CALL FOR PAPERS | Metabolic Syndrome

Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes

Fahim Abbasi,1 Sang-Ah Chang,2 James W. Chu,1 Theodore P. Ciaraldi,2 Cindy Lamendola,1 Tracey McLaughlin,3 Gerald M. Reaven,1 and Peter D. Reaven3

1Department of Medicine, Stanford University School of Medicine, Stanford; 2Medical Research Service, Veterans Affairs San Diego Healthcare System and Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Diego, California; and 3Medical Research Service, Division of Endocrinology and Metabolism, Department of Medicine, Carl T. Hayden Veterans Affairs Medical Center, Phoenix, Arizona

Submitted 22 April 2005; accepted in final form 30 August 2005

Abbasi, Fahim, Sang-Ah Chang, James W. Chu, Theodore P. Ciaraldi, Cindy Lamendola, Tracey McLaughlin, Gerald M. Reaven, and Peter D. Reaven. Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. Am J Physiol Regul Integr Comp Physiol 290: R139–R144, 2006; doi:10.1152/ajpregu.00287.2005.—It has been suggested that changes in adiponectin levels may contribute to improved insulin sensitivity in insulin-resistant individuals both after weight loss and after treatment with thiazolidinedione compounds. If this is correct, then changes in total circulating adiponectin and/or distribution of its multimeric complexes should coincide with improvements in insulin sensitivity after both interventions. To address this issue, fasting adiponectin concentrations and distribution of adiponectin complexes were measured in plasma samples in 24 insulin-resistant, nondiabetic subjects before and after 3–4 mo of treatment with either rosiglitazone or caloric restriction. The degree of insulin resistance in each group of 12 subjects was equal at baseline and improved to a similar extent (~30%) after each therapy. Whereas total adiponectin levels increased by nearly threefold and the relative amount of several higher molecular weight adiponectin complexes increased significantly in the rosiglitazone treatment group, there were no discernible changes in adiponectin levels or in the distribution between high or low molecular weight complexes in the weight loss group. These data indicate that, although changes in total adiponectin and several specific adiponectin complexes paralleled improvements in insulin resistance in thiazolidinedione-treated subjects, neither circulating adiponectin concentrations nor multimeric complexes changed in association with enhanced insulin sensitivity after moderate weight loss in 12 insulin-resistant, obese individuals.

thiazolidinedione; adiponectin multimers; high molecular weight complexes

ADIPONECTIN is a ~30-kDa protein produced by adipocytes, which circulates in plasma in multimeric aggregates of different sizes and has been closely associated with insulin action (7, 25). Total circulating adiponectin levels have been shown to be lower in insulin-resistant individuals (1, 27), and both over- and underexpression of adiponectin alters insulin sensitivity in animal models of insulin resistance (4, 29). Furthermore, in vitro and in vivo studies have demonstrated several possible mechanisms for the beneficial actions of adiponectin, including enhanced hepatic insulin sensitivity (4), an increase in fatty acid oxidation (9), and decreased inflammatory activity (20). In light of these observations, it has been suggested that interventions that improve insulin sensitivity may accomplish this through modulation of adiponectin levels, and this notion is consistent with evidence that plasma adiponectin concentrations increase when insulin-resistant individuals, either nondiabetic or with Type 2 diabetes, are treated with thiazolidinedione (TZD) compounds (13, 17, 33).

On the other hand, not all interventions that enhance insulin sensitivity are associated with significant changes in plasma adiponectin concentrations. For example, plasma adiponectin levels do not significantly increase in response to two therapeutic modalities that clearly improve insulin action, namely, moderate weight loss (~8.0 kg) (2, 28) and increases in physical activity (14, 18, 31). There are several possible explanations for these discrepant results. First, a functional peroxisome proliferator-activated receptor-responsive element in the adiponectin promoter has recently been identified that is thought to play a significant role in the transcriptional activation of adiponectin gene in adipocytes (15). Thus it could be argued that the increase in adiponectin concentrations in TZD-treated insulin-resistant individuals was a direct effect of the drug itself, and changes in adiponectin are not responsible for improved insulin resistance resulting from all treatment modalities. Second, the apparent divergent changes in plasma adiponectin concentrations following moderate weight loss and increased physical activity vs. TZD treatment could also be explained by differences in either the baseline degree of insulin resistance and/or the improvement in insulin resistance after each of the interventions. Third, although adiponectin is synthesized as a ~30-kDa monomer, it circulates as a variety of multimeric forms, including homotrimer (70–90 kDa) and larger oligomers, and a high molecular weight (HMW) form.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
containing six trimers (21). Evidence indicates that these multimers vary in their biologic activities, and it has been suggested that the proportion of adiponectin in the HMW form is a more accurate indicator of insulin sensitivity than total adiponectin levels (21, 22). Therefore, there may be changes in the absolute or relative levels of various adiponectin multimers that explain the improvement in insulin resistance accompanying weight loss. Finally, because moderate weight loss (unlike TZD treatment) is not associated with changes in circulating levels of adiponectin (2, 28), other factors may contribute to improvements in insulin sensitivity in this setting. The present study was initiated to evaluate these various possibilities and involved directly comparing the changes in total plasma adiponectin concentrations and the distribution of adiponectin multimers that were seen in response to TZD treatment with those before and after weight loss in insulin-resistant subjects that were matched for both baseline insulin resistance and the degree to which the defect in insulin action improved with each therapeutic intervention. If variations in adiponectin are largely responsible for improvements in glucose metabolism following most typical therapeutic interventions, then changes in plasma adiponectin and/or distribution of its complexes should reflect improvements in insulin sensitivity after both moderate weight loss and TZD treatment.

MATERIALS AND METHODS

The study included 24 subjects selected from a larger group of volunteers who had participated in studies at Stanford University’s General Clinical Research Center examining the effect of weight loss (19) and treatment with rosiglitazone (5) on insulin resistance and cardiovascular disease risk factors. Participants for these studies were recruited from the San Francisco Bay area through advertisements in local newspapers. Each volunteer signed a written informed consent before admission to the General Clinical Research Center. All study participants were determined to be nondiabetic according to the criteria of the American Diabetes Association (5a) and were required to have normal findings on history, physical examination, and routine chemical screening battery, including hematocrit, creatinine, and alanine transaminase levels. Degree of adiposity was assessed by body mass index (BMI), and all subjects with BMI >35.0 kg/m² were excluded from the studies. Volunteers selected for the weight loss study were required to have a BMI of 30.0–34.9 kg/m². There were no specific BMI inclusion values for the rosiglitazone-treated group, and BMI ranged from 21.9 to 34.8 kg/m². The study protocol was reviewed and approved by the Institutional Review Board at Stanford University.

Study subjects underwent an insulin suppression test to quantify insulin-mediated glucose disposal as originally described (10) and validated by our research group. Briefly, after a 12-h overnight fast, subjects were infused for 180 min with octreotide acetate (0.27 µg·m⁻²·min⁻¹), insulin (32 mIU·m⁻²·min⁻¹), and glucose (267 mg·m⁻²·min⁻¹). Blood was drawn at 10-min intervals from 150 to 180 min of the infusion to measure plasma glucose and insulin concentrations, and the means of these four values were used as the steady-state plasma insulin and glucose (SSPG) concentrations for each individual. Because steady-state plasma insulin concentrations were similar in all subjects during these tests, the SSPG concentration provided a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG concentration, the more insulin resistant the individual. On the basis of the results of the insulin suppression test, participants with SSPG concentrations >180 mg/dl were defined as being insulin resistant and were eligible for this study. This cutoff point was chosen based on the results of a previously reported distribution of SSPG concentrations (32) where approximately one-third of the 490 nondiabetic subjects had SSPG concentration values above 180 mg/dl.

To accomplish the goals of our study, we selected 24 nondiabetic insulin-resistant subjects, matched for age and gender distribution, who had similar SSPG concentrations before and after administration of rosiglitazone (n = 12) or weight loss (n = 12). Rosiglitazone-treated subjects received 4 mg/day for 4 wk, followed by 8 mg/day for 8 wk, while maintaining their usual diet. All baseline measurements were repeated at the end of 12 wk of treatment. The weight loss group included volunteers with a BMI between 30.0 and 34.9 kg/m². All subjects were instructed by a certified dietitian on calorie-restricted diets calculated to lead to a weight loss of 0.5 kg/wk. The period of weight loss was 4 mo in duration, during which time subjects were seen bimonthly to be weighed and receive dietary advice. At the completion of the weight loss phase, subjects were instructed on a weight-maintenance diet. After 2 wk of stable weight, all measurements performed at baseline were repeated.

Plasma glucose and insulin levels were measured as described previously (32). Plasma adiponectin levels were measured on blood samples drawn before the initiation of the insulin suppression test with a radioimmunoassay established by Linco Research (St. Charles, MO). This assay has a sensitivity of 1 ng/ml, a range of 500 ng/ml to 100 µg/ml, when samples are diluted 1/500 as per manufacturer’s instructions, and intra- and interassay coefficient of variation of <8%.

Analysis of the multimerization status of circulating adiponectin was performed by size fractionation of plasma samples using SDS-PAGE under nonreducing, nondenaturing conditions, as described (with minor modifications) by Waki et al. (26). Plasma was combined with a 4 × Laemmlli sample buffer, prepared without β-mercaptoethanol. Sample preparation was performed at room temperature. Samples, equivalent to 2 µl of plasma, were size fractionated on 3–8% polyacrylamide gradient gels in a Tris-acetate system. Proteins were transferred to nitrocellulose membranes and blocked for 3 h at room temperature with 5% milk in Tris-buffered saline, pH 7.5. Membranes were incubated with a monoclonal antibody against human adiponectin (BD Biosciences, Palo Alto, CA), at a 1:500 dilution for 1 h at room temperature. The secondary antibody was anti-mouse IgG conjugated with horseradish peroxidase (Amersham Biosciences, Buckinghamshire, UK). Bands were detected using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL) and captured on Hyperfilm ECL film (Amersham Biosciences). Quantitative densitometry was performed using ChemiImager software (Alpha Innotech, San Leandro, CA); and, after subtracting the background density, data were presented as average density per band. Each subject sample was run at least two or three separate times and yielded consistent results. Bands were compared with HiMark Unstained HMW protein standards from Invitrogen (cat. no. LC5688). Manufacturer’s instructions were followed to visualize the bands using Coomassie blue. A single lot of standards was used for all the gels. One limitation to the SDS-PAGE is the semiquantitative nature of the results. However, the ability to assess multiple pre- and posttherapy samples on the same gels makes this approach an excellent method to directly compare the effects of different interventions.

Summary statistics are described as means ± SD. The two study groups were compared using Student’s unpaired t-test and χ²-square test. Within each study group, changes in clinical and metabolic variables were compared using Student’s paired t-test. Finally, Pearson’s correlation coefficients were calculated between the changes in SSPG and adiponectin concentrations in response to the interventions.

RESULTS

Baseline characteristics of the study population are given in Table 1. The groups were similar in terms of age, gender distribution, SSPG, and fasting plasma glucose and insulin concentrations. However, based on the inclusion criteria for the
two different intervention arms, BMI was greater in those who participated in the weight loss program.

Table 2 compares the effects of the two experimental interventions on body weight, BMI, SSPG concentrations, and fasting plasma glucose and insulin concentrations. Participants in the weight loss group lost an average of 7.4 kg (8% of initial weight), whereas rosiglitazone-treated subjects gained 1.3 kg ($P = 0.03$). As a consequence of these changes in opposite directions, weight at the end of the study was nearly identical in the two groups.

SSPG concentrations were essentially identical in the two groups before weight loss (Table 2), declined significantly and to a comparable degree after each intervention (29% vs. 30%), and were again quite similar at the end of the study. In association with the improvement in insulin sensitivity, fasting plasma glucose and insulin concentrations decreased significantly ($P < 0.05$) and to a similar extent in both groups.

Figure 1 depicts plasma adiponectin concentrations before and after the two experimental interventions. Mean baseline adiponectin levels were not statistically different ($P = 0.30$) between the rosiglitazone-treated (10.9 ± 5.0 μg/ml) and weight loss (9.0 ± 3.2 μg/ml) groups. Plasma adiponectin concentration increased in every rosiglitazone-treated subject, with a tendency for the increment to be greatest in individuals with the highest baseline values. The overall increase in adiponectin concentration after rosiglitazone treatment (to 24.8 ± 11.6 μg/ml) was highly statistically significant ($P < 0.001$), and there was no relationship between the modest change in weight seen in these subjects and the increase in adiponectin after administration of rosiglitazone.

In marked contrast, plasma adiponectin concentrations remained unchanged in association with the enhanced insulin sensitivity following weight loss, with values increasing slightly in five subjects, decreasing slightly in six others, and not changing in one subject. As a consequence, plasma adiponectin concentrations at the end of the study were approximately threefold greater (24.8 ± 11.6 μg/ml, 95% confidence interval of 17.4–32.1, vs. 8.9 ± 3.1 μg/ml, 95% confidence interval of 7.0–10.8; $P < 0.001$) in rosiglitazone-treated subjects, despite the fact that both the improvement and the final level of insulin sensitivity were similar in the two groups. Furthermore, there was no correlation in the entire group between the improvement in insulin sensitivity and increases in plasma adiponectin concentration in response to the experimental treatments ($r = 0.12, P = 0.59$).

### Table 1. Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Rosiglitazone Treatment Group</th>
<th>Weight Loss Group</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>56±10</td>
<td>51±7</td>
<td>0.19</td>
</tr>
<tr>
<td>Gender, female/male</td>
<td>9/3</td>
<td>8/4</td>
<td>0.65</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.2±4.6</td>
<td>32.7±1.7</td>
<td>0.02</td>
</tr>
<tr>
<td>SSPG, mg/dl</td>
<td>250±40</td>
<td>242±37</td>
<td>0.65</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>103±11</td>
<td>102±12</td>
<td>0.86</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>22±12</td>
<td>18±13</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values are means ± SD for 12 subjects in each group. BMI, body mass index; SSPG, steady-state plasma glucose.

### Table 2. Effect of rosiglitazone treatment and weight loss on body weight and metabolic variables

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Rosiglitazone Pretreatment</th>
<th>Rosiglitazone Posttreatment</th>
<th>Weight Loss Pretreatment</th>
<th>Weight Loss Posttreatment</th>
<th>$P$*</th>
<th>$P$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>82.0±19.5</td>
<td>83.3±19.8</td>
<td>90.9±11.0</td>
<td>83.5±10.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.2±4.6</td>
<td>29.6±4.7</td>
<td>32.7±1.7</td>
<td>30.0±2.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SSPG, mg/dl</td>
<td>250±40</td>
<td>178±61</td>
<td>242±37</td>
<td>169±61</td>
<td>&lt;0.001</td>
<td>0.93</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>103±11</td>
<td>95±11</td>
<td>102±12</td>
<td>95±6</td>
<td>0.04</td>
<td>0.86</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>22±12</td>
<td>15±6</td>
<td>18±13</td>
<td>12±5</td>
<td>0.03</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are means ± SD for 12 subjects in each group. RGZ, rosiglitazone treatment. *Baseline variables compared with those after the intervention; †changes in the rosiglitazone treatment group compared with those in the weight loss group.
shown in Table 3, and it can be seen that several differences between the two groups were observed. First, the relative amount or distribution of each adiponectin complex, expressed as percentage of total adiponectin for each individual, varied between groups at baseline. The major difference was that the weight loss group had a lower percentage of the HMW complex, whereas percentages of other complexes did not differ between the two groups before the interventions. Second, whereas there were increases in the relative amounts of the HMW adiponectin band and the 200-kDa band in the rosiglitazone group after treatment (Table 3), there was no such shift in the distribution among adiponectin multimers from the plasma of the weight loss group. Representative examples of this are shown in Fig. 2. In support of this overall finding was the observation that the percentage of total adiponectin present in the HMW complex increased after treatment in all 12 of the subjects receiving rosiglitazone (with or without accompanying weight gain), whereas this only occurred in 3 of the 12 subjects in the weight loss group. There was also a significant decrease in the percentage of total adiponectin present in the ~170-kDa band in the rosiglitazone group. There were no other consistent changes in adiponectin multimer distribution in rosiglitazone subjects, and there were no consistent changes in the relative amounts of any adiponectin multimers in the weight loss group.

DISCUSSION

To assess whether changes in specific adiponectin multimeric complexes, rather than total plasma adiponectin concentration, may have accounted for enhanced insulin sensitivity after either intervention, we compared relative levels of each of the major adiponectin forms present in plasma as determined by density measurement of each band identified on the gradient gels. This analysis revealed seven or eight different bands ranging in size from ~90 kDa, most probably the trimeric form, to a HMW form, >400 kDa (Fig. 2). If samples were more extensively heated and denatured before addition to gels, the number of bands were typically reduced to the 30- and 90-kDa forms (data not shown), consistent with a reduction in multimerization of adiponectin as previously described (21, 26). The averaged results from analysis of multiple gels are shown in Table 3, and it can be seen that several differences in the distribution among adiponectin multimers from the plasma of 2 subjects that were in the rosiglitazone (RGZ) treatment group and 2 subjects in the weight loss (WL) group. B, baseline; RGZ or WL, posttreatment. Under reducing conditions, the middle and higher molecular weight bands disappeared, consistent with the notion that these bands represented higher-order multimers of adiponectin. Bands for the posttherapy rosiglitazone group appear darker in general because there were overall increases in the total amount of adiponectin. Relative distribution, as presented in Table 3, adjusts for this difference. MW, molecular weight (as measured by molecular mass in kDa).

A major goal of this study was to clarify the nature of the relationship between plasma adiponectin concentrations and resistance to insulin-mediated glucose disposal. Perhaps the most useful way to view our results from this perspective is to begin by identifying areas of common agreement concerning this issue. In this context, several previous studies have shown that improved insulin sensitivity following TZD treatment is associated with increases in plasma adiponectin concentrations (3, 12, 22, 23, 33). More recently, Pajvani et al. (22) have shown, using velocity sedimentation methodology to identify circulating adiponectin complexes, that the increase in plasma adiponectin following TZD therapy in subjects with Type 2 diabetes or insulin resistance is also associated with greater amounts of larger multimeric forms of adiponectin. Similar to Waki et al. (26), we used a SDS-PAGE method to measure

### Table 3. Effect of rosiglitazone treatment and weight loss on relative amount of individual adiponectin multimers in plasma

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Rosiglitazone</th>
<th>Weight Loss</th>
<th>RGZ vs. Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Posttreatment</td>
<td>P*</td>
</tr>
<tr>
<td>HMW</td>
<td>11.8±4.5</td>
<td>15.1±3.8</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt;300 kDa</td>
<td>15.6±4.8</td>
<td>15.1±2.0</td>
<td>0.76</td>
</tr>
<tr>
<td>~240 kDa</td>
<td>15.2±2.4</td>
<td>15.1±1.6</td>
<td>0.94</td>
</tr>
<tr>
<td>~200 kDa</td>
<td>9.2±2.2</td>
<td>11.6±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>~170 kDa</td>
<td>18.3±4.6</td>
<td>14.8±2.1</td>
<td>0.003</td>
</tr>
<tr>
<td>~130 kDa</td>
<td>2.2±5.1</td>
<td>1.8±4.2</td>
<td>0.17</td>
</tr>
<tr>
<td>~120 kDa</td>
<td>12.0±3.3</td>
<td>11.7±1.5</td>
<td>0.78</td>
</tr>
<tr>
<td>~90 kDa</td>
<td>15.8±4.3</td>
<td>14.7±1.9</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Values are means ± SD and depict the amount of adiponectin multimer levels as % total adiponectin for each individual in the 12 subjects in each group. Analysis of the multimerization status of circulating adiponectin was performed by size fractionation of plasma samples using SDS-PAGE under nonreducing, nondenaturing conditions, as described in MATERIALS AND METHODS. Bands were detected using a chemiluminescent substrate and compared with molecular weight standards. Quantitative densitometry comparisons were performed using ChemiImager software. Each gel included pre- and postsamples from individuals from both groups, as illustrated in Fig. 3. Although bands were relatively consistent in their distribution (and reproducible on gels run under similar conditions), some bands such as the 130-kDa band were present in very low in amounts and were only seen in some subjects. * and †, see Table 2.

AJP-Regul Integr Comp Physiol • VOL 290 • JANUARY 2006 • www.ajpregu.org
adiponectin complex distribution in plasma, and our results are quite similar to those of Pajvani et al. (22) in that we also observed an increase in the very highest molecular mass band and, to a lesser extent, in a 200-kDa band, whereas they described an increase in adiponectin complexes that appears to include a range of HMW adiponectin complexes (22). The most likely explanation for the relatively minor discrepancies between the results of the two studies is the difference in the methods used to measure adiponectin multimers.

Thus there is general agreement that TZD treatment is associated with enhanced insulin sensitivity, higher plasma adiponectin concentrations, and an increase in HMW forms of adiponectin. Although there is accumulating evidence that TZD-associated changes in adiponectin concentration and/or multimer distribution may mediate improved insulin sensitivity, it remains possible that the changes in insulin action and adiponectin concentration and size distribution represent separate and unrelated TZD effects. Consistent with this possibility was our inability to discern a significant relationship following TZD administration between the enhanced insulin sensitivity and the changes in plasma adiponectin concentration. The results of the recent report by Hammarstedt et al. (12) also show that insulin sensitivity improves, associated with increases in plasma adiponectin concentration and a shift to a HMW form, subsequent to pioglitazone administration in insulin-resistant, nondiabetic individuals. However, as was the case with our study, there was no relationship between the enhanced insulin sensitivity and the changes in adiponectin amount or multimer distribution. It is possible that the lack of a relationship between changes in insulin action and adiponectin concentration is due to the relatively few number of patients in both studies and/or the fact that they were all insulin resistant. In any event, there appears to be relative unanimity concerning the fact that administration of TZD compounds to insulin-resistant individuals leads to enhanced insulin sensitivity, increases in total adiponectin concentration, and a shift to HMW forms. What is not so clear is the nature and strength of the relationship between these metabolic changes.

There is less agreement concerning the effect of weight loss on plasma adiponectin concentrations. The results of the present study, as well as previous findings of our research group (2) and those of Xydakis et al. (28), indicate that insulin sensitivity improves after relatively moderate weight loss in the absence of any change in plasma adiponectin concentration. In contrast, evidence has also been published that the improvement in insulin action associated with weight loss is accompanied by higher plasma adiponectin concentrations (6, 11, 30). However, these studies differed from ours in two important ways. First, the magnitude of obesity at baseline in these latter studies was much greater; bariatric surgery was used to induce weight loss, resulting in relatively massive amounts of weight loss, i.e., 23 kg to almost 57 kg (6, 11, 30). This approach is quite different from ours in which moderate calorie restriction was instituted to bring about weight loss of 8–9 kg. Furthermore, we directly measured the improvement in insulin sensitivity after moderate weight loss, whereas surrogate measures, based on changes in plasma insulin concentration, were used to assess insulin action in those studies in which massive weight loss was associated with increases in plasma adiponectin concentration. Although these estimates of insulin action are correlated with specific measures of insulin-mediated glucose disposal, they account for < 40% of the variability from person to person when insulin-mediated glucose disposal is measured directly (32). The limitation of insulin concentrations as indicators of insulin action is accentuated in obese individuals because obesity, per se, results in decreased insulin clearance (8, 16). Thus large amounts of weight loss will result in lower insulin concentrations, in excess of any associated improvement in insulin sensitivity, and their use in this situation cannot provide reliable estimates of insulin action. It is also worth noting that the increment in adiponectin concentration following massive weight loss was substantially less than that described following TZD administration (13, 17, 33). Thus, although changes in plasma adiponectin concentration may vary as a function of the amount of weight lost, insulin sensitivity can improve after moderate weight loss in the absence of any change in plasma adiponectin, and the increase in adiponectin concentration with even massive weight loss is attenuated compared with the changes seen after TZD treatment.

In contrast to previous studies, ours was explicitly designed to elucidate the relationship between adiponectin and treatment-induced changes in insulin sensitivity. Furthermore, ours is the only study that directly compared the effects of rosiglitazone administration vs. those of moderate weight loss on both insulin action and adiponectin concentration and its multimeric complex distribution. Finally, the impact of the two treatments was compared in insulin-resistant, nondiabetic subjects, well matched at baseline for age, gender, and degree of insulin resistance. The similarity of the two groups before treatment permits us to more confidently compare the impact of the two interventions on the relationship between treatment-induced changes in insulin action and plasma adiponectin concentrations. The results presented clearly demonstrate that the relationship between these variables varied dramatically as a function of the intervention. Despite essentially identical improvement in insulin sensitivity, the changes in plasma adiponectin concentrations were quite disparate. Whereas increases in total adiponectin and several higher molecular weight complexes paralleled improvements in insulin resistance in TZD-treated subjects, we report the novel finding that no changes in adiponectin concentrations or molecular weight complexes were observed when insulin sensitivity improved to a similar degree after weight loss. As indicated above, exercise-mediated improvements in insulin sensitivity also appear independent of changes in total adiponectin levels (14, 18, 31). Thus it is possible to dissociate improvements in insulin sensitivity from increases in adiponectin concentration, supporting the notion that changes in adiponectin may not be instrumental in improving insulin resistance in all therapeutic settings. As a corollary, it appears that other factors (e.g., other adipokines or cytokines produced in adipose tissue or elsewhere) may play a role in the improvement in insulin-mediated glucose disposal that occurs when insulin-resistant individuals lose a moderate amount of weight.

**GRANTS**

This work was, in part, supported by the office of Research and Development, Medical Research Service, Department of Veterans Affairs, and by research grants from the Department of Veterans Affairs, the National Institutes of Health Grants RR-00070 and HL-067690, and the American Diabetes Association (to T. P. Ciaraldi).
REFERENCES


