An adipose tissue-independent insulin-sensitizing action of telmisartan: a study in lipodystrophic mice

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ABBREVIATIONS: ACC, acetyl-CoA carboxylase; ARB, angiotensin II type 1 receptor blocker; AT1, angiotensin II type 1 receptor; AUC, area under curve; FAS, fatty acid synthase; G6P, glucose-6-phosphatase; HOMA-IR, the homeostasis model assessment of insulin resistance; ITT, insulin tolerance test; NASH, non-alcoholic steatohepatitis; NEFA, non-esterified fatty acids; OGTT, oral glucose tolerance test; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; SBP, systolic blood pressure; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; WT, wild type
ABSTRACT

Adipose tissue plays an important role in energy balance and metabolism and is the major target for insulin-sensitizing peroxisome proliferator-activated receptor (PPAR)gamma agonists. The angiotensin II type 1 receptor blocker telmisartan, a partial agonist of PPARgamma, has been demonstrated to improve insulin sensitivity. However, there is uncertainty about the sites of its action. Here, we demonstrate that treatment with telmisartan (3 mg/kg, p.o.) for 7 weeks decreased plasma glucose levels in oral glucose and insulin tolerance tests and the index of the homeostasis model assessment of insulin resistance (HOMA-IR) in A-ZIP/F-1 transgenic mice, an animal model of lipodystrophy. These effects were accompanied by decreases in circulating triglyceride and fatty acid levels. However, this treatment did not affect body weight and plasma adiponectin, leptin and corticosterone levels. In A-ZIP/F-1 mouse liver the transcripts encoding PPARgamma and its downstream lipogenic genes were highly upregulated, consistent with increased hepatic triglyceride content and lipid droplet accumulation. Telmisartan reversed these effects and also downregulated mRNAs encoding gluconeogenic genes. Thus the present findings are consistent with a novel mode of insulin-sensitizing action of telmisartan, involving an adipose tissue-independent pathway. Telmisartan-elicited downregulation of hepatic
expression of PPARgamma-regulated lipogenic genes is associated with amelioration of fatty liver.
Introduction

Insulin resistance is a key component of the metabolic syndrome and both precedes and predicts the development of type 2 diabetes. Moreover, even in the absence of diabetes, insulin resistance increases the risk of non-alcoholic steatohepatitis (NASH) and cardiovascular disease (Prasad and Quyyumi, 2004).

Telmisartan is a well-established angiotensin II type 1 receptor (AT1) blocker (ARB). It has been demonstrated that telmisartan improves insulin sensitivity in rodents that have received high fat-containing diets (Benson et al., 2004; Sugimoto et al., 2006; Araki et al., 2006) and in diabetic and non-diabetic patients (Pershadsingh and Kurtz, 2004; Honjo et al., 2005; Miura et al., 2005). However, there is uncertainty about the sites of its action.

As the primary site for energy storage, adipose tissue plays an important role in energy balance and metabolism and also produces bioactive substances that regulate insulin sensitivity (Engeli et al., 2003). For example, adipocyte-derived leptin regulates food intake and energy expenditure and, in conjunction with the adipokine adiponectin, modulates insulin sensitivity. The peroxisome proliferator-activated receptor (PPAR)γ is a member of the ligand-activated nuclear receptor superfamily and is expressed at high levels in adipose tissue (Evans et al., 2004). PPARγ regulates genes that modulate lipid utilization and storage, and lipoprotein metabolism and
adipocyte differentiation and insulin action (Evans et al., 2004). Thus, PPAR\(\gamma\) is the master regulator of adipogenesis and is activated by the thiazolidinediones that are used clinically to stimulate the action of insulin in adipose tissue (Evans et al., 2004). In addition to its actions at AT1, telmisartan has been shown recently to facilitate the differentiation of 3T3-L1 preadipocytes and to activate PPAR\(\gamma\)-responsive genes in adipocytes in vitro (Benson et al., 2004, Fujimoto et al., 2004). In light of these findings we speculated that the antidiabetic effects of telmisartan might be dependent on PPAR\(\gamma\) in adipose tissues.

The A-ZIP/F-1 transgenic mouse is an animal model of lipodystrophy in which a dominant-negative protein that impairs gene activation by leucine zipper transcription factors results in the absence of adipose tissue (Moitra et al., 1998). The mice eat, drink and urinate copiously, grow heavier than their littermates and exhibit decreased fecundity (Moitra et al., 1998). A-ZIP/F-1 mice are profoundly insulin resistant, hyperlipidemic, hepatosteatotic and hypertensive (Chao et al., 2000; Ebihara et al., 2001; Gavrilova et al., 2003; Takemori et al., 2007). In the present study, we tested the role of adipose tissue in the insulin-sensitizing effect of telmisartan in A-ZIP/F-1 mice.
Methods

Animals and treatments. The ‘Principles of laboratory animal care’ were followed in the present study. All procedures were conducted in accordance with institutional guidelines and were approved by the Animal Ethics Committee, Kyoto University, Japan. Mice were housed in a temperature controlled facility (21±1°C, 55±5% relative humidity) with a 12-h light/dark cycle.

The generation and characterization of the “fatless” A-ZIP/F-1 mice have been reported previously (Ebihara et al., 2001). A-ZIP/F-1 mice were on the FVB/N background, produced by breeding of A-ZIP/F-1 males and wild type (WT) females. Female A-ZIP/F-1 mice and their WT littermates were used in experiments because the females are sterile, possibly as a consequence of leptin deficiency, whereas the males were used for breeding purposes. The animals received a standard diet (CLEA Tokyo, Japan) and water ad libitum. At 16-18 weeks of age, systolic blood pressure (SBP) and body weight were measured. For determination of non-fasting plasma levels of glucose, triglyceride and non-esterified fatty acids (NEFA) by enzymatic methods, five small droplets of blood were collected carefully by orbital puncture under light ether anesthesia. The A-ZIP/F-1 mice were selected and grouped (n=7 each group) for experiments on the basis of these parameters; these parameters did not differ between animals in the vehicle- and telmisartan-treated groups. Two
experiments were performed. 1) Higher-dosage experiment: telmisartan (3 mg/kg, a generous gift from Boehringer Ingelheim Co., Ltd, Japan, suspended in 5% Gum Arabic) was administered by oral gavage, once daily (11:00-12:00) for 7 weeks, whereas controls received 5% Gum Arabic alone. Mice were weighed every 3-4 days and food intake was estimated weekly. SBP was measured at Week 2. Eight small droplets of non-fasted and/or fasted (12 h) blood were collected for determination of plasma levels of adiponectin, leptin, corticosterone, glucose and lipids at week 4. Oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (ITT) were performed at weeks 5 and 6, respectively. Liver was dissected and weighed after animals were killed, and a segment of liver was immediately frozen in liquid nitrogen and stored at -80°C for subsequent determination of triglyceride and gene expression.

2) Lower-dosage experiment: the protocol was the same as that of the higher-dosage experiment except for replacement of 3 mg/kg with 1 mg/kg of telmisartan.

**SBP measurement.** SBP was measured in conscious mice by a tail-cuff method 2-5 h after administration of drug or vehicle (MK-2000ST; Muromachi Kikai Co Ltd, Tokyo, Japan). At least six readings were taken for each measurement.

**OGTT and ITT.** Mice were fasted for 12 h with free access to water. In the OGTT, mice received a glucose solution (2 g/kg in 10 ml) by the oral route and in the ITT mice received aqueous insulin (0.75 IU/kg in 10 ml by i.p. injection). Two-three
small droplets of blood were collected at each time-point for determination of plasma glucose levels using a commercial kit (Wako, Osaka, Japan) prior to and 20, 60 and 120 min after administration of glucose or insulin (Humulin R-Insulin, Eli Lilly & Co., Indianapolis, IN), respectively. The area under the curve (AUC) of glucose was calculated from the plasma concentration-time relationships. Plasma insulin concentrations at 0 min were also measured by ELISA (Morinaga, Tokyo, Japan). The index of the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as an indicator of insulin sensitivity according to the following formula:

$$\text{[insulin (\mu IU) } \times \text{ glucose (mM)}]/22.5.$$

**Histological examination.** A portion of liver was fixed with 10% formalin and embedded in paraffin. Twenty-micron sections were cut and stained with hematoxylin and eosin for examination of liver histology (IX-81, Olympus Corporation, Tokyo, Japan).

**Determination of blood biochemistry and liver triglyceride content.** Plasma triglyceride and NEFA levels were determined using commercial kits (Wako, Osaka, Japan). Plasma adiponectin (Otsuka Pharmaceutical, Tokushima, Japan), leptin (Morinaga, Tokyo, Japan) and corticosterone (Cayman, MI, USA) levels were also assayed by ELISA.
Tissue triglyceride content was determined as described previously (Oakes et al., 2001). Briefly, 100 mg of liver was homogenized and extracted with 2 ml of isopropanol. After centrifugation, triglyceride content in the supernatant was determined with an enzymatic colorimetric method (Wako, Osaka, Japan).

**Gene expression analysis.** RNA was extracted from the livers of individual mice using TRIzol (Invitrogen, Osaka, Japan). Single-stranded cDNA was synthesized from 1 μg of total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen, Osaka, Japan). Quantitative real-time PCR was performed with an AB 7300 Real-Time PCR System using TaqMan (Applied Biosystems, USA). The primers and probes (Sigma-Genosys, Hokkaido, Japan) used were shown in Table 1. Mouse mitochondrial subunit 18s rRNA was selected as the endogenous control gene.

**PPARγ** protein was quantified by Western blotting (Lorenzo et al., 2002). Tissue proteins were resolved on 4-12% polyacrylamide gels in the presence of sodium dodecylsulfate, transferred electrophoretically to polyvinylidene difluoride membranes, blocked (in buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 5% bovine serum albumin, 0.1% Tween-20), and incubated at 4°C for 18 h with **PPARγ**-specific antibody (1:800; Cell Signaling, MA, USA). Detection was performed with peroxidase-conjugated secondary antibody, by enhanced chemiluminescence.
Immunoblotting with a monoclonal anti-β-actin antibody (Cell Signaling, MA, USA) was conducted to ensure equal protein loading.

**Data analysis.** All results are expressed as means ± SEM. Data from more than two experimental groups were analyzed by one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was performed to identify the differences between groups. Data from two experimental groups (Fig 6) were analyzed using the Student's t-test. *P*<0.05 was considered significant.
Results

Higher-dosage experiment. In accordance with the previous reports (Moitra et al., 1998; Takemori et al., 2007), A-ZIP/F-1 mice that received a standard diet exhibited higher SBP (Fig 1A), increased food intake (Fig 1B), heavier body weights (Fig 1C), compared to WT mice. It has been reported that there is a 90% reduction in brown adipose tissue and the remaining 10% is inactive in A-ZIP/F-1 mice (Moitra et al., 1998). In contrast to WT mice (Fig 1D) in the present study, A-ZIP/F-1 mice showed minimal adipose tissue (Fig 1E). We first tested the effects of telmisartan at higher dosage (3 mg/kg), although this was lower than the dose used in previous studies (5 mg/kg) (Benson et al., 2004; Sugimoto et al., 2006; Araki et al., 2006). Telmisartan treatment lowered SBP to the level observed in WT mice (Fig 1A), but exerted minimal effects on parameters relating to food intake (Fig 1B), body weight (Fig 1C) or adipose tissue (Fig 1F, by gross necropsy) in A-ZIP/F-1 mice.

At week 4, plasma glucose levels in A-ZIP/F-1 mice were significantly increased over those in WT mice under non-fasted conditions, but not after a 12 h fast (Fig 2A). Fasted plasma insulin levels (Fig 2B) and the HOMA-IR index (Fig 2C) were much higher in A-ZIP/F-1 than in WT mice. Telmisartan treatment markedly decreased plasma insulin concentrations (Fig 2B) and the HOMA-IR index (Fig 2C) in A-ZIP/F-1 mice but was without effect on plasma glucose concentrations (Fig 2A).
In further studies the responses of plasma glucose to challenge with exogenous glucose and insulin were assessed. Plasma glucose concentrations and glucose AUC did not differ significantly between A-ZIP/F-1 and WT mice either before or after OGTTs were conducted (2 g/kg) at week 5 (Fig 2D and 2E). Telmisartan treatment prevented the increases in plasma glucose concentrations at 20 min and in glucose AUC in OGTT. In contrast, the decrease in plasma glucose concentrations elicited by insulin was minimal in A-ZIP/F-1 mice compared with WT control (Fig 2F), but was more pronounced when telmisartan was coadministered. Consistent with these findings, the glucose AUC after insulin was higher in A-ZIP/F-1 than in WT mice (Fig 2G), and was decreased by telmisartan treatment.

Plasma adiponectin and leptin levels were extremely low in A-ZIP/F-1 mice and were unaffected by telmisartan treatment (Fig 3A and 3B). In contrast, plasma corticosterone in A-ZIP/F-1 mice was slightly, but not significantly, increased over WT control (Fig 3C); telmisartan treatment did not affect plasma corticosterone levels in A-ZIP/F-1 mice. The generalized lipodystrophy also led to redistribution of lipids. Non-fasted plasma levels of triglyceride and NEFA were elevated in A-ZIP/F-1 mice compared with WT mice (Fig 3D and 3E). Treatment with telmisartan for 4 weeks decreased plasma triglyceride and NEFA levels by 42% and 28%, respectively.

The hepatomegaly exhibited by A-ZIP/F-1 mice (Fig 1E) relative to control
animals (Fig 1D) was partially reversed by telmisartan (Fig 1F). Indeed, the increase in hepatic triglycerides in A-ZIP/F-1 mice to 7-12 fold of WT control was substantially ameliorated by telmisartan (Fig 4B and 4C). In accord with these findings, the extensive fatty infiltration in A-ZIP/F-1 livers (Fig 4E) compared with control (Fig 4D), was also partially reversed by telmisartan (Fig 4F).

Expression of the mRNAs encoding the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P) was similar in liver of A-ZIP/F-1 and control mice; both mRNAs were decreased by treatment with telmisartan by 62% and 61%, respectively (Fig 5A and 5B). In liver of A-ZIP/F-1 mice PPARγ mRNA expression was increased relative to WT control, which was substantially resolved by telmisartan treatment (Fig 5C). In accord with these findings, PPARγ2 immunoreactive protein was upregulated in A-ZIP/F-1 mouse liver and normalized by telmisartan treatment, but PPARγ1 protein expression was very low and unaltered (Fig 5D). Hepatic stearoyl-CoA desaturase (SCD)1, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) 1 mRNA levels in the A-ZIP/F-1 mice were markedly increased over those in WT mice (Fig 5E-G), but the slight increase in sterol regulatory element-binding protein (SREBP)1c mRNA did not attain statistical significance (Fig 5H). Consistent with the effect of telmisartan on PPARγ gene expression, treatment of A-ZIP/F-1 mice with the ARB markedly suppressed
hepatic mRNAs corresponding to the PPARγ-regulated downstream genes SCD1, FAS, ACC1 and SREBP1c.

**Lower-dosage experiment.** We also tested the effects of telmisartan at lower dosage. Treatment with 1 mg/kg of telmisartan significantly decreased SBP in A-ZIP/F-1 mice (Fig 6A). However, this treatment did not affect plasma glucose levels under fasting conditions (Fig 6B) and during OGGT (Fig 6C) and ITT (Fig 6D); similarly, non-fasting triglyceride and NEFA (Fig 6E), liver weight and triglyceride content (Fig 6F) remained unchanged. Real time PCR analysis demonstrated no difference between the vehicle- and telmisartan-treated groups in the expression of hepatic PPARγ (Fig 6G), SCD1 (Fig 6H), SREBP1c (Fig 6I), FAS (Fig 6J) and ACC1 (Fig 6K) mRNAs.


Discussion

PPARγ is expressed predominantly in adipose tissue, which facilitates lipid uptake and storage in that tissue and alters the release of leptin and adiponectin, that modulate insulin sensitivity. Adipose PPARγ is the molecular target for the insulin-sensitizing thiazolidinediones (Evans et al., 2004). The principal findings to emerge from the present study were that telmisartan treatment (3 mg/kg) enhanced insulin sensitivity and improved the abnormalities of lipid metabolism observed in A-ZIP/F-1 mice. Thus, the present findings indicate that telmisartan has additional beneficial effects that are distinct from adipose tissues. It appears that the improvement in insulin sensitivity in adipose tissue-deficient mice effected by telmisartan is independent of PPARγ agonism.

Although the mechanisms underlying insulin resistance in patients with lipodystrophies are unclear, accumulation of triglycerides in liver and skeletal muscle is probably important (Garg, 2004). Intracellular accumulation of fatty acids and triglycerides in nonadipose tissues has been implicated in insulin resistance (Shimabukuro et al., 1997). It has been demonstrated that a decrease in hepatic triglyceride pools leads to improved insulin sensitivity (Neschen et al., 2005, Savage et al., 2006). On the other hand, an increase in blood lipid levels, especially NEFA, modulates the action of insulin. A high plasma NEFA concentration is a risk factor for
deterioration of glucose tolerance that is independent of the other parameters relating
to insulin resistance or insulin secretion (Charles et al., 1997). Chronically elevated
plasma NEFA concentrations stimulate gluconeogenesis, exacerbate hepatic/muscle
insulin resistance, and also impair insulin secretion in genetically predisposed
individuals (Boden 1997; Bergman and Ader, 2000). Decreased availability of
precursor substrates, including free fatty acids and glucose, also diminishes hepatic
synthesis and export of esterified lipids (Ran et al., 2004). In the present study,
amelioration of insulin resistance in the lipodystrophic A-ZIP/F-1 mice by telmisartan
treatment was accompanied by a decrease in hepatic triglyceride accumulation and
circulating lipid concentrations.

PPARγ is expressed normally at low level in liver, but is strongly upregulated in
liver of A-ZIP/F-1 mice and appears to contribute to the development of hepatic
steatosis (Chao et al., 2000; Gavrilova et al., 2003). Although treatment of A-ZIP/F-1
mice with rosiglitazone, a thiazolidinedione that acts as a PPARγ agonist, lowered
circulating lipid levels, hepatic steatosis was exacerbated (Chao et al., 2000).
Moreover, ablation of hepatic PPARγ decreased hepatic steatosis in A-ZIP/F-1 mice
and promoted hyperlipidemia (Gavrilova et al., 2003). Thus, steatosis appears to be
due to increased triglyceride formation and uptake by liver in a PPARγ-regulated
fashion (Gavrilova et al., 2003). In the present study telmisartan reversed the increase
in hepatic PPARγ expression in A-ZIP/F-1 mouse liver and also normalized the expression of several PPARγ-responsive genes that participate in fatty acid and triglyceride synthesis. Whereas thiazolidinediones decreased serum triglycerides and free fatty acids, glucose and insulin regulation was not restored (Chao et al., 2000). Thus, the present findings clearly distinguish the effects of telmisartan from PPARγ agonist thiazolidinediones. The precise underlying molecular mechanism by which telmisartan modulates hepatic PPARγ activity should now be explored further.

Energy homeostasis is regulated at the level of food intake, overall activity, sympathetic tone, energy expenditure and insulin sensitivity (Flier, 1997). Serum leptin concentrations in A-ZIP/F-1 mice are ~5% of those in WT mice (Moitra et al., 1998). The adipokine adiponectin acts in concert with leptin to enhance insulin sensitivity and regulate glucose metabolism (Berg and Scherer, 2005). Leptin is secreted by white and brown adipose tissue in proportion to tissue mass (Moitra et al., 1998). Leptin acts through the hypothalamic-pituitary-adrenal axis to regulate insulin sensitivity indirectly (Cusin et al., 1998; Liu et al., 1998). Hepatic leptin overexpression decreased substantially the steatosis in A-ZIP/F-1 mice and also normalized muscle lipid and serum concentrations of glucose and insulin, as well as triglycerides and free fatty acids (Ebihara et al., 2001). Thus, restitution of leptin compensated for the lack of adipose tissue and prevented the hyperglycemia,
hyperinsulinemia, hypertriglyceridemia and increased free fatty acid levels that are characteristic of the A-ZIP/F-1 mouse. In the present study telmisartan did not affect plasma adiponectin and leptin levels, which indicates that the primary defect due to adipocyte deletion was not overcome.

While disturbance of the regulatory actions of adipose tissue-derived mediators is important, adrenocortical dysregulation may contribute to insulin resistance (Roberge et al., 2007). Thus, glucocorticoids regulate food intake and metabolism that influences energy utilization (Haluzik et al., 2002) and also stimulate gluconeogenesis via PEPCK and G6P (Pilkis and Granner, 1992). Leptin deficiency may contribute to hypercorticosteronemia in A-ZIP/F-1 mice (Haluzik et al., 2002). Indeed, combined leptin infusion and adrenalectomy decreased plasma corticosterone levels and improved diabetes in A-ZIP/F-1 mice (Haluzik et al., 2002). In the present study, the small increase in plasma corticosterone levels in A-ZIP/F-1 mice was not statistically significant and was minimally affected by telmisartan. Thus it is unlikely that telmisartan improves insulin resistance by modulating adrenal corticosterone production in A-ZIP/F-1 mice.

Telmisartan at lower dosage (1 mg/kg) significantly decreased SBP in A-ZIP mice, which is attributed to AT1 inhibition. However, it did not affect insulin resistance, hyperlipidemia or fatty liver, and did not decrease the expression of hepatic PPARγ-
regulated lipogenic genes. These results were distinct from those made after treatment with telmisartan at 3 mg/kg (see above) or rosiglitazone (Chao et al., 2000). Thus, these findings may suggest that AT1 inhibition alone is unlikely to account for the improved insulin sensitivity and fatty liver in the lipodystrophic mice. Genetic blockade of AT1 or PPARγ signaling may be necessary to dissociate the actions of telmisartan at AT1 and PPARγ.

Lipodystrophies, such as the Seip-Berardinelli syndrome (Moitra et al., 1998) are characterized by the selective loss of adipose tissue. These conditions may be genetic in origin or may emerge during the treatment of HIV patients with protease inhibitors. Affected patients are predisposed to insulin resistance and its attendant complications, including diabetes, hypertriglyceridemia, hepatic steatosis and hypertension (Garg, 2004; Agarwal and Garg, 2006). Morbidity and mortality in patients with lipodystrophies are frequently manifested as diabetes mellitus, hepatic cirrhosis as a result of steatosis, atherosclerosis and recurrent acute pancreatitis due to prolonged hypertriglyceridemia (Garg, 2004; Agarwal and Garg, 2006). Current treatments are restricted to managing the metabolic abnormalities of insulin resistance, diabetes, dyslipidaemia and hypertension; to date no specific therapies have emerged. The present study has identified an adipose-tissue-independent insulin-sensitising effect of telmisartan. Amelioration of hepatic steatosis was associated with down regulation of
hepatic expression of PPARγ-mediated genes responsible for lipid synthesis. Therefore, the present finding that telmisartan improves insulin resistance and its associated complications in A-ZIP/F-1 mice may constitute important information for the development of new strategies to manage patients with lipodystrophy.

Taken together, the present findings are consistent with a novel mode of insulin-sensitizing action of telmisartan, involving an adipose tissue-independent pathway. The precise underlying molecular mechanism by which telmisartan modulates hepatic PPARγ activity should now be explored further.
Acknowledgments

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References


Footnotes

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Legends for Figures

**Fig. 1** Systolic blood pressure (SBP) (A), food intake (B), body weight (C) and abdominal appearance (adipose tissues and liver) (D-F) in female wild type (WT) and A-ZIP/F-1 mice. Animals were treated orally with vehicle (control, Tel 0) or telmisatan (3 mg/kg, Tel 3) once daily for 7 weeks. SBP was measured with a tail-cuff method at Week 2 after treatment. 24-h food intake was determined at Week 5. All values are means ± SEM (n=7). *, P<0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA).

**Fig. 2** Non-fasted and fasted (12 h) plasma glucose (A) and fasted insulin (B) levels, the index of the homeostasis model assessment of insulin resistance (HOMA-IR) (C) at Week 4, and plasma glucose response to exogenous glucose and insulin challenge in female WT and A-ZIP/F-1 mice. OGTT (glucose: 2 g/kg, p.o.) (D and E) and insulin (ITT, insulin: 0.75 IU/kg, i.p.) (F and G) tolerance test were performed after fasting (12 h) at Week 5 and 6, respectively. All values are means ± SEM (n=7). *, P<0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA). Tel 3: telmisartan 3 mg/kg.
Fig. 3 Non-fasted plasma adiponectin (A), leptin (B), corticosterone (C), triglyceride (D) and NEFA (E) levels in female WT and A-ZIP/F-1 mice (Week 7). All values are means ± SEM (n=7). vs A-ZIP/F-1 control (Tel 0), *P<0.05 (ANOVA). Tel 3: telmisartan 3 mg/kg.

Fig. 4 Liver weight (A), liver triglyceride (B) and total liver triglyceride (C) contents, and liver histology in female WT and A-ZIP/F-1 mice (Week 7). All values are means ± SEM (n=7). *, P<0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA). Representative liver pathological changes (Hematoxylin and eosin–staining, X200) (D-F). Tel 3: telmisartan 3 mg/kg.

Fig. 5 Hepatic expression of phosphoenolpyruvate carboxykinase (PEPCK) (A), glucose-6-phosphatase (G6P) (B), PPARγ (C), stearoyl-CoA desaturase (SCD)1 (E), fatty acid synthase (FAS) (F) and acetyl-CoA carboxylase (ACC)1 (G), and sterol regulatory element-binding protein (SREBP)1c (H) mRNAs, and PPARγ protein (D) in female WT and A-ZIP/F-1 mice (Week 7). Quantitative Real Time PCR results were normalized to 18s, while the results from Western blot analysis were normalized to β-actin. Levels in WT mice that were arbitrarily assigned a value of 1. All values
are means ± SEM (n=7). *, P<0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA). Tel 3: telmisartan 3 mg/kg.

Fig. 6 SBP (A), fasted (12 h) plasma glucose concentrations (B), plasma glucose response to exogenous glucose (C) and insulin (D) challenge, nonfasted plasma triglyceride and NEFA levels (E), liver weight and triglyceride content (F), and hepatic expression of PPARγ (G), SCD1 (H), SREBP1c (I), FAS (J) and ACC1 (K) in female WT and A-ZIP/F-1 mice. Animal treatments and sample processing were conducted as described except that a dose of 1 mg/kg telmisartan was used instead of 3 mg/kg. All values are means ± SEM (n=7). *, P<0.05 compared with A-ZIP/F-1 control (Tel 0) (Student's t-test). Tel 1: telmisartan 1 mg/kg.
### Table 1. Primer and probe sequences for Real Time RT-PCR assays

<table>
<thead>
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<th>Gene</th>
<th>Probe</th>
<th>Primers&lt;sup&gt;a&lt;/sup&gt;</th>
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| 18s   | CGCGCAAAATTACCCACTCCCGA | f CGGCTACCACATCCAAGGA  
|       |                   | r CCAATTACAGGGCGCTCGAA |
| PPARγ | CTTCCATCGGAGAGGTCCACAGAGC | f AGACATGGTGCTTTCG  
|       |                   | r ATGCTCAAGGAATGGCGAGTG |
| PEPCK | CACTGTGGCTGGCTTACCTAGACC | f GTGTCATCCGCAAGCTGAA  
|       |                   | r CTTTCGATCCTGGCCACATCT |
| G6P   | CTCTGTATGGGAACCTCGCCACG | f GAGGCCTTTGAGAAAGCTTG  
|       |                   | r CCATCCCAGCCATCGTACG |
| SCD1  | CCACCACCACCATCAGCTACCTC | f ATGCTCCAAGAGATCTCAGTTCT  
|       |                   | r CTTCACTTCTCCGTTTCACCTT |
| SREBP1c | CAGCTCATCAACAACCAAGACAGTGACTT | f GGAGCCATGGATGCACATT  
|       |                   | r CCTGCTCAACCACAGCAT |
| FAS   | ACCACCATCTCGGCGATGTACATCTTCTTCT | f GGCTCAGCATGGTCGCTT  
|       |                   | r CTCACCAGCCAGCTGATT |
| ACC1  | CTCAACCTGGATGTACTTTGTCCAGC | f GCCATTTGATTTGAGGGCTTAC  
|       |                   | r CCCGACCAAGGACTTTTG |

Sequences: 5’ to 3’. <sup>a</sup>Forward primers are designated by f and reverse primers by r.
Figure 1

A. SBP (mmHg)

B. Food intake (kcal/mouse/day)

C. Body weight (g)

D. WT Tel 0
E. A-ZIP Tel 0
F. A-ZIP Tel 3
Figure 3

A. Plasma triglyceride (mg/dl)

B. Plasma leptin (ng/ml)

C. Plasma corticosterone (ng/ml)

D. Plasma triglyceride (mg/dl)

E. Plasma NEFA (mEq/L)
Figure 4

A. Liver weight (g) per treatment group (WT, A-ZIP) with Tel 0, 0, 3.

B. Liver triglyceride (mg/g) per treatment group (WT, A-ZIP) with Tel 0, 0, 3.

C. Total liver triglyceride (mg/liver) per treatment group (WT, A-ZIP) with Tel 0, 0, 3.

D. WT Tel 0, A-ZIP Tel 0, A-ZIP Tel 3.

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Figure 5

A. Hepatic PEPCK/18s

B. Hepatic G6P/18s

C. Hepatic PPARγ/18s

D. PPARγ2/β-actin protein

E. Hepatic SCD1/18s

F. Hepatic FAS/18s

G. Hepatic ACC1/18s

H. Hepatic SREBP1c/18s

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Figure 6

A. SBP (mmHg)

B. Plasma glucose (mg/dl)

C. Plasma glucose (%)

D. % Plasma glucose

E. Plasma triglyceride levels (mg/dl)

F. Liver weight (g)

G. Liver triglyceride content (mg/g)

H. Hepatic PPARγ/18s

I. Hepatic SCD1/18s

J. Hepatic SREBP1c/18s

K. Hepatic ACC1/18s

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