OBJECTIVE — Adiponectin, a protein specifically synthesized and secreted by adipose tissue, has recently been shown to have anti-inflammatory, antiatherogenic properties in vitro and beneficial metabolic effects in animals. Lower plasma levels of adiponectin have been documented in human subjects with metabolic syndrome and coronary artery disease. We investigated whether the level of this putative protective adipocytokine could be increased by treatment with a peroxisome proliferator–activated receptor-γ (PPAR-γ) agonist in diabetic patients.

RESEARCH DESIGN AND METHODS — Type 2 diabetic patients (30 in the treatment group and 34 in the placebo group) were recruited for a randomized double-blind placebo-controlled trial for 6 months with the PPAR-γ agonist rosiglitazone. Blood samples were collected and metabolic variables and adiponectin levels were determined in all patients before initiation of the study.

RESULTS — In the rosiglitazone group, mean plasma adiponectin level was increased by more than twofold \( (P < 0.0005) \), whereas no change was observed in the placebo group. Multivariate linear regression analysis showed that whether rosiglitazone was used was the single variable significantly related to the changes of plasma adiponectin. The amount of variance in changes of plasma adiponectin level explained by the treatment was \( \sim 24\% \) \( (r^2 = 0.24) \) after adjusting for age, sex, and changes in fasting plasma glucose, HbA\(_1c\), insulin resistance index, and BMI.

CONCLUSIONS — Rosiglitazone increases plasma adiponectin levels in type 2 diabetic subjects. Whether this may contribute to the antihyperglycemic and putative antiatherogenic benefits of PPAR-γ agonists in type 2 diabetic patients warrants further investigation.

Diabetes Care 25:376–380, 2002

A diponectin is an adipose tissue-specific plasma glycoprotein with various homology to collagen, complement proteins, and hibernation-associated proteins (1–4). The human adiponectin (also called apM1 or GBP-28) gene and its mouse homolog (adipoQ or ACRP30) have been identified (1–4). This protein is strictly expressed in adipose tissue and secreted by adipocytes and adipose tissue–specific anti-inflammatory and antiatherogenic molecule and may be a bridge between obesity and atherosclerosis. Furthermore, adiponectin is detectable only in the walls of catheter-injured vessels but not in the intact vascular walls in an animal model and in humans (8,9).

Lower plasma levels of adiponectin have been documented in human subjects with obesity, type 2 diabetes, or coronary artery disease (5,10,11). Plasma levels of adiponectin were shown to correlate negatively with plasma glucose, insulin, triglyceride levels, and BMI but positively with plasma levels of HDL cholesterol (11). Plasma adiponectin levels were also demonstrated to correlate positively with insulin-stimulated glucose disposal measured by hyperinsulinemic-euglycemic clamp (12). Taken together,
Table 1—Baseline characteristics of 30 patients treated with rosiglitazone and 34 patients treated with placebo

<table>
<thead>
<tr>
<th></th>
<th>Rosiglitazone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.9 ± 9.4</td>
<td>57.8 ± 8.9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/17</td>
<td>13/21</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>64.9 ± 11.8</td>
<td>65.3 ± 11.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.76 ± 2.87</td>
<td>25.84 ± 3.50</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.8 ± 13.8</td>
<td>134.8 ± 18.8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.5 ± 10.5</td>
<td>80.2 ± 10.0</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>10.7 ± 2.7</td>
<td>11.0 ± 2.9</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>69.6 ± 33.3</td>
<td>75.7 ± 45.2</td>
</tr>
<tr>
<td>Insulin resistance (by HOMA)</td>
<td>1.08 ± 0.61</td>
<td>1.16 ± 0.63</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>9.5 ± 1.1</td>
<td>9.7 ± 1.4</td>
</tr>
</tbody>
</table>

Data are means ± SD.

these suggest that low plasma adiponectin according to levels may be a novel biomarker of insulin resistance syndrome.

Recent animal studies indicate that adiponectin has a wide array of metabolic effects. Injection of recombinant adiponectin in experimental animals was shown to reduce plasma fatty acid and glucose levels, increase fatty acid β-oxidation and decrease triglyceride content in skeletal muscles, improve insulin sensitivity, and reduce body weight (13–15). These studies further support that a low plasma adiponectin level may play a role in the pathophysiology of type 2 diabetes.

The expression of adiponectin is developmentally regulated and is activated by day 4 during adipocyte differentiation in cultured 3T3-L1 cells (1). Furthermore, we have tested whether proliferator-activated receptor-γ (PPAR-γ), the master regulator of adipocyte differentiation, might upregulate its expression in fully differentiated 3T3-L1 adipocytes. We showed that the steady-state mRNA of adiponectin in differentiated 3T3-L1 adipocytes was increased by administration of rosiglitazone for 24 h (16). Rosiglitazone is a synthetic PPAR-γ agonist and is now widely used to treat patients with type 2 diabetes through enhancing insulin sensitivity (17). Therefore, we asked in this study whether plasma adiponectin levels might increase in type 2 diabetes patients treated with rosiglitazone.

**Research Design and Methods** — In a phase IIib, double-blind, placebo-controlled, parallel-group comparative study of rosiglitazone (BRL 49653C) and concurrent sulfonylurea therapy, patients who met the inclusion criteria (aged 30–80 years, type 2 diabetic according to diagnostic criteria of the World Health Organization, fasting plasma glucose 7–13 mmol/l, and HbA₁c >7.5%) and exclusion criteria (other severe medical problems and severe microvascular complications requiring immediate medical attention) and who had been stable on sulfonylurea therapy for at least 2 months before the screening visit were recruited for the study. During the screening visit, patients entered a single-blind placebo/sulfonylurea run-in period for 4 weeks to establish baseline characteristics. Patients were then randomly double-blind phase. Each patient received two tablets of either rosiglitazone (Avandia, 2 mg/tablet) or placebo in a dosing regimen of one tablet twice daily for 6 months. A total of eight follow-up visits were arranged in these 6 months. After evaluation of the safety and efficacy of rosiglitazone (manuscript in preparation), 30 patients in the rosiglitazone group and 34 patients in the placebo group (of 38 and 39 recruited, respectively) who were observed for 6 months were included in this follow-up study. The average age at onset of diabetes was ~50 years. The baseline characteristics of these subjects are shown in Table 1. This protocol has been approved by the Institutional Review Board and Department of Health of Taiwan. Written informed consent was obtained from each participant.

**Anthropometric and biochemical measurements**

Body weight, height, blood pressure, and heart rate were measured at the time points specified. The concentrations of plasma glucose, total cholesterol, and triglyceride were measured in fasting blood samples by an autoanalyzer (Hitachi 7250 Special; Hitachi, Tokyo, Japan). Serum insulin levels were determined by a microparticle enzyme immunoassay using the AxSYM system (Abbott Laboratories, Dainabot, Tokyo, Japan). HbA₁c was measured with DCA2000 (Bayer Sankyo, Tokyo, Japan). The homeostasis model assessment was applied to estimate the degree of insulin resistance (homeostasis model assessment [HOMA]-IR = Insulin/22.5e−[Glucose/3.5]), where insulin is expressed in μU/ml and glucose is expressed in mmol/l (18). Plasma levels of adiponectin were determined by an enzyme-linked immunosorbent assay system as described previously (10). All data except those for adiponectin were presented in SI units, because the molecular weight of adiponectin has not been precisely determined.

**Statistical analyses**

Data were presented as means and SDs. Statistical analyses, including Student’s t test and multivariate linear regression analysis, were performed using Statistical Analysis System software (Edition 6.12; SAS Institute, Cary, NC). Differences in clinical characteristics between those treated with rosiglitazone and those treated with placebo were tested by Student’s t test. Several multivariate linear regression models were performed that included age, sex, changes in fasting plasma glucose level, HbA₁c, insulin resistance index by HOMA, BMI, and treatment status as independent variables and changes in plasma adiponectin levels as dependent variables. The amount of variance explained by treatment status was indicated by the $r^2$ or partial $r^2$ in the models.

**RESULTS** — At baseline, there was no difference in general characteristics between rosiglitazone and placebo groups (Table 1). Three months after treatment, there were significant differences in mean plasma adiponectin levels, fasting plasma glucose level, and HbA₁c between the groups (data not shown). After 6 months of treatment, the subjects in the rosiglitazone group had significant weight gain as well as better glycemic control compared with those in the placebo group (Table 2).
Insulin resistance was reduced in the rosiglitazone group after 6 months. However, the change was not significant when compared with that of the placebo group.

The increase of plasma adiponectin was more than twofold in the rosiglitazone group, whereas in the placebo group, there was virtually no change of plasma adiponectin (Fig. 1). Treatment with rosiglitazone for 6 months did not further increase the plasma adiponectin level from that measured at 3 months, suggesting that the maximal effect of rosiglitazone on adiponectin levels had been attained by the 3rd month.

Using the changes in adiponectin as the dependent variable in multivariate linear regression models, we found that changes in HbA1c, BMI, and treatment status (use of rosiglitazone or placebo), but not fasting plasma glucose level and insulin resistance, were significantly related to adiponectin after adjusting age and sex (data not shown). After further adjustment of treatment status in each analysis, none of these variables remained significantly related to adiponectin (data not shown). Stepwise multivariate linear regression analyses showed that treatment status was the single significant variable related to the changes in plasma adiponectin (Table 3), although both the changes in insulin resistance and BMI were significantly related to that of plasma adiponectin before treatment status was adjusted. Without adjusting other variables, treatment status alone contributed to ~38% of variance in the changes of plasma adiponectin ($r^2 = 0.38$). After adjusting the other variables in model 5, treatment status was attributed to ~24% of variance ($r^2 = 0.24$), whereas the $r^2$ of the model was 0.44. These results suggest that the increase in plasma adiponectin level may primarily be the result of rosiglitazone treatment.

**CONCLUSIONS**—Adiponectin is an adipose tissue–derived plasma protein, the functions of which have not been clear until recent years. In vitro studies have shown antiatherogenic, anti-inflammatory, and apoptotic effects of adiponectin (5–8). However, strong evidence linking adiponectin to these biological functions in vivo is still deficient. In many human studies, low plasma levels of adiponectin were shown to be associated with insulin resistance and various clinical manifestations of metabolic syndrome (5,10–12). Whether low plasma adiponectin levels may directly contribute to accelerated atherosclerosis in patients with metabolic syndrome merits intensive investigation.

Recently, adiponectin also has been shown to have various metabolic effects. It was reported that the decrease in plasma adiponectin paralleled the development of insulin resistance and diabetes among monkeys in a longitudinal observation study (19). Plasma level of adiponectin was also related to the direct measurement of insulin sensitivity by clamp studies in humans (12). Furthermore, injection of recombinant adiponectin in experimental animals was shown to increase fatty acid oxidation in muscle, improve insulin sensitivity, and decrease hepatic glucose output (13–15). This indicates that low plasma adiponectin may contribute to the pathophysiology of insulin resistance among patients with type 2 diabetes.

The mechanism of adiponectin in improving insulin sensitivity has been elucidated, at least in part. Recombinant adiponectin injection in experimental animal models showed a decrease in plasma fatty acid levels (13,14). Fatty acid β-
oxidation in skeletal muscle increased in these animals, probably secondary to enhanced expression of genes involved in β-oxidation and energy dissipation, such as acyl-CoA oxidase and uncoupling protein-2 (14). As a result, adiponectin also decreased triglyceride content in muscle. Both the increased fatty acid levels in plasma and increased triglyceride content in muscle have been shown to cause insulin resistance (20). In addition, insulin-stimulated tyrosine phosphorylation of signaling molecules, including insulin receptor, insulin receptor substrate-1, and Akt in skeletal muscle, was also enhanced by adiponectin (14).

In view of its potential beneficial effects, any measures that could increase plasma adiponectin levels would likely have some clinical significance. We recently also showed that weight reduction with gastric partition surgery significantly increased plasma adiponectin levels and insulin sensitivity among severely obese individuals (21). Whether certain beneficial metabolic effects, such as improvements in insulin sensitivity by weight reduction or by treatment with PPAR-γ agonists, were at least partially mediated by adiponectin remains to be investigated.

Although we did not investigate the expression of adiponectin in adipose tissue in these subjects, it should be plausible to speculate that the mechanism of increased plasma adiponectin by rosiglitazone treatment was secondary to increased transcription of adiponectin gene by activating the transcription factor, PPAR-γ. We have also shown, in cultured mouse adipocytes, that rosiglitazone increased the steady-state mRNA of adiponectin (16). The proximal promoter region (1 kb) of the human adiponectin gene contains only a half-site of PPAR-γ response element (AGGTC between −610 and −605 relative to ATG start codon) (22,23). Its proximal promoter also contains several regulatory elements commonly observed in the promoters of genes expressed in adipose tissue. These cis-elements include C/EBPs, SREBP, E-box, and GATA-1 (22,23). However, it cannot be excluded that rosiglitazone may enhance the stability of adiponectin mRNA, as well as the synthesis, stability, and secretion of adiponectin protein.

In this study, we observed an increase in plasma adiponectin along with weight gain after rosiglitazone treatment. This may seem contradictory to the previously reported negative correlation between plasma adiponectin level and body weight. It is plausible that activation of PPAR-γ by rosiglitazone may, on one hand, promote body weight gain by increasing adipocyte differentiation and the number of small adipocytes as previously shown (24) and, on the other hand, enhance adiponectin gene transcription.

In conclusion, this study clearly demonstrates that treatment with rosiglitazone in type 2 diabetic patients increased plasma adiponectin levels. This effect may potentially protect diabetic patients from macrovascular complications and may improve their insulin sensitivity and glycemic control. Therefore, further studies on its clinical and biological relevance are warranted.

Acknowledgments—This study was supported, in part, by Smith-Kline Beecham Pharmaceuticals and a grant from the Department of Education (89-B-FA01-1-4) of the R.O.C.

We thank Chia-Ling Chao for technical assistance.

References
7. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S,

Table 3—Multivariate linear regression models showing intercepts and regression coefficients ± SEM, using changes of adiponectin as the dependent variable, with age, sex, changes of fasting plasma glucose, HbA1c, insulin resistance, and BMI, and treatment status as independent variables.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−7.7 ± 4.7</td>
<td>−6.3 ± 4.7</td>
<td>−6.1 ± 4.7</td>
<td>−5.7 ± 4.1</td>
<td>−8.9 ± 3.8</td>
</tr>
<tr>
<td>Age</td>
<td>0.15 ± 0.08</td>
<td>0.13 ± 0.08</td>
<td>0.12 ± 0.08</td>
<td>0.12 ± 0.07</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Sex</td>
<td>1.8 ± 1.5</td>
<td>1.7 ± 1.5</td>
<td>1.7 ± 1.5</td>
<td>1.1 ± 1.3</td>
<td>1.5 ± 1.2</td>
</tr>
<tr>
<td>Change in fasting plasma glucose</td>
<td>−0.36 ± 0.18</td>
<td>−0.18 ± 0.18</td>
<td>0.05 ± 0.31</td>
<td>0.14 ± 0.27</td>
<td>0.18 ± 0.18</td>
</tr>
<tr>
<td>Change in HbA1c</td>
<td>−1.12 ± 0.60</td>
<td>−1.22 ± 0.60*</td>
<td>−2.84 ± 1.36*</td>
<td>−2.84 ± 1.36*</td>
<td>−1.7 ± 1.26</td>
</tr>
<tr>
<td>Change in insulin resistance</td>
<td>−2.17 ± 1.53</td>
<td>−2.17 ± 1.53</td>
<td>2.72 ± 0.67†</td>
<td>3.2 ± 0.72</td>
<td>5.5 ± 1.5†</td>
</tr>
<tr>
<td>Change in BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.001.
Rosiglitazone increases plasma levels of adiponectin


