Rosiglitazone Inhibits Expression and Secretion of PEDF in Adipose Tissue and Liver of Male SD Rats Via a PPAR-γ Independent Mechanism

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Pigment epithelium-derived factor (PEDF) plays an important role in insulin resistance (IR). The study aims to investigate the effect of rosiglitazone, an insulin sensitizer, on PEDF production and release both in vivo and in vitro. Male SD rats were divided into normal control group, high-fat group, and rosiglitazone group. Hyperinsulinemic euglycemic clamp was performed to evaluate insulin sensitivity. IR models of 3T3-L1 adipocytes and HepG2 cells were established by the hyperinsulinemic method. Glucose uptake was examined to validate IR of adipocytes, and phosphorylation of protein kinase B and glycogen synthesis kinase 3 were examined to validate IR of HepG2 cells. Rosiglitazone, 2-chloro-5-nitro-N-phenylbenzamide (GW9662, an inhibitor of peroxisome proliferator-activated receptor-γ), and compound C (inhibitor of AMPK) were used for the in vitro intervention. In vivo, the high-fat group showed increased serum PEDF levels, which negatively correlated with insulin sensitivity, whereas the rosiglitazone treatment decreased the serum PEDF and down-regulated PEDF expression in fat and liver of the obese rats, concomitant with significantly enhanced insulin sensitivity. In vitro, the IR cells showed increased PEDF secretion and expression, whereas rosiglitazone lowered PEDF secretion and expression, accompanied with increased insulin sensitivity. Interestingly, combination with 2-chloro-5-nitro-N-phenylbenzamide did not influence the effect of rosiglitazone on PEDF. However, rosiglitazone stimulated AMPK phosphorylation in fat and liver of the obese rats, whereas in vitro, when combined with compound C, the effect of rosiglitazone on PEDF was abrogated. In summary, rosiglitazone inhibits the expression and secretion of PEDF in fat and liver via promoting AMPK phosphorylation rather than peroxisome proliferator-activated receptor-γ, and changes of PEDF induced by rosiglitazone are closely associated with IR improvement. (Endocrinology 155: 941–950, 2014)
AMP-activated protein kinase (AMPK), a fuel-sensing enzyme that has been implicated in the regulation of glucose and lipid homeostasis and insulin sensitivity, has been demonstrated to account for some observed effects of TZDs, implying that AMPK may be another key regulator of the beneficial metabolic effects of TZDs (7–9). In recent years, cross talk between TZDs and cytokines (eg, adiponectin, fibroblast growth factor-21) has been proved to exist and may act coordinately with these factors to promote insulin sensitization (10, 11).

Pigment epithelium-derived factor (PEDF) is a protein mainly produced by the hepatocytes and adipocytes with the property to induce IR and activate inflammatory signaling (12, 13). Serum PEDF level and adipocyte PEDF expression were elevated in obese mice and reduced upon weight loss; prolonged PEDF administration stimulated adipose tissue lipolysis, resulting in ectopic lipid deposition and reduced insulin sensitivity, whereas neutralizing PEDF with PEDF antibody in obese mice enhanced insulin sensitivity (14, 15). In the previous work, we found the serum PEDF level was elevated in women with polycystic ovary syndrome and associated with IR, indicating that PEDF might play a role in the pathogenesis of IR in patients with polycystic ovary syndrome (16).

There are no data on whether rosiglitazone could influence PEDF production and release in liver and adipose tissue or data on whether rosiglitazone could influence PEDF via PPAR-γ. The present study was undertaken to get insight into the effect of rosiglitazone on the expression of PEDF in hepatocytes and adipocytes and whether this effect is associated with the insulin-sensitizing action of this agent, which might help to clarify pharmacological action of rosiglitazone. Our data showed that 1) rosiglitazone inhibits PEDF production and release in liver and adipose tissue; 2) however, its effects on PEDF are mediated by AMPK rather than PPAR-γ; and 3) the changes of PEDF induced by rosiglitazone are closely associated with the improvement of IR.

Materials and Methods

Animals

Animal care and experimental procedures were performed with the approval from the Animal Care Committees of Chongqing Medical University. After 2 weeks of adaptive feeding, the 7-week-old male SD rats were randomly assigned to receive a normal chow diet (15% fat) (normal control group, NC group, n = 6) or a high-fat diet (45% fat) (n = 12) for 15 weeks, then the high-fat diet rats were gavaged saline (high fat diet group, HF group, n = 6) or rosiglitazone (rosiglitazone group, RSG group, n = 6, 4 mg/kg · d; rosiglitazone from Taiji Co) for 4 weeks. At the end of the 19th week, insulin sensitivity was assessed by the hyperinsulinemic-euglycemic clamp, and then the rats were culled with cervical dislocation. Blood and tissue samples were collected for further assessments.

Cell culture

Mouse embryo fibroblast 3T3-L1 cells were obtained from American Type Culture Collection and grown in DMEM containing 10% calf serum and 1% antibiotics (Life Technologies) until confluence and induced to differentiation, as described previously (17). Human hepatoma cells (HepG2 cells) (obtained from American Type Culture Collection) were grown in RPMI 1640 medium supplemented with 10% calf serum and 1% antibiotics. The glucose concentration of the RPMI 1640 medium and the DMEM was 2g/L (11.1 mmol/L) and 4.5g/L (25 mmol/L), respectively.

The IR cell models were established by the hyperinsulinemic method (18–21). Differentiated 3T3-L1 cells seeded on 96-well plates were incubated with 0nM, 0.5nM, 0.5nM, 5nM, and 50nM insulin for 24 hours, and then glucose uptake was examined to validate the establishment of the cell models. HepG2 cells were incubated with 50nM insulin for 24 hours, and the IR in HepG2 cells was validated by measurement of phosphorylation of protein kinase B (Akt) and phosphorylation of glycogen synthase kinase 3β (GSK-3β).

The IR cell models were treated with or without rosiglitazone for 24 hours, and 2-chloro-5-nitro-N-phenylbenzamide (GW9662, Sigma) or compound C (Sigma) was added 1 hour before rosiglitazone addition. Supernatants, RNA, and protein of the cells were collected for PEDF assay.

Measurement of glucose uptake

The tracer used to monitor glucose uptake in cells was 6-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-6-deoxyglucose (2-NBDG) (Sigma), with the method described previously, with some modification (22, 23). After chronic insulin exposure and rosiglitazone treatment, the cell medium was removed, and the cells were washed twice with Krebs-Ringer-HEPES (KRH) buffer (136mM NaCl, 20mM HEPES, 4.7mM KCl, 1.25mM MgSO₄, and 1.25mM CaCl₂ [pH 7.4]), and the plate was measured by a fluorescence microplate reader (SpectraMax M2 multimode plate readers; Molecular Devices) set at an excitation wavelength of 488 nm and an emission wavelength of 520 nm, and the results were expressed as Fc. After exposure to 2-NBDG (50μM) for 15 minutes, the cells were washed twice with KRH buffer and measured by the fluorescence microplate reader again, and the results were expressed as Fb. Later, 2-NBDG was added again, with or without 100nM insulin stimulation for 15 minutes, and then the cells were washed twice with KRH buffer, measured by the fluorescence microplate reader for the third time, and the results were expressed as Fc. Finally, the cell viability was measured by the fluorescence microplate reader for the fourth time, and the results were expressed as an optical density (OD). The 2-NBDG uptake was calculated as (Fc − Fb)/(Fb − Fa)/OD, and the mean (Fc − Fb)/(Fb − Fa)/OD of the 3T3-L1 cells without insulin (no insulin for a 24-h exposure and no insulin for a 15-min stimulation, ie, basal condition) was set to 100%.

PEDF and metabolite analysis

PEDF concentrations in the serum and the supernatants of 3T3-L1 and HepG2 cells were analyzed by the ELISA kits (Uscn...
Life Science, Inc) according to the manufacturer’s instruction. Plasma glucose was assessed by the hexokinase method (Roche). Serum insulin was determined by the ELISA kits (Uscn Life Science, Inc). Serum lipids were measured as follows: total cholesterol using the cholesterol oxidase-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxynaphtaldehyde sodium salt method (Wako); triglycerides using the glycerol-3-phosphate oxidase-N-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxynaphtaldehyde sodium salt method (Wako); high-density lipoprotein cholesterol using the immunoinhibition (direct) method (Wako); and low-density lipoprotein cholesterol using the selective protection enzymatic (direct) method (Wako).

Hyperinsulinemic-euglycemic clamps

Clamp was performed as described previously, with some modifications (24, 25). In brief, after rats were anesthetized with 10% chloral hydrate (0.4 mL/100g body weight), 2 polyethylene catheters were inserted into the right jugular vein and left carotid artery. Patency was maintained by 0.5% heparin (1 mL/kg body weight) administration, and the vein catheter was connected with 2 digital syringe pumps (Medtronic, Inc) by a Y connector. Blood samples of 600 μL were obtained for the measurement of serum insulin, lipids, and PEDF before the infusion of insulin. The infusion rate of insulin (Humulin regular insulin; Eli Lilly), mixed with a variable volume of 0.1% BSA, was set at (10 mU/kg min), and when the plasma glucose was lower than 5.0 mmol/L, 20% glucose infusion began, and euglycemia (5.0–6.0 mmol/L) was maintained by a variable infusion rate that was adjusted according to the serum PEDF concentration and glucose infusion (Figure 1A). In addition, a negative association was found between the serum PEDF concentration and glucose infusion rate (P < 0.05) (Figure 1B).

Real-time PCR

Total RNA was extracted using the TRIzol reagent (Life Technologies) according to the manufacturer’s instruction. The RNA purity was assessed by measuring OD at 260 and 280 nm with the standard of A260/A280 ≥ 1.8. RNA integrity was assessed by agarose gel electrophoresis. To eliminate DNA contamination, RNA was treated with 1 U of deoxyribonuclease I (Life Technologies) for 15 minutes at room temperature. cDNA synthesis was performed with 0.5-mg total RNA treated with Dnase I in a 10-μL reaction system using the First Strand synthesis for RT-PCR kit (Takara), cDNA was stored at −80°C until real-time PCR. Real-time RT-PCR was performed in the Applied Biosystems 7500 Real-Time PCR System, using SYBR Green dye (QIAGEN) according to the manufacturer’s protocol. Each quantitative reaction was performed in duplicate. All the primers were designed by Beacon Designer Software and synthesized by Life Technologies Corp. To normalize expression data, ß-actin was used as the internal control gene. Relative gene expression levels were calculated using the 2^−ΔΔct cycle threshold method.

Western blotting

Proteins were extracted from tissues and cells using cell lysis buffer for Western (Beyotime), and protein concentration was determined using the BCA Protein Assay kit (Beyotime). Sample proteins were separated by SDS-PAGE in a Bio-Rad MiniPROTEAN apparatus. Then protein extracts were transferred to a PVDF membrane. Membranes were blocked with blocking buffer and incubated with primary antibodies, rabbit polyclonal anti-PEDF, rabbit polyclonal anti-phosphorylation of AMPKα Thr172 (p-AMPK), mouse monoclonal anti-AMPKα, rabbit polyclonal anti-ß-actin (Santa Cruz Biotechnology, Inc), rabbit polyclonal anti-Akt (Ser473), rabbit monoclonal anti-Akt, rabbit monoclonal anti-GSK-3β, and rabbit monoclonal anti-p-GSK-3β (Ser9) (Cell Signaling Technology) overnight at −4°C, followed by horseradish peroxidase-labeled secondary antibodies (goat antirabbit IgG [Santa Cruz Biotechnology, Inc] and goat antimouse IgG [Cell Signaling Technology]) for 1 hour at room temperature. Immunoreactive proteins were visualized using enhanced chemiluminescence (Beyotime), and the band intensities were quantified using Quantity One software (Bio-Rad).

Statistical analysis

All statistical analyses were performed using the statistical software SPSS 13.0. Data involving more than 2 groups were assessed by one-way ANOVA (with Games Howell test [in case of heterogeneity of variance] or Tukey’s test [in case of homogeneity of variance] for post hoc analysis). Spearman correlation was used to evaluate the relationship of PEDF with insulin sensitivity. P < 0.05 (2-tailed) was considered statistically significant.

Results

Rosiglitazone alleviates IR and inhibits PEDF expression and secretion in male obese rats

Compared with the NC group, rats in the HF group showed higher levels of body weight, fasting plasma glucose, fasting serum insulin, low-density lipoprotein cholesterol, and a lower level of glucose infusion rate (Table 1).

With rosiglitazone treatment for 4 weeks, the IR of the high-fat diet-induced obese rats was obviously improved, manifested as enhanced glucose infusion rate (8.18 ± 0.53 vs 3.65 ± 0.68 μmol/kg · min, P < 0.05) (Table 1).

The obese rats showed higher levels of serum PEDF levels as compared with the NC group (2.16 ± 0.09 vs 3.65 ± 0.68 μmol/kg · min, P < 0.05) (Table 1). However, after rosiglitazone treatment, the obese rats exhibited decreased serum PEDF levels (1.77 ± 0.14 μg/mL vs 2.16 ± 0.09, P < 0.05) (Figure 1A). In addition, a negative association was found between the serum PEDF concentration and glucose infusion rate (r = −0.66, P < 0.05) (Figure 1B).

The mRNA and protein expression of PEDF in liver and epididymal adipose tissue were significantly up-regulated in the HF group as compared with the NC group. However, after rosiglitazone treatment, the obese rats exhibited significantly down-regulated PEDF expression in liver and epididymal adipose tissue (Figure 1).

Rosiglitazone inhibits the expression and secretion of PEDF in hepatocytes and adipocytes

To be in accordance with the IR condition in vivo, IR models of adipocytes and hepatocytes were established by

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the hyperinsulinemic method (18–21). In 3T3-L1 adipocytes, with low or moderate concentration of insulin (0.05nM–0.5nM) treated for 24 hours, the insulin-stimulated glucose uptake showed no significantly decrease compared with the basal condition. However, when the insulin concentration was as high as 5nM–50nM, an obvious decreased glucose uptake was observed, indicating an IR status in adipocytes (Figure 2A).

Chronic insulin exposure also decreased insulin sensitivity in HepG2 cells. Both the insulin-stimulated phosphorylation of Akt (Figure 2C) and GSK-3β (Figure 2D) were reduced in HepG2 cells incubated with 50nM insulin for 24 hours.

With rosiglitazone treatment, insulin sensitivity was improved, demonstrated as increased glucose uptake in IR adipocytes (Figure 2B) and enhanced phosphorylation of Akt (Figure 2C) as well as GSK-3β (Figure 2D) in IR hepa-

### Table 1. Effects of Rosiglitazone on Metabolic Index in Male SD Rats

<table>
<thead>
<tr>
<th></th>
<th>NC Group (n = 6)</th>
<th>HF Group (n = 6)</th>
<th>RSG Group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at baseline (g)</td>
<td>218.75 ± 16.27</td>
<td>219.60 ± 13.78</td>
<td>219.60 ± 13.78</td>
</tr>
<tr>
<td>Weight at the end (g)</td>
<td>458.50 ± 51.50</td>
<td>573.30 ± 44.57</td>
<td>593.0 ± 28.2</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.40 ± 0.27</td>
<td>6.02 ± 0.29</td>
<td>5.46 ± 0.42</td>
</tr>
<tr>
<td>Fins (mIU/L)</td>
<td>10.98 ± 7.20</td>
<td>33.07 ± 12.66</td>
<td>16.32 ± 9.62</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.26 ± 0.19</td>
<td>1.48 ± 0.15</td>
<td>1.34 ± 0.09</td>
</tr>
<tr>
<td>TGs (mmol/L)</td>
<td>0.57 ± 0.16</td>
<td>0.67 ± 0.24</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>LDL-c (mmol/L)</td>
<td>0.35 ± 0.10</td>
<td>0.48 ± 0.11</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>1.11 ± 0.20</td>
<td>0.97 ± 0.17</td>
<td>0.96 ± 0.07</td>
</tr>
<tr>
<td>GIR (μmol/kg · min)</td>
<td>8.63 ± 0.96</td>
<td>3.65 ± 0.68</td>
<td>8.18 ± 0.53</td>
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</table>

Male SD rats were fed a normal chow diet (NC group, n = 6) or a high-fat diet (n = 12) for 15 weeks, then the high-fat diet rats were gavaged saline (HF group, n = 6) or rosiglitazone (RSG group, n = 6) for 4 weeks. At the end of the 19th week, hyperinsulinemic-euglycemic clamp was performed, and blood samples were taken for analysis. Data are presented as mean ± SD and analyzed by one-way ANOVA with the Games–Howell test (in case of heterogeneity of variance) or Tukey’s test (in case of homogeneity of variance) for post hoc analysis. FPG, fasting plasma glucose; Fins, fasting serum insulin; TC, total cholesterol; TGs, triglycerides; HDL-c, high-density lipoprotein cholesterol; GIR, glucose infusion rate.

*P < 0.05, compared with NC group.

**P < 0.05, compared with HF group.

**Figure 1. Effects of rosiglitazone on the serum PEDF levels and PEDF expression in adipose tissue and liver of the obese rats. Male SD rats were fed a normal chow diet (NC group, n = 6) or a high-fat diet (n = 12) for 15 weeks, then the high-fat diet rats were gavaged saline (HF group, n = 6) or rosiglitazone (RSG group, n = 6) for 4 weeks. At the end of the 19th week, hyperinsulinemic-euglycemic clamp was performed to evaluate the insulin sensitivity, which was expressed as glucose infusion rate. A, Error bar charts showed the serum PEDF concentrations in different groups. B, Scatter plots showed a negatively correlation of serum PEDF levels and insulin sensitivity. C, Real-time PCR assay results of PEDF mRNA levels in WAT and liver. D and E, Western blot analysis of PEDF and AMPK protein expression in WAT and liver. *, P < .05, compared with NC group; #, P < .05, compared with HF group. WAT, white adipose tissue.
The effects of rosiglitazone on PPAR-γ rather than PPAR-γ

As a PPAR-γ ligand, rosiglitazone exerts its actions mainly through PPAR-γ activation. As Figure 4 showed, PPAR-γ downstream target genes, including fatty acid-binding protein 4 and lipoprotein lipase in the adipocytes and fatty acid translocase in hepatocytes, were activated after rosiglitazone treatment. When combined with GW9662, a PPAR-γ inhibitor, changes of PPAR-γ target genes induced by rosiglitazone were abrogated (Figure 4). However, combination with GW9662 did not abolish the suppressive effect of rosiglitazone on PEDF (Figure 5).

Because AMPK may be another key regulator of the beneficial metabolic effects of rosiglitazone (7–9), the phosphorylation at Thr172 of the AMPK and total AMPK protein were examined. As the Figure 1, D and E, showed, total AMPK protein of adipose tissue and liver did not change significantly in the HF group and RSG group. However, rosiglitazone treatment promoted AMPK phosphorylation in adipose tissue and liver of the obese rats, which was accompanied with profound reduction in PEDF protein expression.
In vitro, when the IR adipocytes and hepatocytes were treated with rosiglitazone, expression of p-AMPK was upregulated (Figure 6), accompanied with the profound reduction in PEDF secretion and expression, but when combined with an AMPK inhibitor compound C, the inhibitive effects of rosiglitazone on PEDF expression and secretion were significantly abrogated (Figures 5 and 6).

**Discussion**

PEDF is expressed in various tissues throughout the body, such as the adipocyte, hepatocyte, retinal pigment epithelium cell, and myocyte (13–15). It is reported as a multifunctional, pleiotropic protein with antiangiogenic, antioxidant, neurotrophic as well as both pro- and anti-inflammatory properties (12, 26). Our study has found, for the first time, that rosiglitazone inhibits the expression and secretion of PEDF both in vivo and in vitro, and its effects on PEDF are mediated by AMPK rather than PPAR-γ. In addition, the changes of PEDF induced by rosiglitazone are closely associated with the improvement of IR.

High-fat diet-induced obese rats were used in this study, and their IR was validated by the hyperinsulinemic-
euglycemic clamp. Our data revealed an increased PEDF secretion and expression in liver and fat of the obese rats. Similarly, Crowe et al (14) reported an elevated serum PEDF and expression in adipose tissue in high-fat diet-induced obese mice. In our study, a negative correlation was found between the serum PEDF levels and the insulin sensitivity, which was also confirmed in our previous findings in human (16). Recently, the association between the

![Graphs showing PEDF secretion and mRNA expression changes induced by rosiglitazone in adipocytes (A-C) and hepatocytes (D-F).](image-url)

Figure 5. Effects of compound C and GW9662 on PEDF secretion and mRNA expression changes induced by rosiglitazone in adipocytes (A-C) and hepatocytes (D-F). Insulin-resistant models of 3T3-L1 cell and HepG2 cell (established by 50nM insulin exposure for 24 h) were treated with rosiglitazone, GW9662, and compound C at indicated concentrations for 24 hours (GW9662 or compound C was added 1 h before rosiglitazone addition). Real-time PCR assay was used to analyze the PEDF mRNA expression, and PEDF concentration in the cell supernatants was determined by ELISA. RSG, rosiglitazone; GW, GW9662; CC, compound C. *, P < .05, compared with the insulin-resistant group (with 50nM insulin exposure but no rosiglitazone treatment); #, P < .05, compared with the rosiglitazone group (with both 50nM insulin exposure and rosiglitazone treatment but no GW or CC).
serum PEDF level and IR was also reported in healthy young people (27) and patients with essential hypertension (28). Furthermore, we found that after rosiglitazone treatment for 4 weeks, both the serum PEDF levels and the expression of PEDF in adipose tissue and liver of the obese rats decreased significantly, along with an IR improvement, implying that the effect of rosiglitazone on PEDF is closely associated with the insulin-sensitizing action of the agent.

In vitro, the IR models of 3T3-L1 and HepG2 cells were established by the hyperinsulinemic method. Further, glucose uptake in the 3T3-L1 cells and phosphorylation of Akt and GSK-3β in the HepG2 cells were examined to validate whether the models were successful. It is generally accepted that IR is the most common cause of hyperinsulinemia (29). However, recent studies have revealed that IR might also be a result of prolonged exposure of high concentrations of insulin (30–32). Liu et al (21) found that exposure of cultured hepatocytes to 10nM insulin for 72 hours induced significant IR, which might be associated with disturbed insulin signal transduction and oxidative stress. Another study reported that exposure to 100nM insulin for 24 hours, the 3T3-L1 cell showed impaired capacity for acute stimulation of glucose uptake by insulin (33). Similar to these studies, our data revealed that incubated with 50nM insulin for 24 hours, the adipocytes and hepatocytes were markedly insensitive to insulin. It is noteworthy that although the concentration of insulin (50nM) induced IR in vitro is much higher than in vivo levels as showed in Table 1 (the averaged levels of insulin of the HF rats was 33.07 mIU/L or 0.26nM). In fact, in vitro environment could be quite different from in vivo environment, which is systemic, full of positive and negative feedbacks, and far more complicated; therefore, wide discrepancies might exist between in vivo and in vitro. Besides, the hyperinsulinemia of the HF rat in vivo was established by several months, whereas the in vitro hyperinsulinemia was established in 24 hours; therefore, much higher concentration of insulin might be needed to induce such a pathological condition in vitro.

In accordance with the in vivo findings, insulin-resistant adipocytes and hepatocytes also showed decreased secretion and expression of PEDF after rosiglitazone treatment, accompanied with improved insulin sensitivity, demonstrated as enhanced glucose uptake in adipocytes and enhanced Akt and GSK-3β in hepatocytes. Although no studies report the effect of rosiglitazone on PEDF, Famulla et al (13) found that another insulin sensitizer troglitazone inhibited PEDF secretion in human primary adipocytes.

It is generally acknowledged that rosiglitazone functions as the ligand to PPAR-γ. Therefore, we used
GW9662, an irreversible PPAR-γ antagonist (34), to further investigate the role played by PPAR-γ in the PEDF changes induced by rosiglitazone. Fatty acid-binding protein 4, lipoprotein lipase, and fatty acid translocase are PPAR-γ downstream target genes (35–37), which were found activated after rosiglitazone treatment in our study, whereas GW9662 inhibited the activation. However, combination with GW9662 did not influence the effect of rosiglitazone on PEDF, indicating that this effect of rosiglitazone on PEDF was independent of classic PPAR-γ pathway activation. It is worth noting that although TZDs effects have been thought to be PPAR-γ-dependent, PPAR-γ-independent effects have been widely described and may coexist with the PPAR-γ-dependent effects (5, 6). AMPK, a fuel-sensing enzyme that has been implicated in the regulation of glucose and lipid homeostasis and insulin sensitivity, has been demonstrated to account for some observed effects of TZDs, implying that AMPK may be another key regulator of the beneficial metabolic effects of rosiglitazone (7–9). In this context, the phosphorylation at Thr172 of the α-subunit of AMPK was measured, and down-regulated AMPK phosphorylation was found in liver and adipose tissue of the obese rats, whereas rosiglitazone treatment ameliorated the inhibited p-AMPK. The finding was in line with previous reports, which depicted inhibited AMPK phosphorylation or activity in obese rats (38–40) and enhanced AMPK phosphorylation by pioglitazone in ob/ob mice (41). Further, we discovered that the IR adipocytes and hepatocytes induced by hyperinsulinemia showed decreased p-AMPK in vitro. Previous studies also reported that insulin could inhibit AMPK activation in liver, heart, and adipocytes, which might be associated with the lipogenic effect of insulin and antilipogenic effect of AMPK (42–44). When the adipocytes and hepatocytes were treated with rosiglitazone, enhanced p-AMPK accompanied with inhibited PEDF production was found, whereas when combined with AMPK inhibitor compound C, the suppressive effect of rosiglitazone on PEDF was abrogated. Those results suggested that rosiglitazone might exert the effects on PEDF via stimulation of AMPK phosphorylation. Additionally, our study indicates that AMPK might be a potential regulator of PEDF production, and by which mechanism AMPK might regulate PEDF production is worth further exploration. Wang et al (45) revealed that gene expression of PEDF could be negatively regulated by glucose and insulin via an mammalian target of rapamycin (mTOR)-dependent pathway. Due to a close association between AMPK and mTOR (46), there is a possibility that AMPK regulates PEDF production via mTOR pathway. However, the presumed mechanism needs further study.

In summary, our study reveals that rosiglitazone inhibits expression and secretion of PEDF in adipocytes and hepatocytes. However, this effect is not mediated by activating classic PPAR-γ pathway but via the stimulation of AMPK phosphorylation. Although we found that the effect of rosiglitazone on PEDF is closely associated with the IR-improving action of the agent, the lack of investigation into whether rosiglitazone requires PEDF for its insulin-sensitizing actions is a limitation of this study, and future study should be carried to clarify this question. Further exploration of whether rosiglitazone has such an inhibitive effect on PEDF in human needs to be carried out in the future.

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Rosiglitazone inhibits PDE5 independent of PPARγ.


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