Modulation of Circulating and Adipose Tissue Adiponectin Levels by Antidiabetic Therapy

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The relationship between insulin action and control of the adipocyte-derived factor adiponectin was studied in age- and weight-matched obese individuals with type 2 diabetes failing sulfonylurea therapy. After initial metabolic characterization, subjects were randomized to troglitazone or metformin treatment groups; all subjects received glyburide (10 mg BID) as well. Treatment was continued for 3 months. The extent of glycemic control after treatment was similar in both groups. However, the increase in maximal insulin-stimulated glucose disposal rate was greater following troglitazone therapy (+44%) compared with metformin treatment (+20%). Troglitazone treatment increased serum adiponectin levels nearly threefold. There was no change in serum adiponectin with metformin treatment. A positive correlation was found between increases in whole-body glucose disposal rates and serum adiponectin levels after troglitazone; no such relationship was seen with metformin. The adiponectin protein content of subcutaneous abdominal adipocytes was increased following troglitazone treatment and unchanged after metformin. Adiponectin release from adipocytes was also augmented with troglitazone treatment. Adiponectin was present in adipocytes and plasma in several multimeric forms; a trimer was the major form secreted from adipocytes. These results indicate that increases in adiponectin content and secretion are associated with improved insulin action but are not directly related to glycemic control. Modulation of adipocyte function, including upregulation of adiponectin synthesis and secretion, may be an important mechanism by which thiazolidinediones influence insulin action. Diabetes 52:667–674, 2003

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dipose tissue is now recognized as an important source of metabolically active secretory products (adipocytokines), including leptin, tumor necrosis factor (TNF)-α, interleukin (IL)-6, plasminogen activator inhibitor (PAI)-1, adipisin, and free fatty acids (FFAs), which are capable of affecting peripheral insulin action. Human adiponectin (also known as GBP28 or apM1) and its murine homolog acrp30 (also known as adipQ) are novel fat cell secretory products recently independently identified by a number of investigators (1–4). Adiponectin is abundantly expressed; its levels in plasma account for 0.01–0.03% of total plasma protein (5). Circulating adiponectin levels have been shown to be negatively correlated with BMI (5), plasma glucose, triglyceride, and insulin levels (6). An involvement of adiponectin in regulation of metabolism has been suggested by studies in nonhuman obese primates (7) and an obese Pima Indian population (8), which has shown whole-body insulin sensitivity to be independently associated with reductions in circulating adiponectin levels. Consistent with this finding, two different interventions that improve insulin action, weight loss (9), and thiazolidinedione treatment (10–12) elevate circulating adiponectin levels.

Thiazolidinediones (TZDs) are a new class of insulin-sensitizing agents used in the treatment of type 2 diabetes. In clinical studies they have been shown to reduce plasma glucose and insulin levels and to improve lipid abnormalities (13). Recent studies have shown that TZDs dose-dependently increase the mRNA expression and secretion of adiponectin (10). Metformin, a member of the biguanide class of compounds, is effective at lowering blood glucose in patients with type 2 diabetes (14). A number of studies have shown that metformin exerts its effects primarily on the liver, inhibiting gluconeogenesis and reducing hepatic glucose output (14,15). Metformin has also been reported to improve peripheral insulin sensitivity and increase insulin-mediated glucose uptake in skeletal muscle of patients with type 2 diabetes (16), possibly through stimulation of the AMP-activated protein kinase (17). To understand more about the potential relationships between adiponectin, glucose tolerance, and insulin action, we evaluated the effects of these two different pharmacologic interventions on adiponectin production in obese type 2 diabetic subjects failing sulfonylurea treatment. We report here that subjects with type 2 diabetes treated with TZDs had significantly greater fat cell content, release, and
circulating levels of adiponectin as compared with those with matched glycemic control treated with metformin.

**RESEARCH DESIGN AND METHODS**

**Human subjects and treatment protocol**

**Protocol 1.** Subjects were recruited from diabetes clinics or by advertisement. The subjects were classified as diabetic or non-diabetic by their response to a 75-g oral glucose tolerance test according to American Diabetes Association (ADA) criteria and the absence or presence (BMI ≥30) of obesity. Insulin action was determined with a 3-h hyperinsulinemic (300 mU·m²·min⁻¹) euglycemic (5.0–5.5 mmo/l) clamp; the glucose disposal rate (GDR) was measured during the last 30 min of the clamp (18). Glucose and insulin (10 min) levels were determined by standard techniques. Adiponectin levels were determined by using a commercially available radioimmunossay kit (Linco, St. Louis, MO). Characteristics of the subject groups are summarized in Table 1. Both experimental protocols were approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from all subjects after explanation of the protocol.

**Protocol 2.** Twenty-two male and female subjects with type 2 diabetes (between the ages of 30 and 70 years) who were poorly controlled (HbA₁c >8.5% and fasting plasma glucose >140 mg/dl) on at least half-maximal doses of any sulfonylurea agents were recruited. Except for diabetes, the subjects were healthy and on no other medications known to influence glucose metabolism. After screening, their existing sulfonylurea medication was discontinued and all subjects were uniformly started on glyburide 10 mg b.i.d. for at least 4 weeks. Baseline studies, including biopsies, were then performed and subjects were randomized to either the troglitazone or metformin treatment group. Treatment involved troglitazone titration up to 600 mg per day or metformin to 2,550 mg per day over 4–6 weeks as required to achieve glycemic goals. Following 3–4 months of troglitazone or metformin treatment, subjects were reevaluated for repeat studies. Subjects were counseled to consume a fixed calorie diet for the duration of the study protocol. Data resulting from this protocol has been presented in a series of earlier publications (20–22). Characteristics of the subjects studied in the current report and the effect of the treatments are summarized in Table 2.

**Materials.** Human biosynthetic insulin was kindly supplied by Eli Lilly (Indianapolis, IN). Collagenase was purchased from Worthington (Freehold, NJ). Bovine serum albumin (BSA, Colan fraction V) was obtained from Roche Diagnostic (Indianapolis, IN). A monoclonal antibody against human adiponectin was obtained from BD Bioscience (San Diego, CA). Horseradish peroxidase–conjugated anti-mouse IgG was from Amersham (Arlington Heights, IL), while SuperSignal enhanced chemiluminescence substrate was obtained from Pierce (Rockford, IL). Electrophoresis reagents were purchased from BioRad (Richmond, CA). All other chemicals were reagent grade and purchased from Sigma Chemical (St. Louis, MO).

**Adipose tissue biopsy and preparation of human adipocytes.** Adipose tissue was obtained by needle biopsy of the lower subcutaneous abdominal depot using a 5-mm side-cutting needle. One percent lidocaine was infiltrated in a square field fashion and the biopsy was taken from the center of the field. Isolated adipocytes were prepared by a modification (23) of the method of Rodbell (24). After digestion and filtration, the cells were washed twice in a buffer consisting of 150 mmo/l NaCl, 5 mmo/l KC1, 1.2 mmo/l MgSO₄, 1.2 mmo/l CaCl₂, 2.5 mmo/l NaHPO₄, 10 mmo/l HEPES, 2 mmo/l pyruvate, pH 7.4, and supplemented with 4% BSA. The cells were then divided and the major portion of the cells were washed twice in a buffer (HEPES washing salts [HWS]) consisting of 116 mmo/l NaCl, 5 mmo/l KC1, 0.5 mmo/l MgSO₄, 0.7 mmo/l CaCl₂, 25 mmo/l HEPES, 5 mmo/l glucose, and 2% BSA, pH 7.4, and resuspended at ~1–5 × 10⁶ cells/ml before cell extraction.

**Adipocyte treatment and extraction**. Adipocytes in HEPES washing salts [HWS] were incubated for 50 min at 37°C with gentle shaking. Cells were treated with or without insulin for 15 min at 37°C and then concentrated by centrifugation (50g) and the buffer collected for subsequent assay of adiponectin release. The cells were then rapidly washed twice in 17°C, insulin-free, BSA-free HWS buffer as described previously (26). A twice-concentrated solubilization buffer was then added (final concentrations: 20 mmo/l Tris-HCl, 145 mmo/l NaCl, 10% glycerol, 5 mmo/l EDTA, 1% Triton X-100, 0.5% NP-40, 200 mmo/l sodium orthovanadate, 200 mmo/l PMSF, 1 mmo/l leupeptin, and 10 mmo/l aprotinin, pH 7.5). After lysis/extraction for 30 min at 4°C with repeated vortexing, nonsolubilized material was removed by centrifugation at 14,000g (10 min, 4°C), and the total cell extracts were stored at −70°C before analysis.

**Electrophoresis and Western blotting**. Procedures for the electrophoresis, transfer, and Western blotting of proteins are similar to standard methods (27). Samples were prepared in Laemmli’s sample buffer in the absence or presence of β-mercaptoethanol. Detection was by enhanced chemiluminescence, followed by densitometric analysis. Quantitation of the blots was

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>LND</th>
<th>OND</th>
<th>LT2D</th>
<th>OT2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (F/M)</td>
<td>10/5</td>
<td>9/5</td>
<td>6/3</td>
<td>11/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43 ± 3</td>
<td>46 ± 3†</td>
<td>61 ± 5*</td>
<td>50 ± 3†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 1.3</td>
<td>35.5 ± 1.6*†</td>
<td>22.8 ± 1.6</td>
<td>38.0 ± 2.5*†</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.8 ± 0.2</td>
<td>5.2 ± 0.1*</td>
<td>7.2 ± 0.9*§</td>
<td>7.5 ± 0.6*§</td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
<td>82 ± 18</td>
<td>95 ± 10</td>
<td>151 ± 39*§</td>
<td>196 ± 28*§</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.3 ± 0.1</td>
<td>5.7 ± 0.2*†</td>
<td>7.5 ± 0.4*</td>
<td>7.6 ± 0.4*</td>
</tr>
<tr>
<td>GDR (mg·kg⁻¹·min⁻¹)</td>
<td>10.56 ± 0.96</td>
<td>7.90 ± 0.48*</td>
<td>ND</td>
<td>5.02 ± 0.78*§</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>101 ± 36</td>
<td>163 ± 34</td>
<td>163 ± 34</td>
<td>245 ± 30*</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.40 ± 0.07</td>
<td>0.53 ± 0.09</td>
<td>ND</td>
<td>0.65 ± 0.08§</td>
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<tr>
<td>HDL (F/M)</td>
<td>51 ± 5 (55 ± 9/51 ± 7)</td>
<td>49 ± 4 (53 ± 6/44 ± 2)</td>
<td>50 ± 9 (52 ± 6/66 ± 17)</td>
<td>40 ± 2* (42 ± 3/38 ± 2)</td>
</tr>
</tbody>
</table>

Data are means ± SE, *P < 0.05 vs LND; †P < 0.05 vs LT2D; §P < 0.05 vs OND. TG, triglycerides.

**Table 2**

Effect of antidiabetic therapy on clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>Troglitazone (n = 9)</th>
<th>Metformin (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change from baseline (%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.3 ± 2.2</td>
<td>4 ± 1*†</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>11.6 ± 0.8</td>
<td>−38 ± 7*</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>253 ± 46</td>
<td>−18 ± 9</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>8.6 ± 0.4</td>
<td>−19 ± 4*</td>
</tr>
<tr>
<td>GDR (mg·kg⁻¹·min⁻¹)</td>
<td>5.21 ± 0.71</td>
<td>44 ± 22*†</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>204 ± 19</td>
<td>−25 ± 9</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.58 ± 0.08</td>
<td>−11 ± 11</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>32 ± 3</td>
<td>11 ± 5*†</td>
</tr>
</tbody>
</table>

Data are means ± SE, *P < 0.05 vs. baseline; †P < 0.05 vs. metformin. TG, triglycerides.
performed using ScanAnalysis software (Biosoft, Cambridge, U.K.). An internal standard, purified plasma provided by the antibody supplier, was included in each gel to permit for correction of variability between blots.

**Quantitative assay for adiponectin.** Circulating adiponectin was measured using a commercially available radioimmunoassay kit (Linco, St. Louis, MO). According to the manufacturer’s instructions, plasma or serum samples were diluted before assay. Conditioned media from adipocytes was concentrated twofold by centrifugation (molecular weight [MW] cutoff 10,000) before assay. All samples were assayed in duplicate. The lower limit of detection with this assay was 2 μg/ml; the inter- and intra-assay coefficients of variation were 7% and 11%, respectively.

**Statistical analysis.** Statistical analysis was performed using the GraphPad Prism program (Intuitive Software, San Diego, CA). Statistical significance was evaluated with Student’s $t$ test and repeated-measures ANOVA. Paired analysis was used to determine the effect of treatment in the same subject. Data are presented as mean ± SE. Significance was accepted at the $P < 0.05$ level. Due to limitations in sample availability, not all analyses were performed in every subject. The number of subjects studied is given in the legend for each figure.

**RESULTS**

**Plasma adiponectin levels in diabetic and nondiabetic lean and obese subjects.** The influence of type 2 diabetes and obesity on circulating adiponectin levels was evaluated in subjects stratified on the basis of BMI into one of four groups (Table 1): lean (BMI <25 kg/m²) nondiabetic (LND), obese (BMI >30) nondiabetic (OND), lean type 2 diabetic (LT2D), and obese type 2 diabetic (OT2). Plasma adiponectin levels were highest in LND subjects (9.06 ± 1.77 μg/ml) and similar to values reported by other investigators (5,6,8,28) (Fig. 1). While there was a tendency for levels to be lower in the OND and LT2D groups, the differences did not reach significance. Other investigators, studying larger numbers of subjects, did find adiponectin levels to be significantly reduced in these groups as well (5,6,8). However, circulating levels of adiponectin were significantly reduced in OT2 subjects as compared with the other groups ($P < 0.025$ vs. all three groups).

**Regulation of serum adiponectin levels in metformin- and troglitazone-treated diabetic subjects.** We have recently reported on the comparison of responses to troglitazone and metformin in subjects with type 2 diabetes who were failing control on glyburide alone (20–22). The important observations were that, while glycemic control was matched between the two treatment groups, there were differences in the changes in circulating lipid profile and insulin action (Table 2); the increase in GDR after troglitazone treatment was double that seen with metformin therapy (20). The troglitazone-treated group also displayed increases in weight and circulating leptin levels (22). Serum adiponectin levels were determined in the fasting state at baseline and at study completion in both the metformin and troglitazone study subjects. Sample availability dictated that measurements be performed in serum. Control studies on the subjects described in Table 1 indicated comparability of values determined in plasma and serum from the same individuals (data not shown). Baseline circulating adiponectin levels did not differ significantly between the groups (2.46 ± 0.45 vs. 4.10 ± 0.81 μg/ml for troglitazone and metformin groups, respectively). After 3–4 months of therapy, there was a significant increase in the average serum adiponectin level only in the troglitazone-treated subjects (to 7.24 ± 1.65, $P < 0.005$); the average value in the metformin-treated subjects was unchanged (3.44 ± 0.45, NS). Increases in serum adiponectin levels were recorded in all troglitazone-treated patients over the study period, whereas the majority of metformin-treated subjects had no change or a decrease in serum adiponectin level (Fig. 2).

The magnitude of the change in serum adiponectin level was positively correlated ($r = 0.767$, $P < 0.025$) with the improvement in whole-body insulin action (GDR) seen after troglitazone treatment (Fig. 3). No such relationship was seen after metformin treatment ($r = 0.153$, $P = 0.65$), dissociating changes in glycemic control from regulation of adiponectin levels. Further support for a relationship between circulating adiponectin levels and insulin action following troglitazone treatment were the significant correlations between relative changes in adiponectin levels and either fasting insulin ($r = −0.742$, $P < 0.025$) or fasting glucose ($r = −0.670$, $P < 0.05$). While both adiponectin and HDL levels increased with troglitazone treatment (20 and Table 2), there was no significant correlation between the relative (%) extent of either change.

**Treatment effects on adipocyte adiponectin content and release.** The contribution of adipocytes to the change, or lack of, in serum adiponectin levels following treatment was evaluated. Subcutaneous abdominal adi-
pose tissue biopsy specimens were obtained at baseline and at completion of the study. Fat cells were isolated and adiponectin content was determined. At baseline, the adiponectin content of adipocytes isolated from metformin- or troglitazone-treated subjects were similar (Fig. 4B). After 3–4 months of treatment there was a significant increase in adipocyte adiponectin protein content in the troglitazone-treated subjects (Fig. 4, \( P < 0.05 \)) but no detectable change in adipocyte adiponectin protein isolated from metformin-treated subjects.

Conditioned media was collected from adipocytes following a 65-min incubation. Adipocytes isolated from troglitazone-treated subjects showed increased release of adiponectin (Fig. 5, \( P < 0.05 \) vs. pretreatment) as compared with unchanged release observed from adipocytes isolated from metformin-treated subjects. In six of seven troglitazone-treated subjects fat cell adiponectin release increased from baseline values, whereas in five of seven metformin-treated subjects release decreased from baseline values. These findings remain unchanged if adiponectin release is adjusted for cell number. In troglitazone-treated subjects, release is increased with treatment (3.68 \( \mu g/ml/10^5 \) cells baseline vs. 8.36 \( \mu g/ml/10^5 \) cells posttreatment), and release is decreased in adipocytes from metformin-treated subjects (5.19 \( \mu g/ml/10^5 \) cells baseline vs. 2.77 \( \mu g/ml/10^5 \) cells posttreatment).

**Processing of adiponectin.** Adiponectin has identified in adipocytes as an \( \sim 32 \)-kDa protein (2,4,28) or the 28-kDa globular domain, while multimeric forms have been detected both intracellularly and in the circulation (28,29). To evaluate potential sites of oligomerization of the protein, the electrophoretic mobility of immunoreactive adiponectin was monitored. When electrophoresis was performed under reducing conditions, the presence of the \( \sim 32 \)-kDa band as the predominant form of adiponectin in the adipocyte was confirmed (Fig. 6A). Larger forms were seen only with prolonged exposure of the membranes or in those subjects with the highest level of expression and, even then, never represented \( >10\% \) of the total immunoreactive protein (not shown). The reverse was true in plasma, where larger forms of \( \sim 75–80 \) kDa were most abundant (\( \sim 90\% \) of total immunoreactivity). A similar pattern, predominantly larger MW forms, was observed in skeletal muscle tissue (Fig. 6A). The absence of adiponectin protein in cultured human skeletal muscle cells suggests that other tissues contribute the protein detected in the muscle biopsy material. The presence of the 32-kDa form in muscle tissue could be accounted for by an adipose tissue content of muscle representing \( \sim 5\% \) of total tissue protein. However, adipose tissue contamination cannot account for the amount of the larger MW forms; they may be provided by blood or be present in or on muscle cells.
The tissue distribution of oligomeric forms of adiponectin was investigated in the native state. Under nonreducing conditions, almost all of the adiponectin in adipocytes is present as complexes consistent with trimers (~75 kDa), hexamers, and higher MW forms; a similar pattern was observed in skeletal muscle (Fig. 6B). Monomeric adiponectin was detectable in adipocytes only with prolonged exposure. The predominant form secreted from the adipocyte is the trimer; other size bands were not readily detected. A different pattern was observed in plasma; the predominant forms were mainly trimers and higher MW forms, with fewer hexamers.

**DISCUSSION**

Beyond its importance as a major site for glucose and lipid metabolism, as well as a site for insulin resistance, adipose tissue has also recently been shown to play a critical role in tissue-tissue communication. A number of factors have been identified as originating from adipose tissue (30) that can influence glucose and lipid metabolism as well as vascular function. These factors include leptin, TNF-α, IL-6, PAI-1, adipsin, FFAs, and adiponectin. Adiponectin, also known as GBP28 or apM1 in humans and acrp30 or adipoQ in rodents, is a highly abundant secreted protein that has been associated with insulin sensitivity, as circulating levels are reduced in a number of insulin-resistant conditions in humans and animals, including obesity (3,5), type 2 diabetes (6–8,31), and cardiovascular disease (32). Our current results are in qualitative and quantitative agreement with these reports, with the most insulin-resistant, obese type 2 diabetic subjects having the lowest adiponectin levels (Fig. 1). Meanwhile, improvements in insulin action with weight loss (9) and thiazolidinedione treatment (33) are accompanied by increases in circulating adiponectin levels. More direct evidence for a role of adiponectin in control of metabolism is provided by studies where adiponectin supplementation, either in vivo (34,35) or in vitro (34,36), results in improved glucose tolerance, along with augmented fatty acid metabolism.

The factors responsible for regulation of adiponectin expression and the relationship between adiponectin and insulin action are still unclear. To address these questions, we investigated obese type 2 diabetic subjects who had been treated with combination sulfonylurea and biguanide or thiazolidinedione therapy. As previously reported (20), the extent of glycemic control was matched in these two groups; changes in glucose, insulin, and FFA levels were similar, simplifying comparisons. Important differences between the two groups were that the troglitazone-treated subjects gained weight, had elevated HDL levels, and showed a greater increase in insulin action (20,22, Table 2). A further difference between these treatment regimens, revealed by the current results, is that troglitazone resulted in increases in adiponectin levels, while metformin had no effect. Circulating adiponectin levels have previously been shown to be inversely related to both obesity and fasting insulin levels. Troglitazone treatment results in a disruption of those associations, as it is possible to both gain weight and increase circulating adiponectin levels. Conversely, it is possible to lower insulin levels yet have no effect on adiponectin levels, as is the case with metformin treatment. Thus, the long-term control of adiponectin levels can, under certain circumstances, be made independent of obesity and insulin. The most consistent predictor of adiponectin levels is insulin sensitivity (8) and our current results support that finding, as adiponectin levels are increased only in the presence of a larger increase in whole-body insulin action, following troglitazone. Several laboratories have reported that thiazolidinedione treatment results in elevated circulating adiponectin levels in human subjects. This result was observed with rosiglitazone (11) and pioglitazone (33), as well as troglitazone (10,12), indicating that this is a class effect of these agents. In one study (12), the change in circulating adiponectin levels was not correlated with improvements in GDR, unlike the current results (Fig. 3). That study included both normally insulin-sensitive individuals and type 2 diabetic subjects who failed to respond to troglitazone treatment, suggesting that the relationship between changes in adiponectin levels and insulin action may be apparent only in the most insulin-resistant subjects where there is a therapeutic effect. Consistent with this reasoning, the more modest improvement in GDR with metformin may fall below a threshold needed to trigger increases in adiponectin expression or may possibly act through a different mechanism.

Adiponectin levels have also been shown to be reduced, independent of obesity and diabetes, in individuals with CVD (32). In addition, adiponectin has been reported to have antiatherogenic effects, reducing stenosis either directly with effects on macrophage function (37) or indi-
rectly through control of circulating lipids. Our results are consistent with this behavior, as the same treatment that elevated adiponectin levels, also improved the circulating lipid profile, including increases in HDL (Table 2).

Elevations in adiponectin levels could arise from alterations in synthesis, secretion, and/or clearance. The current results indicate both the adipocyte content (Fig. 4) and release (Fig. 5) of adiponectin are increased with troglitazone treatment. While adiponectin is produced solely in adipocytes (30), the inverse correlation between adiponectin levels and obesity (3,5,8) gives rise to the question of what is the signal that suppresses adiponectin with increasing adiposity? Troglitazone treatment appears to be able to overcome this inhibitory signal. Adiponectin content has been shown to vary between adipose tissue depots (31); the current results are specific only for subcutaneous adipose tissue. Evidence has accumulated that weight gain during TZD therapy is accompanied by a redistribution of adipose tissue from visceral to subcutaneous depots (33,38,39). It is possible that there are also adipose tissue depot-specific responses to TZD treatment, so that the greater relative subcutaneous adipose tissue mass would produce and release more adiponectin. We have shown previously, in these same subjects, that troglitazone treatment has multiple impacts on subcutaneous adipocytes, including improvement in insulin-stimulated glucose transport and insulin signaling (22). Adiponectin synthesis and secretion would be another adipocyte function that is upregulated by TZDs. Thus TZDs, possibly acting through peroxisome proliferator-activated receptor-γ (PPAR-γ) (40), could restore normal functionality of subcutaneous adipose tissue. This would be in addition to demonstrated actions to drive adipocyte differentiation (41,42). However, there is conflicting data about the importance of PPAR-γ in control of apM-1 gene transcription (43–45). The failure of metformin treatment to alter adiponectin expression further suggests that it is not changes in circulating glucose or insulin levels, but rather direct actions on the adipocyte that are driving the regulation of adiponectin, as we found that metformin treatment also did not effect glucose transport or insulin signaling in adipocytes (22).

As with any secreted protein, there are multiple sites and or processes where adiponectin expression and function could be regulated. Beyond gene transcription and protein synthesis, the most extensively studied events to date are other processes that include posttranslational modification, secretion, and oligomerization. Adiponectin is glycosylated on four lysine residues in the collagenous domain (46). This glycosylation, which occurs in mammalian but not bacterial cells, appears to be necessary for full potency of adiponectin to inhibit gluconeogenesis (46). The presence of an O-linked disialic acid residue has also been identified in adiponectin (47), although the functional importance of this modification is unknown. The regulation of adiponectin glycosylation, especially in insulin-resistant states, is currently unknown.

Adiponectin is primarily present, both within cells and in the circulation, in multimeric forms: trimers, hexamers, and high–MW oligomers (2,4,28). The adiponectin monomer is not detected in cultured cells (28). A similar behavior was observed in human subcutaneous adipocytes; although monomeric (~30 kDa) adiponectin was detected, the predominant species was the trimer. It appears that the major form secreted from the adipocyte is also the trimer. The relative distribution of adiponectin between trimers, hexamers, and larger forms appears to differ between adipose tissue and the circulation (Fig. 6). The oligomerization state of adiponectin may be another critical variable since emerging evidence suggests that different forms may mediate different activities; the globular domain being important for stimulation of fatty acid oxidation in muscle (34), while hexamers or larger molecular weight forms activate nuclear factor-κB signaling (28). Thus, the processes by which adiponectin multimers are interconverted may have physiologic significance. Initial studies did not reveal any obvious effect of troglitazone treatment on the oligomerization state of adiponectin in adipocytes, as secreted from adipocytes, and in skeletal muscle tissue. These added levels of complexity, glycosylation, and oligomerization with variable effects on activity, indicate that measures of adiponectin expression alone provide an incomplete picture of the role and regulation of adiponectin in insulin-resistant states and by insulin-sensitizing therapies.

Several mechanisms have been proposed for the impact of adiponectin on glucose disposal and insulin action. In rodents, acute adiponectin injection reduces hepatic glucose output (HGO), with no impact on peripheral glucose uptake (35). Studies in isolated hepatocytes suggest that adiponectin works as an insulin sensitizer for suppression of hepatic glucose output, as the hormone has no direct effect on gluconeogenesis (36). This effect would differ from that attributed to metformin, which suppresses basal gluconeogenesis (16). We found fasting glucose levels, primarily determined by HGO, to be reduced to the same extent by troglitazone and metformin, which would be consistent with a lack of involvement of adiponectin with fasting HGO. In vivo adiponectin administration reduced triglyceride content in muscle and liver while upregulating the expression of genes involved in fatty acid oxidation (34,35). The globular domain of adiponectin was shown, both in vivo and in vitro, to increase fatty acid oxidation in skeletal muscle (34). Attention recently has focused on the strong relationship between intramuscular lipid content and insulin resistance (48,49). Action of adiponectin to increase fatty acid oxidation in skeletal muscle could contribute to a lowering of circulating fatty acid levels with an even more profound effect in muscle to improve insulin action by reducing intramyocellular lipid content. Indeed, we have reported that these same subjects who displayed an increase in adiponectin levels in response to troglitazone treatment also improved insulin signaling in skeletal muscle through the phosphatidylinositol 3-kinase/ protein kinase B pathway (21), as well as reduced intramyocellular lipid content.

Comparison of results between the troglitazone and metformin treatment groups indicates that the upregulation of adiponectin expression is not the result of improving glucotoxicity or reducing hyperinsulinemia. What is uncertain is whether elevation of adiponectin levels is either the cause or the consequence of the greater improvement in insulin action on whole-body insulin action following troglitazone treatment. While the current results
do not establish causation, they suggest that treatments that target adiponectin expression and processing in the adipocyte may have added benefit as anti-diabetic therapies, improving both tissue-specific metabolism and communication between tissues.

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