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

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Abstract

Objective: 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.

Methods: To investigate the possible correlation between glucose production and adiponectin during infections, we measured glucose production as well as plasma adiponectin levels in 7 patients with cerebral malaria, in 6 patients with uncomplicated malaria and in 12 matched controls.

Results: Glucose production was significantly higher in malaria patients compared to healthy controls (p<0.001). 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<0.005). Glucose production and gluconeogenesis were positively correlated to plasma adiponectin in the patients (r=0.835, p<0.001 and r=0.846, p<0.001, respectively), while these correlations were absent in the controls (r= -0.329, NS and r= -0.028, NS, respectively).

Conclusions: Adiponectin levels were not different between malaria patients and their matched controls. However, patients infected with Plasmodium falciparum who have higher glucose production rates, 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.
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 humans, in which adiponectin plasma levels were positively correlated with insulin sensitivity\(^6\) and negatively 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 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, TNF-\(\alpha\) 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 an 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, i.e. malaria, but different degrees of disease severity. Malaria is frequently complicated by disturbances in glucose metabolism. Although hypoglycaemia 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 uncomplicated and severe malaria patients 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.

**Material and methods**

**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 (BMI) 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 malaria patients have been published before 21, 25.

**Study design**

To measure glucose production in our subjects, we used [6,6-2H2]glucose. The fractional contribution of gluconeogenesis was measured using 2H2O, which is presently considered to be the golden 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 two 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 deuterated water. Twelve hours after the last meal, after having a urine sample for determination of background 2H2O enrichment in urine, as well as drawing blood for measurement of background enrichment, patients were given 1g of 2H2O per kg body weight at 30-minute intervals for a total of 5 times. Body water was estimated to be 60 % of body weight in the males and 50 % in the females. Four hours after the 2H2O gift, after drawing blood for background isotope abundances, a primed (3.2 mg/kg), continuous (2.4 mg/kg/h) infusion of [6,6-2H2]glucose, 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). Six hours after the 2H2O-gift, 3 blood samples for 2H enrichment were taken at intervals of 15 min. Urine for 2H enrichment was collected between the 6th and 7th hour after the 2H2O 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 [6,6-2H2]glucose enrichment. Plasma levels of insulin, cortisol, glucagon, catecholamines, TNF-α, FFA’s and adiponectin were also collected at this timepoint. During the study patients could drink ad libitum water, enriched with 0.5% deuterated water.

Blood for measurement of gluconeogenesis was promptly deproteinized by adding an equal amount of 10 % perchloric acid. Blood for [6,6-2H2]glucose enrichment, as well as hormones were 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.

Blood variables

Plasma samples for glucose enrichments of [6,6-2H2]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 (30m x 0.25 mm, d 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 (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 0.25 μm). 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 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 measured 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 analyzer (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. Catecholamines were measured by an in-house HPLC method; norepinephrine: inter-assay CV 6-8%, intra-assay CV 7-10%, detection limit 0.05 nmol/l, and epinephrine:
inter-assay CV 6-8%, intra-assay CV 7-12%, detection limit 0.05 nmol/l. Plasma FFA’s were measured by an enzymatic method (NEFAC, Wako Chemicals), intra-assay CV 2-4%, inter-assay CV 3-6%, detection limit 0.02 mmol/l. TNF-α was measured by ELISA (CLB) with a detection limit of 2 pg/ml. Plasma adiponectin concentrations were measured in duplicate by RIA (LINCO Research, St. Charles, Missouri): intra-assay CV 4-6 %; inter-assay CV 6-9 %; detection limit 0.5 μg/ml.

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% x ([2H2] enrichment on C5 of glucose)/ ([2H2] enrichment in urinary water). The rationale has been discussed in detail by Landau 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’s exact test (sex) or the Mann-Whitney test (other parameters). Correlations between adiponectin and other parameters were analyzed by Spearman’s correlation coefficient. All tests were 2-tailed and a p-value of <0.05 was considered to be statistically significant. The SPSS statistical software program version 11.5 was used for statistical analysis.
Results

Baseline characteristics (table 1)
The main characteristics of the control and the malaria groups are shown in Table 1.

Table 1 Characteristics of the study subjects

<table>
<thead>
<tr>
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<th>Malaria patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>UM</td>
<td>CM</td>
</tr>
<tr>
<td>Number</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26 ± 4</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>5:1</td>
<td>6:1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.4 ± 0.7</td>
<td>19.3 ± 0.7</td>
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</tbody>
</table>

Values are given as mean ± SEM. UM= uncomplicated malaria, CM= cerebral malaria.
No significant differences were found between UM vs. CM, nor between all malaria patients (UM+CM) vs. controls.

Glucose metabolism
Glucose production was much higher in patients than in controls (24.5 ± 1.5 vs. 16.5 ± 0.5 μmol/kg/min; p<0.001). 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 cerebral malaria patients than in patients with uncomplicated malaria (28.2 ± 1.9 vs. 20.2 ± 0.6 μmol/kg/min; p=0.004).

Table 2 Hormones, TNF-α and FFA’s

<table>
<thead>
<tr>
<th></th>
<th>Malaria patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>UM</td>
<td>CM</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>35 ± 9</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>82 ± 9</td>
<td>132 ± 33</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>343 ± 62*</td>
<td>1080 ± 163</td>
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<tr>
<td>Epinephrine (nmol/l)</td>
<td>0.22 ± 0.04</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td>0.91 ± 0.3</td>
<td>4.77 ± 3.5</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>6.7 ± 5.7</td>
<td>6.6 ± 2.0</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.99 ± 0.15</td>
<td>0.80 ± 0.06</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. UM= uncomplicated malaria, CM= cerebral malaria.
* P<0.05 vs. CM
† P<0.05 vs. all malaria patients (UM+CM)
‡ P<0.005 vs. all malaria patients (UM+CM)
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 malaria patients 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= 0.004).

Glucoregulatory hormones, TNF-α and FFA’s (table 2)
There were no significant differences in plasma levels of insulin, epinephrine, norepinephrine and FFA’s 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.

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

Correlates of plasma adiponectin (fig. 1 and 2)
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.

Cortisol was the only hormone that significantly correlated to adiponectin levels in the patients (r=0.78; p=0.002). No correlations were found between adiponectin and insulin, glucagon, epinephrine, norepinephrine, FFA, and TNF-α levels.

Figure 1
Correlation between the glucose production and the plasma adiponectin levels in patients and in controls. Correlation with Spearman’s correlation coefficient.
- ● = cerebral malaria patient, ■ = uncomplicated malaria patient and ○ = control subject
Discussion

Our data show that, although patients infected with Plasmodium 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 Plasmodium 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 Plasmodium falciparum are novel. Neither are comparable data in other infectious diseases available.

Adiponectin plasma levels in our control group were a little lower compared to most of the previously reported levels in healthy persons, with values between 1.9 and 17 and a mean of 8.9 μg/ml. This could be due to racial differences, as lower adiponectin plasma levels have been described in subjects from Asian background. Another explanation is the duration of time of fasting. In most studies, samples are obtained after an overnight fast, while our subjects were fasting for ±18 hours. Fasting for a short time decreases adiponectin production in adipose tissue.

Although adiponectin levels were not statistically different between the malaria and the control group, we did find significantly higher levels in the cerebral malaria vs. the uncomplicated malaria patients. This could point to an increase in adiponectin levels in severe infections. This assumption is supported by data in literature obtained in a model.

Figure 2 Relationship between the gluconeogenesis and the plasma adiponectin levels in patients and in controls. Correlation with Spearman’s correlation coefficient.

○ = cerebral malaria patient, ■ = uncomplicated malaria patient and ○ = control subject
of sepsis. Relative high adiponectin levels were reported in healthy subjects who were injected with endotoxin compared to saline injected subjects 35.

Besides the degree of severity, the location of the infection could play a role in the enhanced adiponectin levels in the cerebral malaria patients as well. Compared to 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. Since 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 adiponectin’s suppressing effect on hepatic glucose production in mice 3. The explanation for the positive correlation between plasma adiponectin levels and glucose production in the malaria group can not be deduced from our study. Adiponectin is an adipocyt-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 adipocyt-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 i.e. catecholamines, glucagon, FFA’s 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 cerebral malaria patients, 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 NF-κβ in C2C12 cells 48 and 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 malaria patients.

In conclusion: plasma adiponectin levels were not statistically different between malaria patients and their matched controls. However patients infected with Plasmodium 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.

Acknowledgments
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