Importance of the high-molecular-mass isoform of adiponectin in improved insulin sensitivity with rosiglitazone treatment in HIV disease

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ABSTRACT

The present study was designed to investigate the relationship of isoforms of adiponectin to insulin sensitivity in subjects with HIV-associated insulin resistance in response to treatment with the thiazolidinedione, rosiglitazone. The two isoforms of adiponectin, HMW (high-molecular-mass) and LMW (low-molecular-mass), were separated by sucrose-gradient-density centrifugation. The amount of adiponectin in gradient fractions was determined by ELISA. Peripheral insulin sensitivity (Rd) was determined with hyperinsulinaemic–euglycaemic clamp, whereas hepatic sensitivity [HOMA (Homoeostasis Model Assessment)]%S] was based on basal glucose and insulin values. Treatment with rosiglitazone for 3 months resulted in a significant improvement in the index of hepatic insulin sensitivity (86.4 ± 15 % compared with 139 ± 23; P = 0.007) as well as peripheral insulin sensitivity (4.04 ± 0.23 compared with 6.17 ± 0.66 mg of glucose/kg of lean body mass per min; P < 0.001). Improvement in HOMA was associated with increased levels of HMW adiponectin (r = 0.541, P = 0.045), but not LMW adiponectin. The present study suggests that the HMW isoform of adiponectin is important in the regulation of rosiglitazone-mediated improvement in insulin sensitivity in individuals with HIV-associated insulin resistance, particularly in the liver.

INTRODUCTION

Adipose tissue plays an important role in the regulation of insulin action. Not only is the obesity-related increase in adipose tissue associated with insulin resistance [1,2], but loss of body fat or lipoatrophy is also associated with insulin resistance [3,4]. The discovery of a number of physiologically active proteins secreted by adipose tissue, adipokines, suggests a possible mechanism by which adipose tissue has an impact on insulin sensitivity. Since its discovery in 1995 [5], plasma concentrations of the adipokine, adiponectin (or ACRP 30) have been associated with insulin sensitivity. Adiponectin levels are lower in obese individuals [6,7] and also lower in subjects with diabetes [8]. Not only are adiponectin levels lower in individuals with insulin resistance, but improvement in insulin sensitivity through weight loss results in increased circulating concentrations of adiponectin [8]. A close relationship of the levels of circulating adiponectin with the degree of insulin resistance has been demonstrated in populations with diminished insulin sensitivity, including obese and diabetic subjects [9], Pima Indians with an increased incidence of diabetes and obesity [10] and individuals with HIV-associated insulin resistance [11]. Evidence of a more direct, causal role of adiponectin in the regulation of insulin sensitivity has

Key words: adiponectin, HIV, insulin resistance, thiazolidinedione.
Abbreviations: BMI, body mass index; HMW, high-molecular-mass; HOMA, Homoeostasis Model Assessment; HOMA-IR, HOMA-insulin resistance; LMW, low-molecular mass; TZD, thiazolidinedione.
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been provided by animal studies demonstrating a direct effect of adiponectin on insulin sensitivity in wild-type mice and in mouse models of insulin resistance [12–14]. Although adiponectin levels have been associated with insulin sensitivity, this ability has been linked to distinct macromolecular forms. Adiponectin has a monomeric structure that exists in a LMW (low-molecular-mass) form consisting of six monomers which readily trimerizes to a HMW (high-molecular-mass) form consisting of eighteen monomers [5]. The majority of adiponectin in the serum has been shown to be the HMW form [15], with a sexual dimorphism in distribution with greater circulating levels of the HMW form in females [16]. When insulin sensitivity is improved by treatment with TZDs (thiazolidinediones), circulating levels of adiponectin are increased [17,18]. Scherer and co-workers have demonstrated that improved insulin sensitivity is associated specifically with increased blood levels of the HMW form of adiponectin [19,20]. Moreover, direct application of the TZD pioglitazone to human adipose tissue in vitro results in increased levels of HMW adiponectin specifically [21]. Further confirmation of the physiological importance of the HMW form of adiponectin in the regulation of insulin action is provided by the demonstration that genetic mutations preventing HMW formation are associated with an increased risk of diabetes [22].

In light of the importance of HMW adiponectin in improving insulin sensitivity [19–21], the present study was undertaken to examine the relationship of the HMW form of adiponectin to insulin sensitivity in subjects with HIV-associated insulin resistance and further to examine the changes in adiponectin associated with improvement in insulin sensitivity following treatment with the TZD rosiglitazone.

MATERIALS AND METHODS

Subject characteristics

Subjects with HIV-associated insulin resistance were enrolled into the study if they had demonstrable insulin resistance but not overt diabetes. Subjects were not enrolled if they had had any acute illness in the 3 months preceding the study, a random (non-fasting) glucose level of greater than 200 mg/dl, liver function assessment three times normal or a change in HIV medication in the 3 months prior to the study.

Following assessment of baseline insulin sensitivity, subjects received 4 mg of rosiglitazone (Avandia; GlaxoSmithKline) twice daily for 12 weeks. Safety evaluations included assessment of liver function, complete blood counts, CD4 counts and viral load during the treatment period. The study was approved by the Committee on Research Involving Human Subjects at Stony Brook University Medical Center and all subjects gave their written, informed consent. The improvement in insulin sensitivity in a subgroup of these subjects has been described previously [23].

Insulin sensitivity

Peripheral insulin sensitivity (glucose disposal or Rd) was determined as the rate of glucose infused to maintain euglycaemia during an insulin infusion (hyperinsulinemic–euglycaemic clamp, [24]). Patients were admitted to the General Clinical Research Center the night before the study. Beginning at 07:00 hours, study subjects received an intravenous infusion of 1.2 m-units of insulin (Humulin; Eli Lilly)/kg of body weight per min. Dextrose (10 %) was administered intravenously to maintain plasma glucose at 90 mg/dl.

Insulin sensitivity was also assessed with HOMA (Homoeostatis Model Assessment), which is based on fasting insulin and glucose [25,26]. HOMA is more a measure of hepatic sensitivity to insulin rather than insulin sensitivity of peripheral tissues. HOMA%S (HOMA insulin sensitivity) was calculated from fasting blood glucose and insulin with a computer model (http://www.dtu.ox.ac.uk/) and expressed as a percentage [27]. HOMA-IR (HOMA-insulin resistance) is the reciprocal of %S, and therefore similar conclusions are reached if the results are expressed as HOMA-IR.

The plasma glucose levels were analysed by the glucose oxidase method, assessed with a Beckman Glucose Analyzer II. Insulin levels were analysed by radioimmunoassay (Diagnostic Products).

Viral load

Plasma samples of HIV RNA were frozen and analysed by the New York state-approved Department of Pathology at Stony Brook. The licensed assay has a lower limit of detection of 400 copies of RNA/ml of plasma. Samples with less than 400 copies of RNA/ml of plasma were assayed with an ultra-sensitive assay with a lower limit of less than 40 copies of RNA/ml of plasma.

Adiponectin isolation and analysis

The HMW and LMW forms of adiponectin from 20 μl of plasma were determined by centrifugation (55 000 rev./min for 8 h at 4 °C in a Beckman SWT155 rotor and L8-60M ultracentrifuge) on a step-wise sucrose density gradient (5–20 %). Twelve gradient fractions of 400 μl each were harvested from the bottom of the gradient. The amount of adiponectin in gradient fractions was determined by ELISA (R&D Systems). The concentration of HMW adiponectin in plasma was determined by combining the amount (concentration × volume) in fractions 2–5. LMW adiponectin was determined in a similar manner from fractions 6–12 (Figure 1).

Statistical analysis

Shapiro–Wilks test was used to test the normality of the data and the data were log-transformed as needed.
Figure 1 Adiponectin concentration in sucrose density fractions

Adiponectin concentration (μg/ml) in fractions from 0.02 ml of plasma separated by sucrose-density-gradient ultracentrifugation and assayed by ELISA, as described in the Materials and methods section. The profiles are from one subject before and after treatment with rosiglitazone. The adiponectin content of fractions 2–5 was taken to be the HMW isoform and the adiponectin content of fractions 6–12 was taken to be the LMW isoform.

to ensure normality. Comparisons between pre- and post-values were assessed with a paired t test. All data are reported as means ± S.E.M., except as noted, and P < 0.05 from a two-sided test was considered significant. Pearson correlation coefficients were calculated to determine the relationship of changes in variables such as insulin sensitivity with changes in adiponectin with rosiglitazone treatment. The changes in each variable with treatment were assessed as the log of the value following rosiglitazone treatment minus the log of the value before rosiglitazone treatment. Statistical analysis was carried out with SAS statistical software, version 9.1.

RESULTS

Seventeen subjects with HIV-associated insulin resistance completed the study (Table 1). The study cohort of seven males and ten females had a mean age of 44 ± 2 years and a BMI (body mass index) of 27 ± 1 kg/m². The number of CD4 cells in these subjects ranged from 47–1330 cells/mm³ and the viral burden ranged from less than 40 to 42 000 copies of RNA/ml of plasma. Fifteen subjects were on highly active antiretroviral treatment, whereas two of the subjects were not on any antiretroviral medications. Because the study was designed to test the ability of rosiglitazone to improve insulin resistance, all of the subjects enrolled in the study were insulin resistant, i.e. had glucose disposal rates (Rd) of equal to or less than 6 mg of glucose/kg of lean body mass per min as assessed with the hyperinsulinaemic–euglycaemic clamp [24].

Prior to treatment with rosiglitazone, the mean fasting glucose in these subjects was 104 ± 3 mg/dl and fasting insulin was 13.5 ± 2.9 (Table 2). Peripheral insulin sensitivity (Rd) was 4.04 ± 0.23 mg of glucose/kg of lean body mass per min and insulin sensitivity from the HOMA%S was 86.4 ± 15 %. In the insulin-resistant-state (pre-rosiglitazone), there was a gender difference in the concentrations of both isoforms: HMW adiponectin (males, 0.50 ± 0.22 μg/ml and females, 1.56 ± 3.5 μg/ml; P = 0.037) and LMW adiponectin (males, 2.12 ± 0.87 μg/ml and females, 5.46 ± 2.25 μg/ml; P = 0.0015) were significantly higher in females.

Table 1 Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Gender</th>
<th>BMI (kg/m²)</th>
<th>Viral burden (copies/ml)</th>
<th>CD4 (cells/ml)</th>
<th>HIV medication</th>
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<td>1</td>
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<td>400</td>
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<td>PI, NNRTI, NRTI</td>
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<td>47</td>
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<td>1094</td>
<td>48</td>
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</table>

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Changes in insulin, glucose, insulin sensitivity and relationship of HMW adiponectin and hepatic insulin sensitivity following treatment with rosiglitazone

Table 2 Changes in insulin, glucose, insulin sensitivity and adiponectin following treatment with rosiglitazone

Values are expressed as means ± S.E.M. The percentage of HMW is the proportion of HMW adiponectin in the plasma, i.e. HMW adiponectin divided by the total (HMW + LMW). P values are from the paired t test of log-transformed data and are for the comparison of pre- and post-rosiglitazone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-rosiglitazone</th>
<th>Post-rosiglitzone</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (μg-units/ml)</td>
<td>14</td>
<td>13.5 ± 2.9</td>
<td>8.75 ± 2.0</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>17</td>
<td>104 ± 3</td>
<td>99 ± 2.3</td>
</tr>
<tr>
<td>Rd (mg of glucose/kg of lean body mass per min)</td>
<td>16</td>
<td>4.04 ± 0.23</td>
<td>6.17 ± 0.66</td>
</tr>
<tr>
<td>HOMA (%)</td>
<td>14</td>
<td>86.4 ± 15</td>
<td>139 ± 21</td>
</tr>
<tr>
<td>HMW adiponectin (μg/ml)</td>
<td>17</td>
<td>1.12 ± 0.26</td>
<td>3.90 ± 0.79</td>
</tr>
<tr>
<td>LMW adiponectin (μg/ml)</td>
<td>17</td>
<td>2.34 ± 0.36</td>
<td>4.71 ± 0.64</td>
</tr>
<tr>
<td>Total adiponectin (μg/ml)</td>
<td>17</td>
<td>3.46 ± 0.57</td>
<td>8.60 ± 1.27</td>
</tr>
<tr>
<td>Percentage of HMW adiponectin</td>
<td>17</td>
<td>0.278 ± 0.03</td>
<td>0.395 ± 0.04</td>
</tr>
</tbody>
</table>

1.54 ± 0.29 μg/ml and females, 2.89 ± 0.49 μg/ml; P = 0.053).

Treatment with rosiglitazone resulted in a significant decrease in both mean fasting glucose (99.2 ± 2 mg/dl; P = 0.025) and insulin (8.75 ± 2.0 μ-units/ml; P = 0.016, Table 2). Peripheral insulin sensitivity (Rd) improved by 51% with rosiglitazone treatment (from 4.03 ± 0.26 mg of glucose/kg of lean body mass per min to 6.17 ± 0.66; P < 0.001, Table 2) and HOMA%S, an index of hepatic insulin sensitivity, was also improved by a similar amount (by 76%; from 86.4 ± 15% to 139 ± 23%; P = 0.007).

The improvement in insulin sensitivity was accompanied by increases in both isoforms of adiponectin; the HMW isoform increased from 1.12 ± 0.26 μg/ml to 3.90 ± 0.79 μg/ml (P < 0.001) and the LMW isoform increased from 2.34 ± 0.36 μg/ml to 4.71 ± 0.64 μg/ml (P < 0.001). ANOVA modelling indicated both gender (HMW, P = 0.0008; LMW, P = 0.0023) and drug (HMW, P < 0.0001; LMW, P < 0.0001) effects, but no gender/drug interaction (HMW, P = 0.5377; LMW, P = 0.35) of log-transformed variables, suggesting that although the isoforms differed by gender in the pre-rosiglitazone condition, there was no significant difference in the response to rosiglitazone between the genders. Therefore the values on the response to treatment for males and females has been combined in subsequent analyses.

Although the blood levels of HMW adiponectin increased following rosiglitazone treatment for the group as a whole, there was individual variation in the response of HMW adiponectin to rosiglitazone. The variation in response of adiponectin and a variable response in insulin sensitivity allowed an assessment of whether or not these variables were related. There was no significant relationship between the improvement in peripheral insulin sensitivity (Rd) and the increase in adiponectin levels following treatment with rosiglitazone (r = 0.39, P > 0.05), but the improvement in hepatic insulin sensitivity, HOMA%S, was significantly associated with increased levels of HMW adiponectin (r = 0.54, P = 0.045; Figure 2). In contrast, increased levels of LMW adiponectin were not significantly associated with improvement in either peripheral insulin sensitivity, Rd (r = 0.41, P = 0.11) or hepatic insulin sensitivity, HOMA%S (r = 0.34, P = 0.24). The data were also examined for the relationship between the increase in insulin sensitivity and the proportion of the HMW isoform of adiponectin in plasma, i.e. HMW divided by HMW plus LMW adiponectin following rosiglitazone treatment (Table 2). The correlations of the change in HMW with the improvement in peripheral insulin sensitivity (r = 0.243, P = 0.37) and HOMA (r = 0.44, P = 0.11) were not as strong as the correlations with the changes in plasma levels of the HMW isoform. Interestingly the improvement in peripheral insulin sensitivity (Rd) following treatment was highly correlated with changes in liver sensitivity (HOMA, r = 0.66, P = 0.01; Figure 3).

**DISCUSSION**

Subjects recruited into the present study all had HIV-associated peripheral insulin resistance with a mean glucose disposal rate (Rd) of 4.04 ± 0.26 mg of glucose/kg of lean body mass per min. For comparison, the Rd for a group of subjects of similar age and BMI without HIV infection was 11.1 ± 1.1 [23]. Insulin resistance is
accompanied by lower plasma concentrations of adiponectin in HIV disease [11] and other insulin resistant states [9,10]. Although the adiponectin levels in blood are reduced in subjects with HIV-associated insulin resistance, in the present study there was still evidence of significant gender dimorphism in the isoforms of adiponectin, with significantly higher levels of the HMW form of adiponectin in females compared with males. This is consistent with the gender differences in HMW and LMW isoforms reported in mice [16], children [28] and older adults [29].

In these subjects with HIV-associated insulin resistance, treatment with rosiglitazone, a TZD, resulted in a significant improvement in insulin sensitivity assessed either with a hyperinsulinaemic–euglycaemic clamp or with HOMA. During the clamp procedure, exogenous insulin is provided and the response of peripheral tissues is assessed. The rosiglitazone-mediated improvement in peripheral insulin sensitivity of approx. 50% has been previously reported in a subgroup of these subjects [23]. The HOMA model, based on fasting insulin and glucose levels, reflects hepatic insulin sensitivity and was increased by a somewhat larger amount (76%).

The improvement in insulin sensitivity following rosiglitazone treatment was accompanied by significantly increased blood levels of HMW and LMW isoforms. This is in agreement with increased levels of total adiponectin reported in other insulin resistant states [17,18]. However, since the magnitude of the changes in insulin sensitivity in the present study (results not shown) and that of Haider et al. [30] did not correlate with the changes in the levels of total adiponectin, it seems unlikely that total adiponectin is important in the regulation of insulin sensitivity.

In contrast with the changes in total adiponectin, the increase in the amount of the HMW isoform of adiponectin was significantly related to changes in hepatic insulin sensitivity as assessed by HOMA (Figure 2). This is in keeping with reports from others that it is the HMW isoform of adiponectin that is important in the regulation of insulin action [19–21], although in the present study the strongest association was with the plasma concentration of the HMW isoform of adiponectin rather than the proportion of adiponectin present as the HMW isoform reported in other studies [19,20]. In the present study there was an observable gender difference in the concentration of the HMW isoform of adiponectin in subjects prior to rosiglitazone treatment, the changes in levels of HMW in response to rosiglitazone treatment were similar in both males and females. Although there are limitations to the present study, including the lack of a placebo group and the relatively small number of subjects, the present study confirms the importance of the HMW isoform of adiponectin in hepatic insulin sensitivity as reported for humans in other insulin-resistant conditions [19,20] and animals [12,31].

Given the importance of the HMW isoform of adiponectin in assessing insulin sensitivity, different methodologies have been developed to simplify the determination of this isoform. Initially, the different isoforms of adiponectin were separated by sucrose-density-gradient ultracentrifugation [19,20] or gel filtration [15,32] followed by quantitative Western blot analysis. The method employed in the present study separated the adiponectin isoforms by density-gradient centrifugation, but employed ELISA for quantification of the isoforms since ELISA correlates well with quantitative Western blot analysis [33] and provides enhanced sensitivity. There are a variety of other methods for the measurement of HMW adiponectin, including non-reducing SDS/PAGE, which provides both the separation and quantification of the isoforms of adiponectin [22] and ELISA analysis for HMW adiponectin after selective removal of the LMW isoform [34]. However, the most promising technique for the assessment of HMW adiponectin is the commercially available ELISA based on a monoclonal antibody to HMW adiponectin (Fujirebio; [35]).

In conclusion, the present study suggests that the HMW isoform of adiponectin is important in the regulation of the rosiglitazone-mediated improvement in insulin sensitivity in individuals with HIV-associated insulin resistance, particularly in the liver.

ACKNOWLEDGMENTS

This work was supported by U.S. National Institutes of Health grant DK049316 to M. C. G. and #M01RR10710,
which supports the General Clinical Research Center at Stony Brook University Medical Center and the Empire Clinical Research Investigators Program Award (to S.Q. and R.F.). We gratefully acknowledge the General Clinical Research Center nursing, Core Lab staff and the volunteer subjects who assisted in these studies.

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Published as Immediate Publication 5 February 2008, doi:10.1042/CS20070387