients with cerebral malaria than in patients with uncomplicated malaria (28.2 ± 1.9 vs 20.2 ± 0.6 μ mol/kg per minute; *P* = .004).

Table 2

Hormones, TNF- α , and FFAs

	Patients with malaria		Controls
	UM	CM	
Hormones			
Insulin (pmol/L)	35 ± 9	49 ± 11	36 ± 3
Glucagon (ng/L)	82 ± 9	132 ± 33	57 ± 3 [‡]
Cortisol (nmol/L)	343 ± 62*	1080 ± 163	189 ± 13 [‡]
Epinephrine (nmol/L)	0.22 ± 0.04	0.27 ± 0.13	0.19 ± 0.04
Norepinephrine (nmol/L)	0.91 ± 0.3	4.77 ± 3.5	0.99 ± 0.1
Others			
TNF- α (pg/mL)	6.7 ± 5.7	6.6 ± 2.0	1.4 ± 0.3 [†]
FFA (mmol/L)	0.99 ± 0.15	0.80 ± 0.06	0.79 ± 0.07

Values are given as mean ± SEM.

UM indicates uncomplicated malaria; CM, cerebral malaria.

* *P* < .05 vs CM.† *P* < .05 vs all malaria patients (UM + CM).‡ *P* < .005 vs all malaria patients (UM + CM).

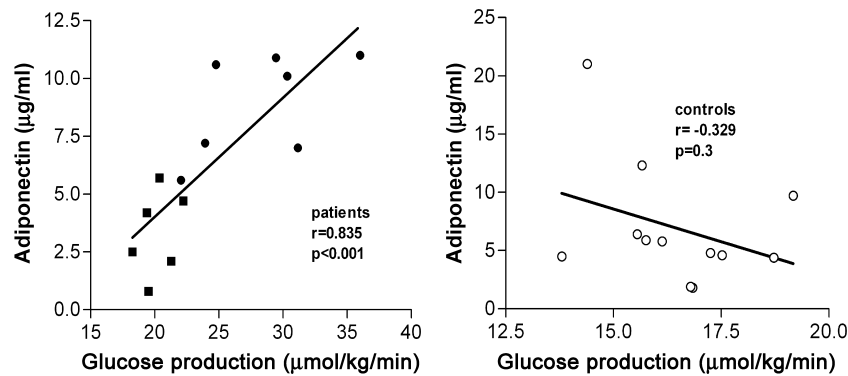


Fig. 1. Correlation between the glucose production and the plasma adiponectin levels in patients and in controls. Correlation with Spearman correlation coefficient. Filled circles indicate patient with cerebral malaria; filled squares, patient with uncomplicated malaria; open circles, control subject.

3.3. Adiponectin

Plasma adiponectin levels were not different between the malaria and the control group (6.3 ± 1.0 vs 6.9 ± 1.5 μg/mL; NS), neither between the different subgroups of the patients with malaria and the healthy controls. In patients with cerebral malaria, plasma adiponectin levels were significantly higher than in patients with uncomplicated malaria (8.9 ± 0.9 vs 3.3 ± 0.8 μg/mL; $P = .004$).

3.4. Glucoregulatory hormones, TNF-α, and FFAs

There were no significant differences in plasma levels of insulin, epinephrine, norepinephrine, and FFAs between the malaria and the control group. Plasma levels of cortisol, glucagon, and TNF-α were significantly higher in the patients compared to the controls (Table 2).

Plasma cortisol levels were higher in the patients with cerebral malaria compared to the patients with uncomplicated malaria.

3.5. Correlates of plasma adiponectin

There was a significant positive correlation between glucose production and plasma adiponectin levels in the patients, whereas this correlation was absent in the control group. Gluconeogenesis was also significantly correlated to adiponectin levels in the patient group (Figs. 1 and 2).

Cortisol was the only hormone that significantly correlated to adiponectin levels in the patients ($r = 0.78$; $P = .002$). No correlations were found between adiponectin and insulin, glucagon, epinephrine, norepinephrine, FFA, and TNF-α levels.

4. Discussion

Our data show that, although patients infected with *P. falciparum* have plasma adiponectin concentrations not different from matched controls, those with a more severe form of malaria had significantly higher adiponectin plasma levels compared to those with uncomplicated malaria. In addition, patients infected with *P. falciparum* who have higher rates of glucose production also have higher plasma adiponectin levels. In healthy subjects such a correlation was not found.

Our data in patients infected with *P. falciparum* are novel. Neither are comparable data in other infectious diseases available.

Adiponectin plasma levels in our control group were a little lower compared with most of the previously reported levels in healthy persons, with values between 1.9 and 17 and a mean of 8.9 μg/mL [8,9]. This could be due to racial differences, as lower adiponectin plasma levels have been described in subjects from Asian background [34]. Another

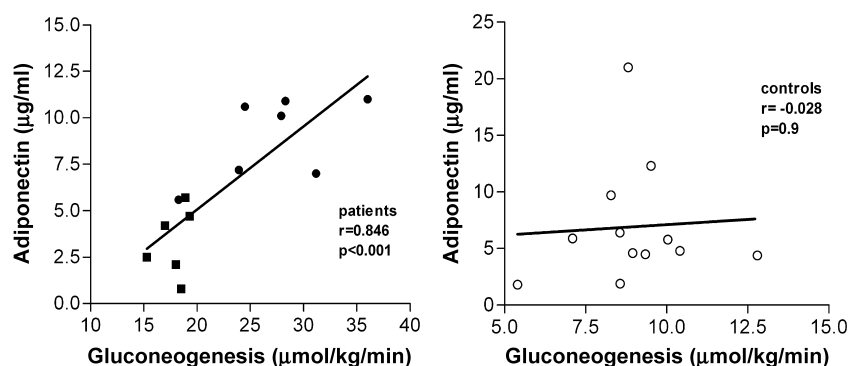


Fig. 2. Relationship between the gluconeogenesis and the plasma adiponectin levels in patients and in controls. Correlation with Spearman correlation coefficient. Filled circles indicate patient with cerebral malaria; filled squares, patient with uncomplicated malaria; open circles, control subject.

explanation is the duration of time of fasting. In most studies, samples are obtained after an overnight fast, whereas our subjects were fasting for ± 18 hours. Fasting for a short time decreases adiponectin production in adipose tissue [35,36].

Although adiponectin levels were not statistically different between the malaria and the control group, we did find significantly higher levels in the patients with cerebral malaria vs the patients with uncomplicated malaria. This could point to an increase in adiponectin levels in severe infections. This assumption is supported by data in literature obtained in a model of sepsis. Relative high adiponectin levels were reported in healthy subjects who were injected with endotoxin compared to saline-injected subjects [36].

Besides the degree of severity, the location of the infection could play a role in the enhanced adiponectin levels in the patients with cerebral malaria as well. Compared with uncomplicated malaria, cerebral malaria is characterized by higher levels of cytokines in the brain, subsequent up-regulation of adhesion molecules on the vascular endothelium, sequestration of blood cells in the cerebral microvasculature, and consequently lower cerebral blood flow [37]. Because adipose tissue is controlled by the autonomic nervous system [38], cerebral inflammation or ischemia could lead to autonomic dysregulation and consequently changes in metabolism of adipocytes.

In our small control group, there tended to be a negative correlation between adiponectin plasma levels and glucose production. This correlation was found before in a large group of healthy controls [7] and is in harmony with the suppressing effect of adiponectin on hepatic glucose production in mice [3]. The explanation for the positive correlation between plasma adiponectin levels and glucose production in the malaria group cannot be deduced from our study. Adiponectin is an adipocyte-specific, secreted hormone with an important positive role in metabolism. This adipocytokine decreases glucose plasma levels and glucose production by sensitizing the liver and muscle to the actions of insulin [3–5]. Data about the regulation of its secretion are less thoroughly studied. It has been shown that in insulin-resistant states, low plasma adiponectin levels are found, suggesting that chronic hyperinsulinemia inhibits the secretion of this adipocyte-specific, secreted hormone [8–10]. However, experiments indicate that short-term elevation of insulin stimulates adiponectin secretion [39–42], indicating a differential regulation of adiponectin secretion by insulin, depending on the duration of the increase in secretion of insulin. As in our patients no correlation was found between plasma adiponectin and insulin levels; insulin does not seem to be the regulator of adiponectin in malaria. The same seems to be true for other known regulators of adiponectin secretion, that is, catecholamines, glucagon, FFAs, and TNF- α .

Another regulator of adiponectin secretion is cortisol. Adiponectin was positively correlated to plasma cortisol concentrations in our patients. As cortisol has been reported to decrease adiponectin, both its plasma concentration and

its production in adipocytes [40,43,44], cortisol will probably not increase the secretion of adiponectin during infection, but this remains to be elucidated.

Although the regulators of adiponectin secretion during infection are unclear, high rates of glucose production and gluconeogenesis coincided with higher adiponectin levels. As adiponectin has been reported to decrease hepatic glucose production [3], this correlation and the high levels of adiponectin in patients with cerebral malaria, who had higher rates of glucose production, could represent an adaptive mechanism. Except for insulin and adiponectin, all the other hormones and cytokines, known to be involved in the regulation of metabolism during infection, stimulate glucose production. Insulin resistance is a well-known feature of infection, making its role in the regulation of glucose production during infection less prominent. Unopposed stimulation of glucose production will result in major dysregulation of glucose metabolism. Concomitant stimulation of a hormone, counteracting this effect, will restrain this process. Our data suggest such a role for adiponectin during infection. Such a compensatory role for adiponectin in the regulation of disturbed metabolism has been suggested before in patients with anorexia nervosa and acromegaly [45,46].

Recently, several studies demonstrated that adiponectin exists in 3 forms in serum, as a high-molecular-weight (HMW) complex, a hexamer, and a trimer [47–49]. These different forms of adiponectin activate different signal transduction pathways. The HMW and the hexamer forms have been described to activate nuclear factor κ B in C2C12 cells [48] and adenosine monophosphate-activated protein kinase (AMPK) in hepatocytes [49], whereas the trimers activated AMPK in myocytes [48,49]. In our study, only total plasma adiponectin levels were measured. However, as the HMW form has been suggested to be more active in hepatocytes [47,49], we hypothesize that the secretion of this form is stimulated to decrease glucose production in our patients with malaria.

In conclusion, plasma adiponectin levels were not statistically different between patients with malaria and their matched controls. However, patients infected with *P. falciparum* who have higher glucose production also have higher plasma adiponectin levels.

As adiponectin is known to inhibit glucose production, stimulation of adiponectin secretion during infection could be intended to restrain the glucose production stimulating properties of hormones and cytokines, secreted during infection.

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