A rosiglitazone-induced increase in adiponectin does not improve glucose metabolism in HIV-infected patients with overt lipoatrophy

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COMBINATION ANTIRETROVIRAL TREATMENT (cART) has remarkably improved the prognosis of patients with HIV-1-infection (25) but is associated with metabolic disturbances and changes in body fat distribution or lipodystrophy (5). The metabolic disturbances include dyslipidemia and alterations in glucose metabolism, ranging from insulin resistance at the level of peripheral glucose disposal, hepatic glucose production, and lipolysis to overt diabetes mellitus type 2 (15, 35). The pathogenesis of these perturbations is likely multifactorial. Dysfunction of adipose tissue has been implicated to contribute to the disturbances in glucose metabolism (18, 33).

In addition to being a fat storage depot, adipose tissue has been shown to be an endocrine organ that synthesizes and secretes several biologically active molecules that influence glucose metabolism. Among these adipocytokines is adiponectin, a relatively abundant plasma protein that is produced and secreted predominantly by adipocytes (2). In healthy rodents and in animals with lipodystrophy or obesity, administration of adiponectin ameliorates glucose metabolism by enhancing peripheral glucose uptake and suppressing hepatic glucose output (10, 38, 40). Probably, these effects occur via AMP-activated protein kinase-dependent stimulation of fat oxidation (38, 39). In plasma, adiponectin circulates as several different entities, including a high-molecular-weight (HMW), a hexameric (medium-molecular-weight), and a trimeric (low-molecular-weight) form. The HMW oligomer has been implicated as the major active form responsible for the insulin-sensitizing effects of adiponectin (37). Plasma levels of adiponectin, primarily of the HMW isoform, are reduced in insulin-resistant subjects with type 2 diabetes and HIV-associated lipodystrophy (3, 23). In addition, adiponectin mRNA expression in subcutaneous adipose tissue of HIV-lipodystrophic patients is lower compared with cART-treated HIV-infected patients without lipodystrophy (32). Considering the insulin-mimetic properties of adiponectin, the reduction of (HMW) adiponectin could play a role in the pathogenesis and perseveration of insulin resistance in HIV-associated lipodystrophy. Indeed, in HIV-infected, lipodystrophic patients, plasma adiponectin levels have been negatively associated with markers of insulin resistance (23, 33). Therefore, it can be hypothesized that upregulation of plasma adiponectin could result in improved glucose metabolism in HIV-associated lipodystrophy. Because adiponectin has not (yet) been administered to human subjects, currently, adiponectin can only be increased in an indirect manner. The most potent enhancers of (HMW) adiponectin are the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (27). Although several studies have reported on the effects of PPAR-γ agonists in HIV-associated lipodystrophy (6, 7, 12–14, 22, 31, 36), only one non-placebo-controlled study addressed the role of (HMW) adiponectin in glucose metabolism (26). To obtain more insight into the role of adiponectin in the perturbations of glucose metabolism in HIV-lipodystrophy, we conducted a randomized, double-blind placebo-controlled clinical trial of 16 wk duration, using the PPAR-γ agonist rosiglitazone to enhance (HMW) adiponectin levels. The study was performed in HIV-1-infected patients with clinically overt...
PATIENTS AND METHODS

Subjects. Male subjects with a documented HIV-infection and ≥18 yr of age were recruited from the Academic Medical Center (Amsterdam, The Netherlands) or from neighboring centers. Participants had to be on the same cART regimen for ≥4 mo before study entry. Additionally, their antiretroviral treatment could not have included any HIV PI for ≥9 mo nor d4T for ≥6 mo. The effects of rosiglitazone on insulin sensitivity at the level of peripheral glucose disposal, endogenous glucose production, and lipolysis were assessed by performing hyperinsulenic-euglycemic clamps using stable isotopes. Body fat distribution was determined by abdominal computerized tomography (CT) and dual-energy X-ray absorptiometry (DEXA).

Calculations and statistical analysis. The rate of appearance ($R_a$) of glucose, peripheral glucose disposal, and $R_g$ of glycolcerol were calculated with a modified form of Steele equations, as described before. Resting energy expenditure as well as glucose and fat oxidation rates were calculated from $\text{O}_2$ consumption and $\text{CO}_2$ production as reported previously. Changes within groups were analyzed by paired Student’s $t$-tests. In both groups, for each parameter, the difference was calculated between the value at week 16 and at baseline. Subsequently, the mean differences between the two arms were analyzed by Wilcoxon tests. Correlations between changes in adiponectin levels and changes in glucose metabolism parameters were analyzed by the Spearman correlation coefficient. A $P$ value <0.05 was considered statistically significant. Data are presented as median and interquartile range. SPSS statistical software version 12.0.1 (SPSS, Chicago, IL) was used for all analyses. Assuming a mean glucose disposal of 21.3 $\mu$mol·kg$^{-1}$·min$^{-1}$ with a standard deviation of 4.9 $\mu$mol·kg$^{-1}$·min$^{-1}$ in male patients with HIV-associated lipodystrophy and ≥50 yr of age (34), a sample size of six patients was considered sufficient to allow detecting a change over time of ≥33% with $\alpha=0.05$ and 80% power.

RESULTS

Patient characteristics. Between November 2003 and March 2006, 13 male patients were included. Eight patients were randomized to rosiglitazone and five to placebo (Table 1). Their demographic and clinical characteristics are shown in Table 1. In the placebo group, the following regimens were used in the usual recommended doses: 1) lamivudine + tenofovir + nevirapine; 2) zidovudine + lamivudine + abacavir; 3) lamivudine + didanosine + nevirapine; 4 and 5) lamivudine + didanosine + efavirenz. In the rosiglitazone arm, patients used: 1) lamivudine + abacavir + nevirapine; 2) zidovudine + lamivudine + nevirapine; 3) lamivudine + tenofovir + efavirenz; 4) zidovudine + lamivudine + didanosine + efavirenz; 5) zidovudine + lamivudine + abacavir + nevirapine; 6–8) zidovudine + lamivudine + abacavir. One patient in the placebo arm switched from abacavir (regimen of lamivudine + abacavir + tenofovir) to nevirapine after...
between groups.

molecular weight; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

basal plasma adiponectin levels by 107% (P = 0.02; Table 2). Neither the patients nor the investigators reported subjective improvements of lipodystrophy (data not shown).

Table 2. Body composition, glucoregulatory hormones, lipids, and immunological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rosiglitazone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age, yr</td>
<td>45 (40–50)</td>
<td>43 (42–58)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 (22–27)</td>
<td>23 (22–26)</td>
</tr>
<tr>
<td>Duration of ART, yr</td>
<td>8.8 (5.1–11.6)</td>
<td>7.5 (5.1–9.6)</td>
</tr>
<tr>
<td>Duration of current regimen, mo</td>
<td>22 (7–47)</td>
<td>20 (13–39)</td>
</tr>
<tr>
<td>Duration after stop PI, mo</td>
<td>52 (42–64)</td>
<td>48 (30–84)</td>
</tr>
<tr>
<td>Duration after stop dd4T, mo</td>
<td>36 (7–48)</td>
<td>39 (18–47)</td>
</tr>
<tr>
<td>Lipoadynetin, %</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fat accumulation, %</td>
<td>88</td>
<td>60</td>
</tr>
<tr>
<td>HIV-1 RNA &lt;50 c/ml, %</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CD4-cell count, ×10⁹ cells/l</td>
<td>565 (488–663)</td>
<td>540 (425–825)</td>
</tr>
</tbody>
</table>

Data represent medians with interquartile ranges (IQR) in parentheses; n, no. of subjects. BMI, body mass index; ART, antiretroviral treatment; PI, protease inhibitors; dd4T, stavudine.

11 wk of study duration because of emerging concern that this regimen might be associated with an increased risk of virological failure. None of the patients started medication that could be expected to influence glucose metabolism during the study.

Body composition. There were no significant changes in any of the body composition parameters over the course of 16 wk (Table 2). Neither the patients nor the investigators reported subjective improvements of lipodystrophy (data not shown).

Plasma adiponectin levels. Rosiglitazone increased total basal plasma adiponectin levels by 107% (P < 0.02; Table 2 and Fig. 1). The ratio of HMW to total adiponectin increased by 73% (P < 0.001). In the placebo group, neither total adiponectin levels (P = 0.62) nor the ratio of HMW to total adiponectin changed (P = 0.94). As a result, both the changes in total plasma adiponectin levels and in the ratio of HMW to total adiponectin were significantly different when comparing the study arms (both P < 0.01).

Glucose and lipid metabolism. In the rosiglitazone group, there were no significant changes in basal plasma glucose (P = 0.22) or insulin levels (P = 0.23) after 16 wk (Table 3). Rosiglitazone had no effect on insulin-mediated peripheral glucose disposal (P = 0.13 if expressed in μmol·kg⁻¹·min⁻¹ and P = 0.12 if expressed in μmol·kg lean body mass⁻¹·min⁻¹), endogenous glucose production or glucose oxidation, neither basally (glucose production: P = 0.90; glucose oxidation: P = 0.77) nor during hyperinsulinemia (glucose production: P = 0.17; glucose oxidation: P = 0.66). Additionally, there were no differences over time in the rosiglitazone arm considering lipolysis or fat oxidation, neither after a 12-h fast (lipolysis: P = 0.90; fat oxidation: P = 0.64) nor during the clamp (lipolysis: P = 0.54; fat oxidation: P = 0.37). Rates of resting energy expenditure did not change either (basal: P = 0.83; clamp: P = 0.39). Rosiglitazone treatment did not change basal FFA levels (P = 0.11) but decreased FFA levels during hyperinsulinemia (P < 0.05).

In the placebo arm, there was a small but significant decline over time in glucose production rates both basally and during the clamp (both P < 0.05). Regarding all other parameters of glucose metabolism, there were no significant changes over the course of 16 wk in the placebo arm. When comparing the two study arms, there were no significant differences in the changes in parameters of glucose metabolism during the study.

Glucoregulatory hormones, lipids, and immunological parameters. There were no significant differences in basal plasma concentrations of cortisol, epinephrine, norepinephrine, or...
sTNFR 1 and 2 over time in either treatment group (Table 2). Rosiglitazone significantly increased basal plasma glucagon levels ($P < 0.05$), whereas glucagon was not affected by placebo. There was no difference in the change in basal glucagon between the two arms. Plasma total and low-density lipoprotein (LDL) cholesterol levels significantly increased during treatment with rosiglitazone (both $P < 0.05$), resulting in significantly increased total and LDL cholesterol in patients randomized to rosiglitazone vs. placebo (both $P < 0.05$). Levels of high-density lipoprotein cholesterol and triglycerides did not change in either arm.

CD4 cell count and HIV-1 RNA remained unchanged over the study course in both arms.

Correlates of (HMW) adiponectin. In the rosiglitazone arm, there were no significant correlations between the changes over time in either total plasma adiponectin levels (data not shown) or the ratio of HMW to total adiponectin and the changes over time in basal plasma glucose levels ($r=0.522, P = 0.19$), basal plasma insulin levels ($r=0.133, P = 0.75$), insulin-mediated peripheral glucose disposal ($r= -0.289, P = 0.49$), glucose production, or lipolysis, neither basally ($r= -0.193, P = 0.65$ and $r= -0.265, P = 0.53$, respectively) nor during hyperinsulinemia ($r=0.145, P = 0.73$ and $r=0.205, P = 0.63$, respectively) (Fig. 2).

Study extension. After the randomized study period of 16 wk, patients were offered to participate in an open-label study of rosiglitazone for another 16 wk. Three patients who had been receiving placebo during the randomized phase accepted to be treated with rosiglitazone during 16 wk. Inclusion of the data from these three patients ($n = 11$) resulted in an increase in trunk fat [change 0.3 (0.0–0.9) kg, $P < 0.05$] and in total body fat [change 0.4 (0.0–1.8) kg, $P < 0.05$]. The results concerning adiponectin levels and glucose metabolism did not change: rosiglitazone significantly increased total basal plasma adiponectin levels and the ratio of HMW to total adiponectin. Despite this increase in (HMW) adiponectin, there were no improvements in any of the parameters of glucose metabolism (data not shown).

Six patients who had already been receiving rosiglitazone during the placebo-controlled study phase consented to participate in the study extension and thus eventually received rosiglitazone for a total of 32 wk. Inclusion of their data in the analysis showed an increase in trunk fat [change 1.2 (0.8–1.9) kg, $P < 0.02$], limb fat [change 0.5 (0.2–1.1) kg, $P < 0.05$], and total body fat [change 1.8 (1.3–2.6) kg, $P < 0.02$]. Despite a further increase in plasma (HMW) adiponectin (data not shown) after 32 wk of rosiglitazone, likewise no improvements in glucose metabolism were observed.

DISCUSSION

HIV-infected patients treated with cART frequently develop changes in body fat distribution and disturbances in glucose metabolism, including insulin resistance at the level of peripheral glucose disposal, hepatic glucose production, and lipolysis (15, 35). Plasma levels of adiponectin, primarily the HMW form, are reduced in insulin-resistant patients with type 2 diabetes and HIV-associated lipodystrophy (3, 23, 26) and have been associated with markers of insulin resistance (23, 33). The present study, to the best of our knowledge, is the first...
prospective, placebo-controlled clinical trial that describes the effects of a rosiglitazone-induced increase in plasma (HMW) adiponectin levels on the perturbations of glucose and lipid metabolism in HIV-associated lipodystrophy by conducting hyperinsulinemic-euglycemic clamps using stable isotopes. Our study suggests that, although rosiglitazone markedly increased both total adiponectin levels and the ratio of HMW to total adiponectin in HIV-infected lipodystrophic patients, this did not result in an improvement of glucose and lipid metabolism.

In animal experiments, administration or overexpression of adiponectin ameliorates glucose metabolism by enhancing peripheral glucose uptake and suppressing hepatic glucose production (8, 10, 27, 38). Because adiponectin has not (yet) been administered to human subjects, information on the effects of this hormone on human glucose metabolism is limited. Several studies have investigated the influence of adiponectin in humans by increasing the levels of this adipocytokine indirectly via administration of PPAR-γ agonists. In patients with type 2 diabetes, PPAR-γ agonists enhanced insulin sensitivity both at the level of the liver and peripherally (11, 24, 28). The improvements in insulin sensitivity were associated with an increase in total adiponectin levels. More recently, it was shown that, in patients with type 2 diabetes, this association could be further strengthened by taking the HMW-to-total adiponectin ratio into account (24, 27). These data suggest that adiponectin, in particular the HMW form, has a major role in improving disturbances in glucose metabolism.

Several studies investigated the effects of PPAR-γ agonists in HIV-associated lipodystrophy (6, 7, 12–14, 22, 31, 36). Most (6, 12, 13, 22, 31, 36) but not all (7, 14) of these studies demonstrated an improvement in the derangements of glucose metabolism. However, the majority of these reports focused on the effects of PPAR-γ agonists on body composition and therefore did not examine glucose homeostasis in detail. Only two studies investigated glucose metabolism more thoroughly by performing hyperinsulinemic-euglycemic clamps (13, 26). These clamp studies reported an improvement in whole body insulin sensitivity 3 mo after starting rosiglitazone in HIV-infected patients with insulin resistance and lipatrophy. In addition, hepatic insulin sensitivity as measured by the Homeostatic Model Assessment (HOMA) index improved as well (11, 24, 28). Concomitantly with these ameliorations in insulin sensitivity, there was a significant increase in total plasma adiponectin levels (13, 26), in the plasma levels of the adiponectin HMW form as well as in the ratio of HMW to total adiponectin (26). The change in HMW adiponectin significantly correlated with the increase in hepatic insulin sensitivity. These data implicate that (HMW) adiponectin may be important in the regulation of insulin sensitivity in HIV-lipodystrophic patients.

In the present study, 16 wk of treatment with rosiglitazone resulted in a marked increase in plasma adiponectin levels, primarily of the HMW form as indicated by the increased HMW-to-total adiponectin ratio. These elevations are in accordance with the results of other studies performed in HIV-associated lipodystrophy (13, 26) and type 2 diabetes (11, 24, 27). Despite the increase in (HMW) adiponectin levels, however, we did not find an improvement in any of the parameters of glucose metabolism. This is in contrast with the earlier described studies in HIV-lipodystrophic patients (13, 26). These different results may be related (partly) to differences in study design. Compared with the other trials, we used lower
insulin infusion rates (40 mU/m² in Refs. 13 and 26 vs. 20 mU/m² in our study) to investigate hepatic and peripheral insulin sensitivity. Additionally, instead of the HOMA index, we measured (hepatic) insulin sensitivity by utilizing stable isotopes, which is considered to be the golden standard. Besides these differences in experimental design, there were also differences in the study population. Basal plasma insulin levels were slightly lower in the present study compared with in the other trials (13, 26), which may indicate a lesser degree of insulin resistance in our subjects. In addition, we exclusively examined HIV patients who were not or no longer receiving PI for ≥9 and d4T for ≥6 mo before randomization. It can be postulated that rosiglitazone merely antagonizes any negative effects of ongoing PI and/or d4T exposure on glucose metabolism and therefore had no effect in our patients. Finally, the different results could be explained by differences in the effects of rosiglitazone on body composition. In contrast to our and several other studies (6, 7, 22, 31), there was an increase in subcutaneous and a decrease in visceral fat mass in the participants of the 2 studies, which showed a positive effect on insulin sensitivity (13, 26). It can be hypothesized that this change in body fat distribution may have been responsible for the observed improvements in glucose metabolism via redistribution of insulin-desensitizing FFA metabolites (17) from the liver and muscle to subcutaneous adipose tissue.

A potential confounding factor in our study might be the increase in basal plasma glucagon levels in the rosiglitazone group. Because glucagon is known to enhance hepatic glucose production, the increased basal glucagon levels in the rosiglitazone arm could have counteracted potential positive effects of adiponectin on basal glucose production. However, because we only found a minimal increase in glucagon levels of 7 ng/l, we find it an unlikely confounding factor (21). Additionally, we do not believe glucagon has affected our data during hyperinsulinemia, since we did not find a significant increase in glucagon levels during the clamp after 16 wk of treatment with rosiglitazone (data not shown).

Adiponectin, primarily the HMW form, has been suggested to play an important role in the regulation of glucose metabolism in insulin-resistant, HIV-associated lipodystrophic patients (13, 26, 27, 32, 33, 40). The results of the present study, however, question the importance of (HMW) adiponectin in this setting. We show that rosiglitazone increased plasma concentrations of total adiponectin to levels above those found in ART naive, nonlipodystrophic, HIV-infected patients (4). Moreover, we found that the rise in adiponectin was primarily the result of an increase in the HMW form, which has been suggested to be responsible for the insulin-sensitizing effects (37). However, despite the normalization of (HMW) adiponectin, rosiglitazone did not improve any of the parameters of glucose and lipid metabolism in our HIV-lipoatrophic patients.

Inclusion of the data obtained during rosiglitazone exposure beyond the placebo-controlled phase (n = 11 exposed for 16 wk and n = 6 exposed for 32 wk) likewise did not show a major effect on glucose and lipid metabolism either. Although conclusions should be drawn with caution because of the limited power of our small study, these findings, if confirmed by larger studies, would suggest that, in HIV-lipodystrophic patients, low (HMW) adiponectin levels per se may not play a (major) role in the pathogenesis and/or perseverance of the disturbances in glucose and lipid metabolism but rather reflect dysfunction of adipose tissue. This could imply that therapies, which are solely aimed at enhancing reduced adiponectin levels, could not be expected to be beneficial at counteracting the insulin resistance in HIV-associated lipodystrophy.

A limitation of the present study is the small sample size. Unfortunately, because of our very strict inclusion criteria, inclusion was slow, precluding the inclusion of more subjects within a reasonable time frame. With eight patients in the rosiglitazone group, we would have been able to detect a difference of ~25% in the peripheral glucose disposal rate taking into account an α of 0.05, 80% power, and a within-group standard deviation of 3.4 μmol·kg⁻¹·min⁻¹. Therefore, a smaller difference in glucose disposal rate could have been missed.

In conclusion, rosiglitazone markedly increased both total adiponectin levels and the ratio of HMW to total adiponectin in HIV-lipoatrophic patients, but was not associated with a significant improvement in glucose and lipid metabolism. Acknowledging our limited sample size, these findings, if confirmed by larger studies, could question the role of adiponectin in the regulation of glucose homeostasis in HIV-lipodystrophy.

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