

# PPAR $\gamma$ -Activating Angiotensin Type-1 Receptor Blockers Induce Adiponectin

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**Abstract**—The adipose-specific protein adiponectin has been recently discovered to improve insulin sensitivity. Angiotensin type-1 receptor (AT1R) blockers (ARBs) reduce the incidence of type 2 diabetes mellitus by mostly unknown molecular mechanisms. To identify new antidiabetic mechanisms of ARBs, we studied the regulation of adiponectin by angiotensin II (Ang II) and different ARBs in murine 3T3-L1 adipocytes and obese Zucker rats. Adiponectin protein expression was markedly stimulated by Ang II (5 nmol/L), which was inhibited by blockade of the AT2R, and further enhanced by the ARB irbesartan. Irbesartan-mediated adiponectin upregulation started beyond the concentrations needed for AT1R blockade and was also present in the absence of Ang II, implicating an AT1R-independent mechanism of action. Recently, certain ARBs (irbesartan, telmisartan) were identified as ligands of the peroxisome proliferator-activated receptor (PPAR) $\gamma$ . Telmisartan also stimulated adiponectin protein expression, whereas the non-PPAR $\gamma$ -activating ARB eprosartan had no effect. Blockade of PPAR $\gamma$  activation by the PPAR $\gamma$  antagonist GW9662 markedly inhibited irbesartan-induced adiponectin expression. Cognate mRNA levels of adiponectin were not affected by ARBs. Kinetic studies using the protein synthesis inhibitor cycloheximide showed that irbesartan prevented the cellular depletion of adiponectin protein. Finally, administration of irbesartan to obese Zucker rats improved insulin sensitivity and attenuated adiponectin serum depletion. The present study demonstrates that AT2R activation and certain ARBs induce adiponectin in adipocytes, which was associated with an improvement of parameters of insulin sensitivity in vivo. ARB-induced adiponectin stimulation is likely to be mediated via PPAR $\gamma$  activation involving a post-transcriptional mechanism. (*Hypertension*. 2005;46:1-7.)

**Key Words:** angiotensin antagonist ■ diabetes mellitus ■ insulin resistance ■ adipose tissue

Angiotensin II (Ang II), the main effector peptide of the Renin-angiotensin system, is implicated in the development of vascular, cardiac, and renal pathologies.<sup>1</sup> Ang II exerts its effects through 2 different receptors: angiotensin type-1 (AT1R) and type-2 (AT2R) receptor.<sup>1</sup> The AT1R is the predominant receptor in the cardiovascular system and mediates most of the deleterious effects of Ang II such as vasoconstriction, endothelial damage, and cell growth.<sup>1</sup> The AT2R is now recognized as the counter-regulator of the AT1R, exerting mostly beneficial actions like vasodilatation, anti-proliferation, and tissue regeneration.<sup>1</sup>

AT1R blockers (ARBs) are selective antagonists at the AT1R, thereby preventing the adverse Ang II-mediated effects in the cardiovascular system. Furthermore, selective inhibition of the AT1R not only inhibits these effects but also leaves the AT2R open to stimulation by Ang II, resulting in additional beneficial effects.<sup>2</sup> Clinically, ARBs are widely used in the treatment of hypertension and hypertension-related end-organ damage.<sup>2</sup> In addition to their role in the cardiovascular system, ARBs have been

recognized recently as regulators of glucose and lipid metabolism in adipocytes and adipose tissue. Clinical trials have demonstrated that AT1R antagonism substantially lowers the risk for type 2 diabetes compared with other antihypertensive therapies.<sup>3</sup> In addition, AT1R blockade improved insulin sensitivity in humans and animal models of insulin resistance.<sup>4,5</sup> The underlying mechanisms of the insulin-sensitizing/antidiabetic effect of ARBs are still widely unknown.

Adiponectin belongs to the family of adipocytokines, is exclusively synthesized by white adipocytes, and is induced during adipocyte differentiation.<sup>6</sup> Adiponectin has been shown to be a potent enhancer of insulin sensitivity.<sup>6</sup> Its administration lowers glucose levels and ameliorates insulin resistance in mice.<sup>6</sup>

To identify new insulin-sensitizing/antidiabetic effects of ARBs, we studied the regulation of adiponectin expression by ARBs and Ang II in murine 3T3-L1 adipocytes, which express AT1R and AT2R.<sup>7</sup> In parallel, the regulation of adiponectin serum levels and metabolic parameters by ARB

Received February 11, 2005; first decision March 1, 2005; revision accepted April 19, 2005.

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*Hypertension* is available at <http://www.hypertensionaha.org>

DOI: 10.1161/01.HYP.0000168046.19884.6a

treatment was examined in obese Zucker rats, a model of obesity-induced insulin-resistance.

## Methods

### Cell Culture

The 3T3-L1 preadipocytes were differentiated as previously described.<sup>8</sup> Adipocytes were serum deprived for 16 hours and then treated with vehicle or various effectors as outlined (see <http://hyper.ahajournals.org> for an expanded Methods section).

### Western Immunoblotting

After stimulation with the indicated agents, protein isolation, electrophoresis, and blotting were performed as previously described.<sup>9</sup> Blots were incubated with an anti-adiponectin mouse monoclonal antibody (1:10 000 dilution).

### Quantitative Real-Time Polymerase Chain Reaction

Real-time polymerase chain reaction was performed as previously described with an ABI 7000 sequence detection system.<sup>8</sup> Mouse 18S ribosomal RNA and rat  $\beta$ -actin were chosen as endogenous controls (housekeeping genes) (see <http://hyper.ahajournals.org>).

### Measurement of Proteasome Activity

After stimulation with the indicated compounds for 24-hour and cycloheximide incubation, activity of the proteasome was assessed in cell lysates of 3T3-L1 adipocytes by using the synthetic peptide substrate Suc-Leu-Leu-Val-Tyr linked to the fluorometric reporter aminomethyl coumarin. Lysates were incubated in incubation buffer containing an ATP regenerating system, and 0.2 mmol/L Suc-Leu-Leu-Val-Tyr aminomethyl coumarin. Aminomethyl coumarin hydrolysis was quantified in a Gemini EM microplate spectrofluorometer (Molecular Devices).

### Animals

Male obese Zucker *fa/fa* rats (8 to 9 weeks of age) received either vehicle (saline) or 50 mg/kg per day of irbesartan by gavage for 21 consecutive days. Serum samples were analyzed at day 0 and 21 for glucose, triglycerides, insulin, and adiponectin according to the manufacturer's guidelines, and the Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR index) was calculated. Tissue samples from fat were prepared from 11-week-old male obese Zucker *fa/fa* rats. Explanted epididymal fat samples were treated for 24-hour *ex vivo* with the indicated compounds, and total RNA was isolated.

### Statistical Analysis

For analysis of densitometry results, unpaired *t* tests were performed. In experiments comparing multiple ARBs, 1-way ANOVA with Bonferroni multiple comparison testing was used. In animal experiments, differences between treatment groups were evaluated by unpaired *t* test, and differences from day 0 to day 21 within 1 group were analyzed by paired *t* test. Statistical significance was designated at  $P < 0.05$ . Values are expressed as mean  $\pm$  SEM.

## Results

### Ang II Induces Adiponectin Protein Expression Via AT2R Activation

Adiponectin protein expression was significantly induced by Ang II reaching a maximum after 24 hours at 5 nmol/L ( $1.4 \pm 0.2$ -fold induction versus vehicle-treated cells;  $P < 0.05$ ) (Figure 1A). Blockade of the AT2R by the selective AT2R-antagonist PD123319 led to a dose-dependent, almost complete inhibition of Ang II-induced adiponectin expression, and AT2R stimulation with the selective agonist CGP

42112A markedly induced adiponectin expression, indicating that activation of the AT2R is required for Ang II-induced adiponectin expression (Figure 1B and 1C). The ARB irbesartan prominently enhanced Ang II-induced adiponectin protein expression at 10  $\mu$ mol/L ( $1.6 \pm 0.1$ -fold induction versus Ang II-treated cells,  $P < 0.01$ ;  $2.7 \pm 0.2$ -fold induction versus vehicle-treated cells,  $P < 0.01$ ) (Figure 1D). Irbesartan at concentrations sufficient to completely block AT1Rs (0.1 and 1  $\mu$ mol/L) had no effect on adiponectin protein expression, suggesting that the AT1R is not involved in Ang II-mediated adiponectin induction, whereas irbesartan-stimulated adiponectin expression might be independent of the AT1R (Figure 1D).

### ARB-Induced Adiponectin Protein Expression Is Mediated Via PPAR $\gamma$ Activation

To further study the mechanism of irbesartan-induced adiponectin protein expression, adipocytes were stimulated with Ang II in the presence of irbesartan and the AT2R blocker PD123319. Blockade of the AT2R by PD123319 (5  $\mu$ mol/L) did not affect irbesartan-induced adiponectin protein expression, implicating that AT2R activation is not required for irbesartan's action (Figure 2A). Irbesartan (10  $\mu$ mol/L) induced adiponectin protein expression also in the absence of Ang II, underscoring that the blockade of Ang II-mediated effects (via AT1R) is not involved in irbesartan-stimulated adiponectin expression (Figure 2B).

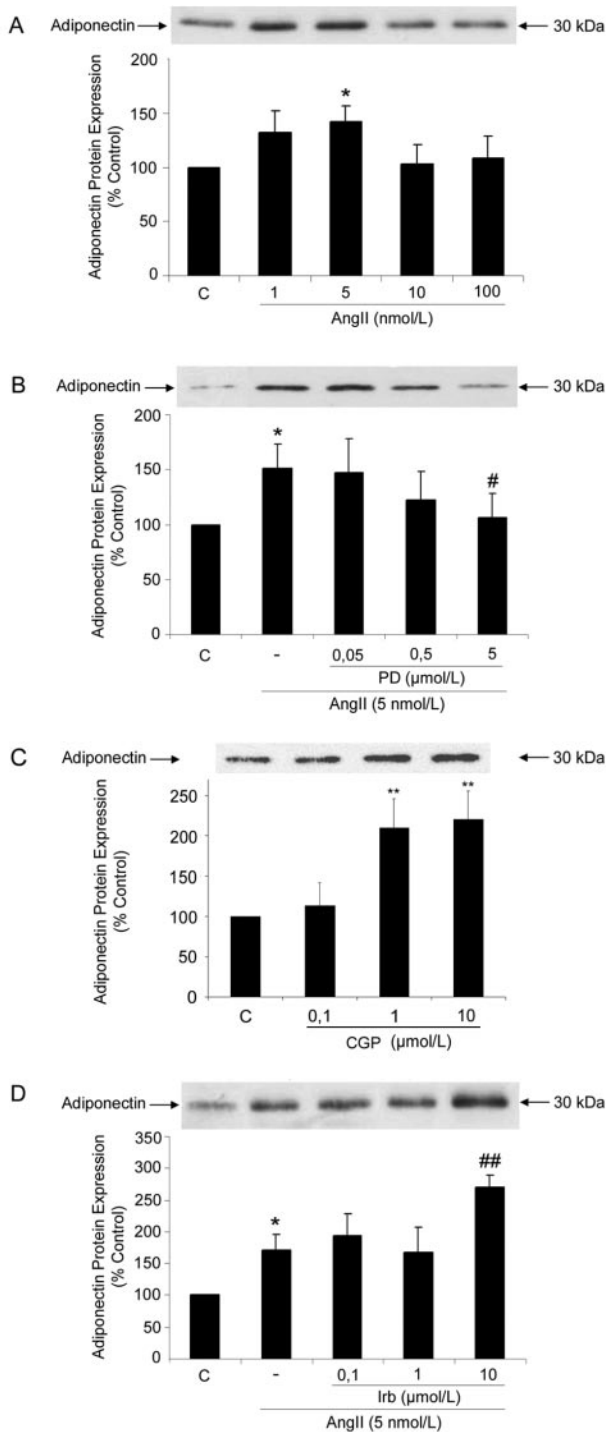
We and others have recently demonstrated that irbesartan induces the activation of the nuclear hormone receptor PPAR $\gamma$  as a partial PPAR $\gamma$  agonist independent of its AT1R blocking properties.<sup>8,10</sup> Antagonizing PPAR $\gamma$  activity with the selective antagonist GW9662 (30  $\mu$ mol/L) potently blocked irbesartan-induced adiponectin protein expression (Figure 2C). Pioglitazone (10  $\mu$ mol/L), a full PPAR $\gamma$  agonist, also stimulated adiponectin protein expression, which was inhibited by the PPAR $\gamma$  antagonist GW9662 (30  $\mu$ mol/L) (Figure 2C). The PPAR $\gamma$ -activating ARB telmisartan also markedly induced adiponectin protein expression, whereas eprosartan, a non-PPAR $\gamma$ -activating ARB, had no effect (Figure 2D). Together these data demonstrate that certain ARBs with PPAR $\gamma$ -activating properties (irbesartan, telmisartan) induce adiponectin protein expression independently of the adipocytic RAS and their AT1R-blocking properties.

### PPAR $\gamma$ -Activating ARBs Induce Adiponectin at a Post-Transcriptional Level

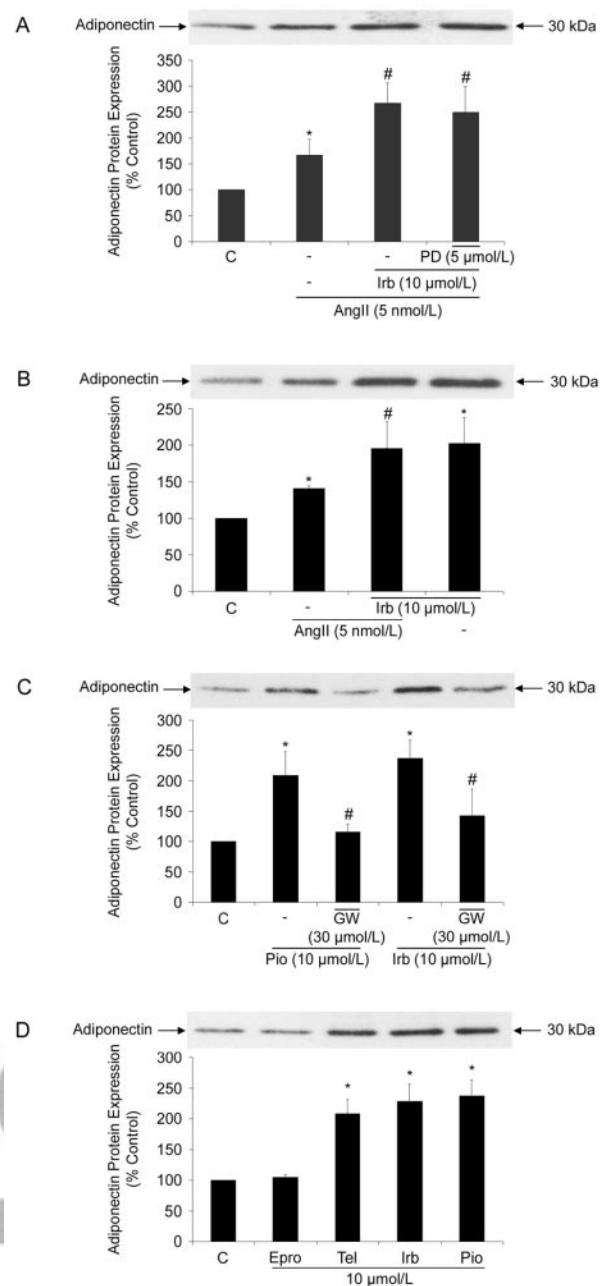
Irbesartan, telmisartan, and pioglitazone did not affect adiponectin mRNA expression in adipocytes (Figure 1).

### The PPAR $\gamma$ -Activating ARB Irbesartan Prevents Cellular Adiponectin Protein Depletion

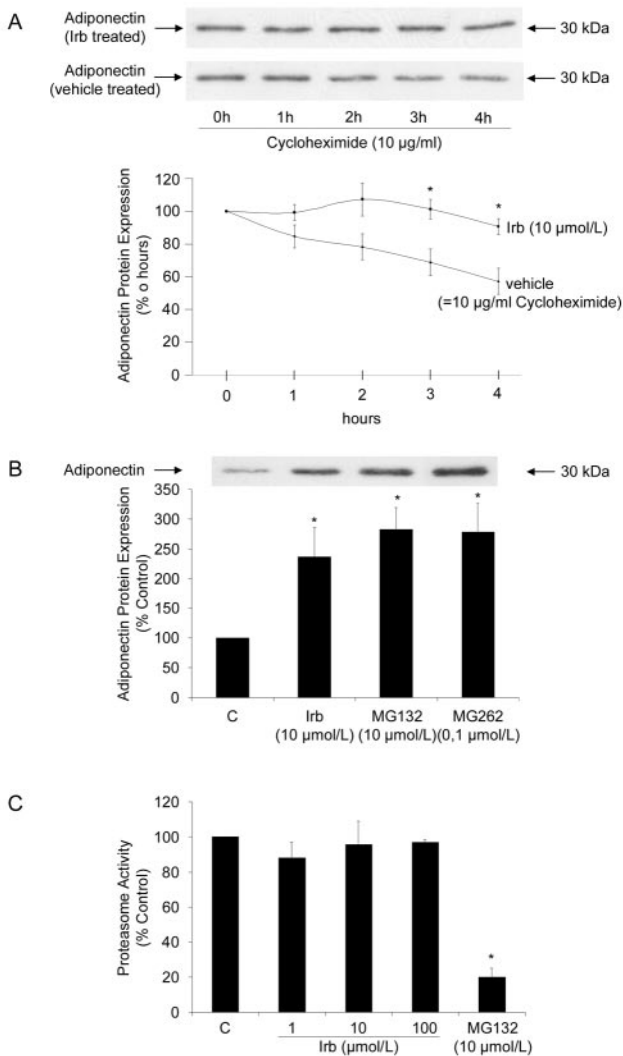
To study potential post-transcriptional mechanisms of adiponectin regulation, we examined protein expression in the presence of 10  $\mu$ g/mL cycloheximide to inhibit *de novo* protein synthesis. At the indicated time, steady-state levels of adiponectin were determined by immunoblotting (Figure 3A). In vehicle treated-adipocytes, adiponectin levels rapidly decreased during the time course of 4 hours. The half-life of adiponectin protein in 3T3-L1 adipocytes was estimated at



**Figure 1.** Ang II and the ARB irbesartan induce adiponectin protein expression. Adiponectin protein expression was measured by Western immunoblotting as described in Methods. Protein expression is shown relative to vehicle-treated controls (100%). A, Quiescent 3T3-L1 adipocytes were treated with Ang II for 24 hours. \* $P < 0.05$  vs vehicle-treated controls. B, Cells were treated with Ang II (5 nmol/L) for 24 hours. The selective AT2R antagonist PD123319 (PD) was added 30 minutes before Ang II. \* $P < 0.05$  vs vehicle-treated controls. # $P < 0.05$  vs Ang II-treated cells. C, Cells were treated with the selective AT2R agonist CGP 42112A (CGP) for 24 hours. \*\* $P < 0.01$  vs vehicle-treated controls. D, Cells were treated as described in (B). The ARB irbesartan (Irb) was added 30 minutes before Ang II. \* $P < 0.05$  vs vehicle-treated controls. ## $P < 0.01$  vs Ang II-treated cells. All experiments were repeated 3 times and densitometry results are presented as mean  $\pm$  SEM.



**Figure 2.** ARB-induced adiponectin protein expression is mediated via PPAR $\gamma$  activation. Adiponectin protein expression was measured by Western immunoblotting as described in Methods. Protein expression is shown relative to vehicle-treated controls (100%). A, Quiescent 3T3-L1 adipocytes were treated with Ang II (5 nmol/L) for 24 hours. The ARB irbesartan (Irb, 10  $\mu$ mol/L), and the selective AT2R-antagonist PD123319 (PD) (5  $\mu$ mol/L) were added 30 minutes before Ang II. \* $P < 0.05$  vs vehicle-treated controls. # $P < 0.05$  vs Ang II-treated cells. B, Cells were treated for 24 hours with/without Ang II in the presence/absence of Irb. \* $P < 0.05$  vs vehicle-treated controls. # $P < 0.05$  vs Ang II-treated cells. C, Cells were treated for 24 hours with the PPAR $\gamma$  agonist pioglitazone (Pio) (10  $\mu$ mol/L) or the ARB irbesartan (Irb) (10  $\mu$ mol/L) in the absence of Ang II. The PPAR $\gamma$  antagonist GW9662 (GW) (30  $\mu$ mol/L) was added 30 minutes before stimulation. \* $P < 0.05$  vs vehicle-treated controls. # $P < 0.05$  vs Pio/Irb-treated cells. D, Cells were treated for 24 hours with eprosartan (Epro) (10  $\mu$ mol/L), telmisartan (Tel) (10  $\mu$ mol/L), and irbesartan (Irb) (10  $\mu$ mol/L), and pioglitazone (Pio) (10  $\mu$ mol/L) in the absence of Ang II. \* $P < 0.05$  vs vehicle-treated controls. All experiments were repeated 3 times and densitometry results are presented as mean  $\pm$  SEM.



**Figure 3.** The PPAR $\gamma$ -activating ARB irbesartan prevents cellular adiponectin protein depletion. **A**, 3T3-L1 adipocytes were pre-treated with the ARB irbesartan (Irb-treated, 10  $\mu$ mol/L) or vehicle for 24 hours, followed by incubation with cycloheximide (10  $\mu$ g/mL). After the indicated time periods, adiponectin protein expression was analyzed. Representatives and densitometry results of 3 separate experiments are presented. \* $P$ <0.05 vs vehicle-treated cells. **B**, Cells were pretreated with the ARB irbesartan (Irb, 10  $\mu$ mol/L) and the proteasome inhibitors MG132 (10  $\mu$ mol/L) and MG262 (0.1  $\mu$ mol/L), followed by incubation with cycloheximide (10  $\mu$ g/mL). After 4 hours, adiponectin protein expression was measured. Representatives and densitometry results of 3 separate experiments are presented. \* $P$ <0.05 vs vehicle-treated controls. **C**, Cells were pretreated with the ARB irbesartan (Irb) and the proteasome inhibitors MG132 (10  $\mu$ mol/L), followed by incubation with cycloheximide (10  $\mu$ g/mL). After 4 hours, proteasome activity was measured as described in Methods. Activity is shown relative to vehicle-treated controls (100%). \* $P$ <0.05 vs vehicle-treated controls.

3.8 hours (Figure 3A). Irbesartan treatment prevented cellular adiponectin protein depletion and markedly prolonged protein half-life (Figure 3A). To clarify whether the decrease of adiponectin protein levels was a result of protein degradation, we studied the involvement of the proteolytic 26S proteasome complex by using the peptide aldehyde proteasome inhibitors MG132 (10  $\mu$ mol/L) and MG262 (100 nmol/L). Pretreatment of adipocytes with both proteasome inhibitors blocked adi-

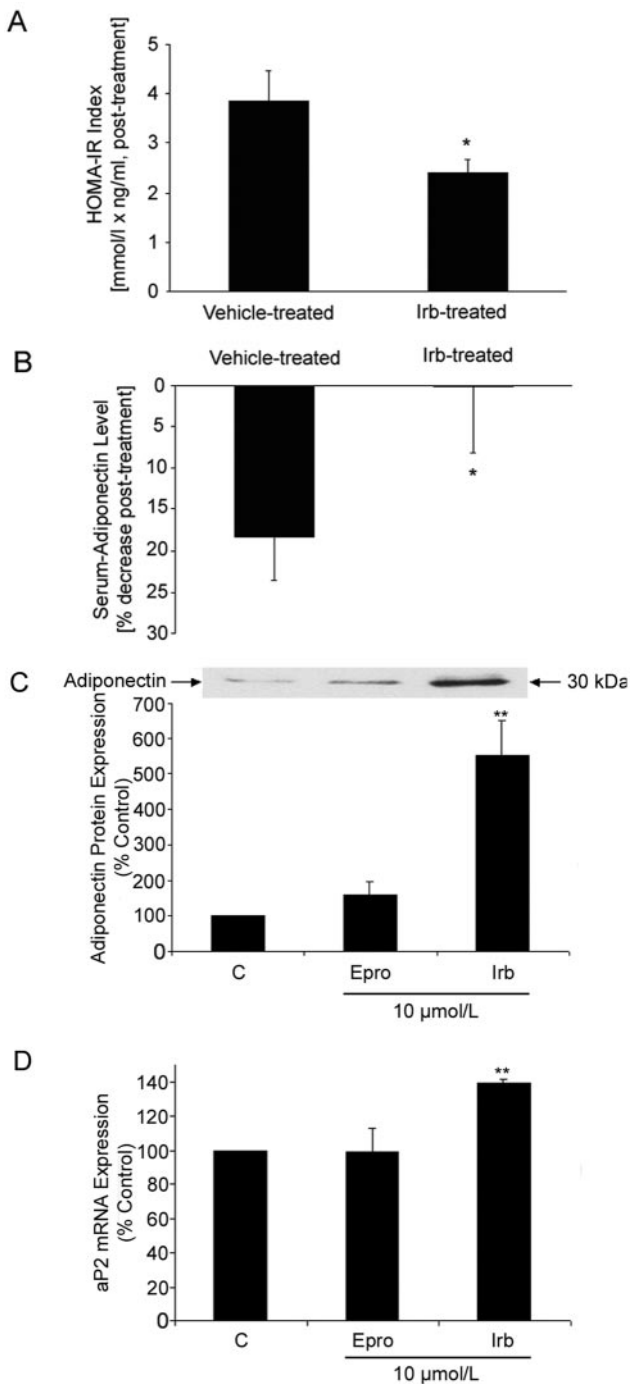
ponectin protein depletion to the same extent as irbesartan (10  $\mu$ mol/L). However, irbesartan did not directly affect proteasome activity, suggesting that the prevention of cellular adiponectin depletion by irbesartan results from a blockade of the ubiquitin-proteasome pathway upstream of the 26S proteasome complex (Figure 3B and 3C).

### Irbesartan Treatment Maintains Adiponectin Serum Levels and Improves Insulin Sensitivity In Vivo

To examine whether irbesartan-mediated adiponectin protein induction in vitro translates into adiponectin regulation and metabolic improvement in vivo, obese Zucker *fa/fa* rats, an animal model of obesity-mediated insulin resistance, were treated for 21 days with irbesartan (50 mg/kg, orally) or vehicle (saline, orally). Adiponectin serum levels and metabolic parameters were analyzed before (day 0) and after (day 21) treatment.

Arterial blood pressure was decreased by irbesartan treatment (mean arterial pressure day 21: vehicle:  $87 \pm 2.5$  mm Hg versus irbesartan:  $70.1 \pm 2$  mm Hg,  $P$ <0.05; systolic arterial pressure day 21:  $110 \pm 3.2$  mm Hg versus irbesartan:  $90.3 \pm 1.4$  mm Hg,  $P$ <0.05). Final body weights increased similarly in both groups during the 21-day treatment period (Table I). Fasting glucose levels did not change during treatment and were similar in both groups (Table I). Compared with the vehicle-treated group, irbesartan treatment resulted in a significant 36% decrease of fasting insulin levels (Table I). In accordance with an improvement of insulin sensitivity, the irbesartan-treated group had a significantly lower HOMA-IR index compared with the vehicle-treated group (HOMA-IR index [mmol/L $\times$ ng/mL] post-treatment: vehicle-treated:  $3.9 \pm 0.9$  versus irbesartan-treated:  $2.4 \pm 0.2$ ,  $P$ <0.05) (Figure 4A). In addition, triglyceride levels increased significantly by  $2.1 \pm 0.2$ -fold in the vehicle-treated group ( $P$ <0.05 versus day 0), whereas irbesartan treatment markedly attenuated this increase ( $1.5 \pm 0.1$ -fold versus day 0; NS; supplemental Table I). Irbesartan treatment prevented adiponectin depletion during the treatment period, whereas in the vehicle-treated group adiponectin serum levels declined by almost 20% (supplemental Table I; and Figure 4B). Absolute adiponectin levels did not differ among the groups at the end of treatment. These data indicate that irbesartan treatment improves metabolic parameters of insulin sensitivity in an in vivo model of obesity-induced insulin resistance, which is associated with the maintenance of adiponectin serum levels.

To analyze differences between PPAR $\gamma$ -activating and non-PPAR $\gamma$ -activating ARBs in their ability to induce adiponectin protein expression in fat tissue, epididymal fat samples from Zucker *fa/fa* rats were stimulated ex vivo with eprosartan and irbesartan. Irbesartan also potently induced adiponectin protein expression in fat tissue, whereas eprosartan had no effect (Figure 4C). Adiponectin mRNA levels were not significantly regulated (data not shown). Consistently with its PPAR $\gamma$ -activating properties, irbesartan also induced adipose expression of the PPAR $\gamma$ -target gene aP2 (Figure 4D).



**Figure 4.** Irbesartan treatment maintains adiponectin serum levels and improves insulin sensitivity in vivo. Male obese Zucker *fa/fa* rats were treated with either vehicle (saline,  $n=7$ ) or 50 mg/kg per day of irbesartan ( $n=7$ ) for 21 consecutive days. **A**, Fasting serum glucose and insulin levels were measured, and the post-treatment HOMA-IR-index was calculated as described in Methods. Results are presented as mean  $\pm$  SEM. \* $P<0.05$  vs vehicle-treated animals. **B**, Fasting serum adiponectin levels were analyzed as described in Methods. Results are presented as percentage decrease of serum adiponectin level during treatment. Results are presented as mean  $\pm$  SEM. \* $P<0.05$  vs vehicle-treated animals. **C**, Epididymal fat tissue samples were prepared as described in Methods. Fat tissue was stimulated ex vivo for 24 hours with irbesartan (Irb, 10  $\mu$ mol/L) and eprosartan (Epro, 10  $\mu$ mol/L), and adiponectin protein expression was measured. Protein expression is shown relatively to vehicle-treated controls (100%). \*\* $P<0.01$  vs vehicle-treated controls. **D**, RNA

## Discussion

The present study demonstrates that PPAR $\gamma$ -activating ARBs induce adiponectin protein expression at a post-transcriptional level, independently of their AT1R-blocking properties. In addition, AT2R activation resulted in adiponectin upregulation. The PPAR $\gamma$ -activating ARB irbesartan improved parameters of insulin sensitivity in obese Zucker rats, which was associated with the prevention of adiponectin serum depletion.

We and others could recently demonstrate that a subset of ARBs including irbesartan has the potential to activate the insulin-sensitizing nuclear hormone receptor PPAR $\gamma$ , completely independent from their AT1R blocking properties.<sup>8,10</sup> PPAR $\gamma$  activation has been shown to stimulate adiponectin expression in adipocytes and to upregulate adiponectin plasma levels in animals and humans.<sup>6,11</sup> In the present study, pharmacological antagonism of PPAR $\gamma$  completely blocked irbesartan-induced adiponectin expression in vitro. In addition, adiponectin expression in adipocytes and fat tissue was solely upregulated by PPAR $\gamma$ -activating ARBs, whereas non-PPAR $\gamma$ -activating ARBs had no effect. These data suggest that PPAR $\gamma$  activation seems to be important for adiponectin induction by ARBs.

Activation of the AT2R also stimulated adiponectin protein expression without affecting mRNA expression suggesting a post-transcriptional mechanism. Consistently, Fasshauer et al reported the absence of Ang II-induced adiponectin mRNA regulation in 3T3-L1 adipocytes.<sup>12</sup> AT2R stimulation might be relevant during selective AT1R blockade under certain pathological conditions in which the available Ang II is redirected to the AT2R.<sup>2</sup> Adiponectin protein expression in adipose tissue was not stimulated by the non-PPAR $\gamma$ -activating ARB eprosartan, indicating that endogenous Ang II effects including AT2R activation are not required for the ARB effects in our system. However, these data do not exclude Ang II-dependent regulation of adiponectin (eg, via AT2R activation) in humans with ARB treatment. Future studies are required to further define the relevance of Ang II-dependent versus PPAR $\gamma$ -dependent regulation of adiponectin during ARB treatment.

Activated by its ligands, PPAR $\gamma$  functions as a transcriptional regulator of genes containing corresponding binding elements, named PPAR response elements (PPRE), in their promoter regions. Surprisingly, PPAR $\gamma$ -activating ARBs and pioglitazone did not induce adiponectin mRNA levels. Transcriptional regulation of adiponectin by PPAR $\gamma$  ligands in adipocytes has been reported controversially. Combs et al showed that PPAR $\gamma$  activation by a full thiazolidinedione agonist did not affect adiponectin mRNA expression in 3T3-L1 adipocytes, whereas Maeda et al detected a stimulation by different glitazones in the same cell model using a different differentiation protocol.<sup>11,13</sup> In addition, the presence of a functional PPRE in the adiponectin promoter also

was isolated from epididymal fat tissue samples (described in C). Expression of aP2 mRNA was assessed by real-time PCR and presented relatively to vehicle-treated controls (100%). Experiments were repeated 3 times and results are presented as mean  $\pm$  SEM. \*\* $P<0.01$  vs vehicle-treated controls.

remains controversial. A “putative” PPRE, which is depending on the presence of the competence factor, liver receptor homolog-1, has been identified in the human adiponectin promoter.<sup>14</sup> However, a “classical” PPRE has not been identified in the human or mouse promoter.<sup>15,16</sup> In conclusion, these studies propose that the transcriptional mechanism of PPAR $\gamma$ -induced adiponectin regulation in adipocytes is still unclear. Our data suggest the presence of additional post-transcriptional mechanisms of adiponectin regulation involving a PPAR $\gamma$ -dependent stabilization of cellular protein levels.

The 26S proteasome, called “the proteasome” is a multicatalytic enzyme complex expressed in the nucleus and cytoplasm degrading multiple proteins to maintain regular cell function.<sup>17</sup> The present study identifies adiponectin for the first time as a target protein for the proteasome pathway. Proteasome inhibition maintained adiponectin protein levels to the same extent as treatment with the PPAR $\gamma$ -activating ARB irbesartan. Consistently, previous reports in hepatocellular carcinoma cells demonstrated that other PPAR $\gamma$  ligands inhibit the ubiquitin–proteasome pathway, resulting in stabilization of the cell cycle inhibitor p27Kip1.<sup>18</sup> However, proteasome activity was not directly inhibited by irbesartan suggesting an upstream mechanism of action. Upstream molecular events within the ubiquitin–proteasome pathway comprise protein modifications (eg, phosphorylation) and conjugation of ubiquitin to the protein, processes that might be targeted by irbesartan.<sup>19</sup>

There is now growing evidence that ARBs, in particular those with PPAR $\gamma$ -activating characteristics, improve insulin sensitivity in diet-induced or genetic animal models of insulin resistance/diabetes.<sup>4,10</sup> Benson et al showed that the potent PPAR $\gamma$ -activating ARB telmisartan improves insulin sensitivity in a model of rats fed a high-fat diet, whereas losartan had no effect.<sup>10</sup> Adiponectin levels usually decline with increasing body weight, which is positively correlated with the degree of insulin resistance.<sup>6</sup> Absolute adiponectin levels and the degree of changes during interventions seem to contribute to the metabolic effects of adiponectin.<sup>6,20</sup> Stabilization of adiponectin serum levels by irbesartan during weight gain in our study provides a new mechanism of insulin sensitization by PPAR $\gamma$ -activating ARBs.

Clinical studies have demonstrated that ARBs (losartan, candesartan, valsartan) reduce the incidence of new onset diabetes.<sup>3</sup> In addition, the ARBs losartan and candesartan increase adiponectin plasma levels in patients with essential hypertension.<sup>5,21</sup> Most of the named ARBs are weak PPAR $\gamma$  activators exerting activation only in very high concentrations.<sup>8,10</sup> This may lead to the following deliberations. High concentrations of these compounds are required for PPAR $\gamma$  activation.<sup>8</sup> These concentrations might be achieved during accumulation in adipose tissue, still allowing PPAR $\gamma$ -mediated insulin sensitization in fat during long-term treatment. Additional mechanism of insulin sensitization by ARBs do exist, eg, AT2R activation, improvement of insulin signaling, or adipose tissue remodeling associated with an improvement of muscular glucose use.<sup>22–24</sup> Future clinical studies comparing PPAR $\gamma$ -activating ARBs with nonactivating ARBs are required to clearly show that PPAR $\gamma$ -activating

substances are superior in the setting of insulin resistance/type 2 diabetes mellitus and hypertension.

## Perspectives

In summary, the present study identifies adiponectin as a new target protein for PPAR $\gamma$ -activating ARBs, providing a new mechanism for their insulin-sensitizing actions. These data will help to understand the differences between ARBs with and without PPAR $\gamma$ -activating properties. Knowledge of molecular processes involved in insulin sensitization by those ARBs will also help to develop new PPAR $\gamma$  modulators, which retain metabolic efficacy together with antihypertensive actions in the absence of side effects.

## Acknowledgments

We thank Andrea Weller for her excellent technical help in the analysis of proteasome activity. We thank Sanofi-Aventis, Berlin, Germany, for kindly providing irbesartan, and Boehringer Ingelheim GmbH & Co, KG, Biebrich an der Rises, Germany, for kindly providing telmisartan. T.U. and U.K. are supported by the Deutsche Forschungsgemeinschaft (GK 754). T.U. is a member of the advisory boards from Boehringer, MSD, Abbot, Novartis, Glaxo-Smith-Kline, Sanofi-Aventis, and Takeda. T.U. has received research grants from Boehringer, MSD, Novartis, and Takeda. U.K. has received research grants from Boehringer, MSD, and Glaxo-Smith-Kline.

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