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Prophylactic treatment with telmisartan induces tissue-specific gene modulation favoring normal glucose homeostasis in Cohen-Rosenthal diabetic hypertensive rats

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ARTICLE INFO

Article history:
Received 31 January 2011
Accepted 9 June 2011

ABSTRACT

The objectives were to assess the potential of long-term prophylactic administration of telmisartan, an angiotensin II receptor antagonist and a partial peroxisome proliferator activator receptor (PPAR)γ agonist, in preventing the development of hypertension and hyperglycemia and to demonstrate the alteration in gene expression associated with the development of hyperglycemia and insulin resistance in Cohen-Rosenthal diabetic hypertensive rat, a unique model of hypertension and type 2 diabetes mellitus comorbidity. Cohen-Rosenthal diabetic hypertensive rats were continuously treated with telmisartan (3 mg/[kg d]) starting at age 6 to 8 weeks before developing hypertension or diabetes. Weight changes, blood pressure, blood insulin, adiponectin, glucose tolerance, and insulin sensitivity were monitored. Fat, liver, and muscle messenger RNAs were examined for the expression of genes potentially involved in the onset of insulin resistance. In addition to the expected antihypertensive effect of prophylactic telmisartan, diabetes was blunted, evidenced at the end of the study by a significantly lower glucose level. This was accompanied by improved glucose tolerance, increased sensitivity to insulin, reduction in fasting insulin levels and homeostasis model assessment index, as well as an increase in serum adiponectin. Telmisartan also prevented the increase in serum triglycerides and the associated appearance of lipid droplets in the liver. Diabetes induced tissue-specific changes in messenger RNAs expression of the following selected genes, which were restored by telmisartan treatment: PPARγ, PPARδ, PPARγ coactivator 1α, adiponectin, adiponectin receptor 1, adiponectin receptor 2, phosphotyrosine binding domain and a pleckstrin homology domain-containing adaptor protein, adenosine monophosphate kinase, and glucose translocator 4. Telmisartan blunted the development of hypertension, insulin resistance, and diabetes in prediabetic Cohen-Rosenthal diabetic hypertensive rats through pleiotropic activity, involving specific gene regulation of target organs.

Authors' contribution: Firas Younis: designed and performed experimental procedures, data analysis, and writing of the paper. Yoram Oron: closely followed each step of the experiment; contributed to data analysis and revising/editing of article writing. Rona Limor: performed part of the experiments; proof-read the paper. Naftali Stern: data analysis and the medical significance of the findings. Talma Rosenthal: closely followed each step of the experiment; contributed to the writing and scientific assessment of the paper.

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0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.06.007

1. Introduction

The co-occurrence of hypertension and diabetes accelerates complications of individual pathologies and constitutes a major independent risk factor for cardiovascular disease [1].

Recent animal model studies of type 2 diabetes mellitus (T2DM) with obesity demonstrated that blockade of the renin/angiotensin system mitigated β-cell damage and improved function [2-4]. Through a different pathway, activation of peroxisome proliferator activator receptor (PPAR)γ by thiazolidinediones was shown to increase adiponectin and concomitantly reduce hyperglycemia and insulin resistance. Hyperinsulinemia and the ensuing insulin resistance due to malfunction of tissue response to insulin and glucose utilization are key factors in the development of diabetes. Insulin-sensitizing properties of adiponectin through adiponectin receptor (AdpR)1 and AdipR2 were demonstrated through glucose uptake and utilization in vivo and in vitro [5-7]. Peroxisome proliferator activator receptor γ is predominantly expressed in fat, where it mediates transcriptional activation of genes involved in the regulation of glucose homeostasis, lipid uptake, and lipogenesis [8]. Telmisartan, in addition to blocking the angiotensin II (Ang II)-1 receptor, activates PPARγ and induces hypoglycemia along with adiponectin upregulation [9-11]. In addition, telmisartan was seen to ameliorate insulin secretion through the reduction of Ang II–induced oxidative stress, thus improving hyperglycemia [12]. The advantage of telmisartan over other Ang II receptor blockers in ameliorating insulin resistance and metabolic parameters, including adiponectin, was demonstrated in clinical and experimental studies [13-15]. In our previous study [16], we have demonstrated the beneficial effects of telmisartan on the putative long-term changes in the physiology and metabolism of animals having both hypertension and diabetes. Thus, it was interesting to further investigate the prophylactic treatment with telmisartan's specific mechanism of action before the onset of hypertension and diabetes, while avoiding the putative changes induced by chronic hypertension and diabetes. The activation of other isoforms of PPARs, PPARα and PPARδ, was recently implicated in telmisartan effects on hyperglycemia, insulin resistance, and fatty acid (FA) accumulation. Peroxisome proliferator activator receptor α is expressed primarily in the liver and plays a critical role in the regulation of uptake, activation, and β-oxidation of FAs [17]. In addition, a recent study showed that telmisartan improved dyslipidemia by increasing the expression of PPARδ and several lipolytic and energy uncoupling-related proteins in fat and muscle [18,19]. Our experimental tool, Cohen-Rosenthal diabetic hypertensive (CRDH) rats, a nonobese animal model that develops both hypertension and type 2 diabetes mellitus after nearly 2 to 3 months of sugar-rich copper-poor diet feeding. [20], is an ideal in vivo model for studying prophylactic/monotherapy treatment of both pathologies. This study was carried out to elucidate the mechanisms of telmisartan-induced hypoglycemic effect by examining changes in messenger RNA (mRNA) expression of genes responsible for energy expenditure and glucose homeostasis in 3 insulin-resistant target tissues: fat, liver, and muscle.

2. Material and methods

2.1. Animals

Two groups of in-house–bred CRDH rats (n = 12 each), 6 to 8 weeks old and weighing 150 to 180 g, were fed a specially prepared sugar-rich copper-poor diet (SD) containing 18% casein, 72% sucrose, 4.5% butter, 0.5% corn oil, 5% salt No. II USP, and nearly 0.5% water and fat-soluble vitamins [20,21]. Along with the introduction of the diet, one group received freshly prepared telmisartan in drinking water adjusted to 3 mg/(kg d) (Tel group); and the second group, a vehicle (Veh group), received tap water only. Animals' weight, blood pressure (BP) and blood glucose levels (BGLs) were monitored at 10-day intervals. The humoral parameters—insulin, homeostatic model assessment (HOMA) index, adiponectin, triglycerides, and oral glucose tolerance test (OGTT)—were monitored at 2 and 4 months posttreatment, and insulin tolerance test (ITT) cytokines levels were assessed at the end of the experiment. A third group (n = 8) of nondiabetic CRDH rats (Control group) was given regular chow ad libitum as control for the gene expression experiments. At the end of the experiment, 4 months posttreatment, animals were euthanized by an overdose (50 mg/kg) intraperitoneal injection of pentobarbital. Rats were maintained on a 14-hour–light/10-hour–dark cycle at 23°C and approximately 50% humidity, in accordance with guidelines approved by the Institutional Animal Care Committee at Tel Aviv University.

2.2. BP and humoral parameters

2.2.1. Blood pressure

Systolic and diastolic BP was measured before treatment and periodically throughout the study using a noninvasive tail-cuff sphygmomanometer (BP-2000 series II; Visitech Systems, Apex, NC). Fully alert animals were placed in a restraining holder on a platform maintained at 37°C while the tail was inserted into an occlusion tail cuff and placed on a light emitter/sensor assembly. Animals were adapted to the setting until calmed. The mean value of at least 10 consecutive measurements for each rat (standard deviation <10%) was used to calculate the overall mean per group at the desired time points. Analysis, processing, and reporting of data were done with BP-2000 software.

2.2.2. Blood glucose level

Animals in all groups were habituated to morning feeding by 3 successive days of overnight fasting and feeding in early hours of the morning. On the fourth day, 2 hours post–SD feeding; the animals' BGL was determined. Starting after 2 months on the SD diet, blood was collected for assessment of glucose level from a punctured distal end of the tail at weekly intervals using a standard glucometer (Bayer, Leverkusen, Germany).

2.2.3. Blood sampling

After 2 and 4 months of treatment, overnight-fasted animals were lightly anesthetized with isoflurane (Rhodia Organique Fine, Bristol, UK); and blood was drawn from the retroorbital

sinus. Collected blood, 1 to 2 mL, was allowed to clot for an hour and then centrifuged at 3500 rpm for 25 minutes. Sera were stored at –80°C.

2.2.4. *Humoral parameters analysis*

Serum adiponectin and insulin levels were determined using rat radioimmunoassay kits (Linco Research, St Charles, MO). Insulin resistance was evaluated using the HOMA index ([fasting insulin {microunits per milliliter} × fasting glucose {milligrams per deciliter}]/405). Triglycerides levels were evaluated spectrophotometrically using glycerol phosphate oxidase/peroxidase reaction (Biosystems Reagents & Instruments, Barcelona, Spain).

2.2.5. *OGTT and ITT*

After 2 and 4 months of treatment, overnight-fasted animals in all groups were gavage-fed glucose solution at 7 mL/kg body weight (final dose, 3.5g/kg) for OGTT assessment. Blood glucose level was determined at 0, 15, 30, 60, 120, and 180 minutes post–glucose loading. At the end of the experiment, at 4 days post-OGTT, insulin tolerance was evaluated on fasted animals of both Tel and Veh groups by intraperitoneal administration of 0.45 IU/kg insulin (Elli & Lilly, Indianapolis, IN); and BGL was determined at 5, 15, 30, 60 and 120 minutes.

2.2.6. *mRNAs expression*

Fat (epididymal), liver, and muscle (diaphragm) samples were snap-frozen in liquid nitrogen and stored at –80°C. Polytron was used for fat and liver homogenization, and precooled mortar and pestle were used for grinding frozen muscle. RNA was extracted by EZ RNA II Total RNA extraction kit (Biological Industries, Beit Haemek, Israel). RNA concentration was determined using ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). One microgram of RNA was reverse transcribed using random hexamers, using a high-capacity complementary DNA reverse transcription kit (Applied Biosystems, Foster City, CA). Messenger RNA expression levels were determined with StepOnePlus real-time polymerase chain reaction (40 cycles) (Applied Biosystems).

Taqman primers and probes were designed and manufactured using Primer Express (Applied Biosystems) for the following: glyceraldehyde 3-phosphate dehydrogenase

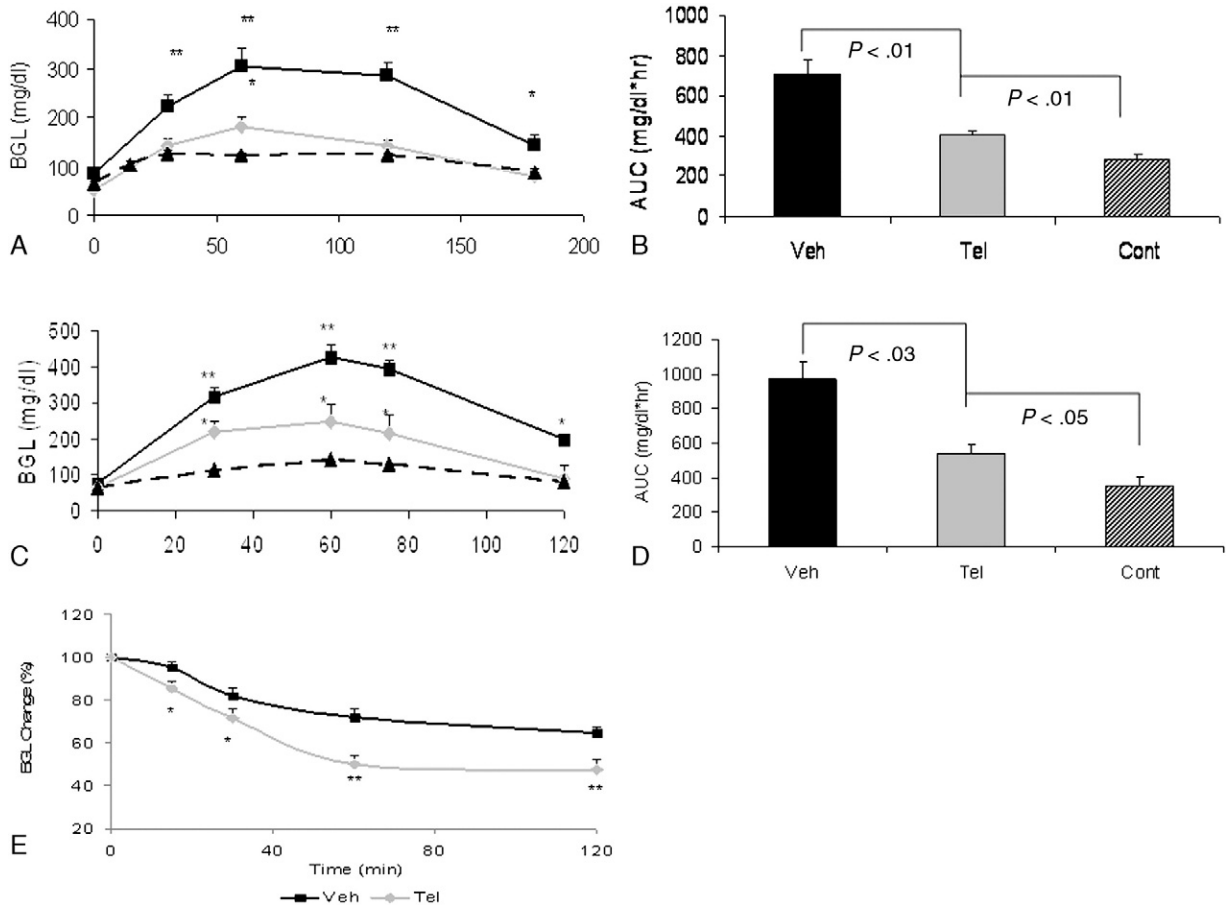

Fig. 1 – Glucose tolerance as measured by OGTT after 2 (A) and 4 (C) months of treatment at specific time intervals following gavage administration of glucose solution. Corresponding area under the curve after 2 (B) and 4 months (D) of SD feeding/ treatment was evaluated by the trapezoidal method of calculation, and significance was determined using 1-way ANOVA with Bonferroni correction test. The ITT (E) presents the percentage decrease of glucose post insulin intraperitoneal administration. Data expressed as percentage relative to baseline (fasting BGL at time 0). Student *t* test was performed, and $P < .05$ was considered significant.

(GAPDH, Rn99999916_sl), adiponectin (Rn00595250_ml), AdpR1 (Rn01483784_ml), AdpR2 (Rn01463117_ml), adenosine monophosphate kinase (AMPK, Rn005576935_ml), glucose translocator 4 (Glut4, Rn00562597_ml), PPARγ (Rn00440945_ml), PPARα (Rn00566193_ml), PPARδ (Rn00565707_ml), PPARγ coactivator 1α (PGC1α, Rn00580241_ml), and phosphotyrosine binding domain and a pleckstrin homology domain-containing adaptor protein (APPL1, Rn01401841_ml). A complementary DNA equivalent to 80 ng of total RNA was used in the polymerase chain reaction. Messenger RNAs expression levels were expressed as comparative amplification threshold cycle (C_T) normalized to the GAPDH C_T in the same sample. The fold changes of the Tel and the Veh groups were assessed relative to the control group. The fold change was expressed on a logarithmic scale.

2.3. *Statistical analysis*

The differences between the treated group and baseline or previous measurements were analyzed by paired Student *t* test and 1-way analysis of variance (ANOVA) followed by the Bonferroni correction test; the differences between the treated group and the control/vehicle groups were analyzed by the unpaired *t* test. Messenger RNA expression data were calculated with DataAssist version 2 (supplied by Agentek, Tel Aviv, Israel) computer software. *P* values < .05 were considered significant.

3. Results

Prophylactic treatment with telmisartan largely prevented the development of hypertension (see online supplementary materials). The present results demonstrated that telmisartan-treated animals, despite normal food intake, achieved reduced weight gain as compared with corresponding nondiabetic controls and reached a plateau of nearly 300 g (see online supplementary materials), a value close to that reported for age-matched diabetic animals [16].

Blood glucose level was assessed 2 hours postprandial at 2 and 4 months on the SD. The prophylactic treatment with telmisartan achieved a significant ($P < .05$) decrease in BGL, which was, however, still higher than in control nondiabetic CRDH rats (see online supplementary materials).

At 2 and 4 months, assessment of glucose homeostasis by OGTT showed marked and increasing glucose intolerance in the Veh group when compared with the telmisartan-treated group (peak BGL, 303 ± 37 vs 181 ± 19 and 428 ± 45 vs 249 ± 30 mg/dL at 1 hour post–glucose loading, respectively; Fig. 1A, C). These values were, however, still higher than those of the control animals (Fig. 1A, C). The area under the curve further emphasized the significant reduction in tolerance to glucose loading in the Veh vs the Tel group and the even lower values in the control nondiabetic group ($P < .05$; Fig. 1B, D). These findings demonstrate a significant improvement in glucose tolerance provided by prophylactic treatment with telmisartan.

To further assess the therapeutic effect of prophylactic telmisartan, we tested the response to intraperitoneal challenge with 0.45 IU/kg insulin at 4 months of treatment (Fig. 1E).

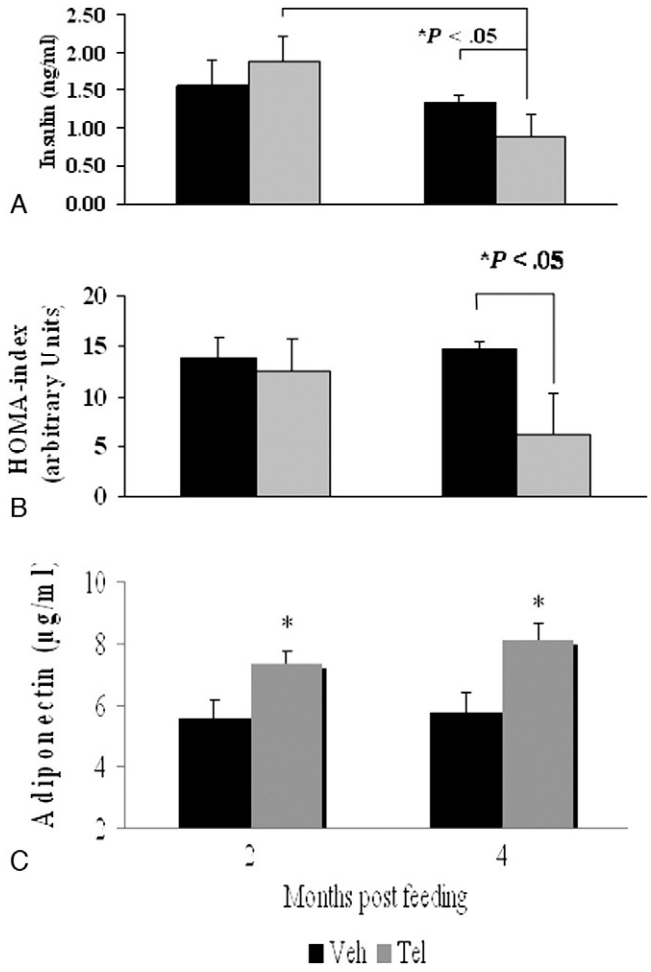

Fig. 2 – Levels of insulin (A) , HOMA index (B), and adiponectin (C) in the Tel and Veh groups after 2 and 4 months of SD feeding and treatment. Data presented as mean ± SEM. Student *t* test was performed, and $P < .05$ was considered significant.

After 1 hour, the BGL in the Tel group dropped to nearly 50% of baseline and remained at the same value for another hour. The response to insulin in the Veh group was less prominent (~25%), and the BGL was significantly higher ($P < .05$) than that in the Tel group at all time points.

Treatment with telmisartan did not affect the high insulin levels at 2 months, but significantly ($P < .05$) blunted the increase in serum insulin after 4 months of treatment (Fig. 2A). This reflected the increased insulin sensitivity evidenced by the significant ($P < .05$) HOMA index between the Veh and the Tel groups at this time point (Fig. 2B). Adiponectin levels (Fig. 2C) were significantly ($P < .05$) higher in the Tel group, reaching 7.33 ± 0.57 and 8.12 ± 0.64 μg/mL at 2 and 4 months of treatment, respectively, compared with the levels in the diabetic Veh group (<6 μg/mL).

Triglycerides serum levels (Fig. 3A) were significantly lower ($P < .05$) in the Tel group compared with the Veh group at 4 months of treatment: 99.83 ± 11.4 vs 150.19 ± 19.7 mg/dL, respectively. The accumulation of lipid droplets observed in the liver sections of the Veh animals was not seen in the Tel group (Fig. 3B, C).

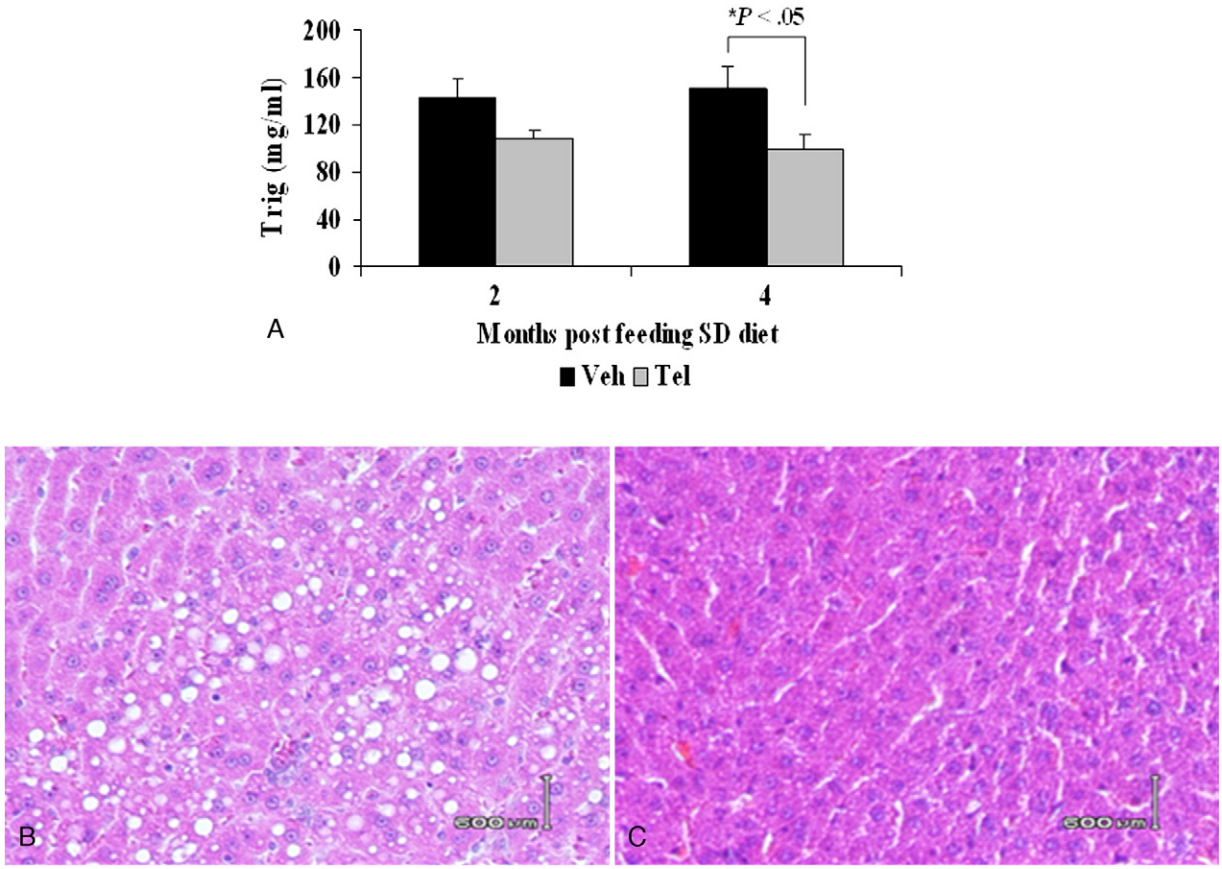

Fig. 3 – Levels of serum triglycerides (A) and histology section of liver from diabetic (B) and telmisartan-treated (C) CRDH rats stained with hematoxylin and eosin. The accumulation of fat/lipid droplets seen in the Veh animals was prevented in the Tel group. Magnification ×40. Data presented as mean ± SEM. Student *t* test was performed, and $P < .05$ was considered significant.

3.1. *mRNA expression of selected glucose homeostasis-related genes*

The relative levels of expression of mRNAs of the following genes were examined: adiponectin, AdpR1, AdpR2, AMPK, Glut4, PPARγ, PPARα, PPARδ, PGC1α, and APPL1. Assay in tissues of control nondiabetic animals clearly indicated that the relative abundance of these mRNAs was tissue specific (Table 1). The mRNAs for adiponectin, PPARγ, PPARδ, and APPL1 were more abundant in fat; AMPK and PPARα were more abundant in liver, whereas muscle exhibited no specific abundance of any of the tested mRNAs, suggesting a different role of each tissue in the regulation of glucose and insulin sensitivity.

To assess the effect of diabetes and of telmisartan treatment, we compared expression levels of these mRNAs in fat, liver, and muscle tissues of Veh and Tel animals. Gene expression in the different tissues was divided into 3 categories: mRNAs of proteins involved in transcription regulation (Fig. 4A-C), mRNAs of proteins whose transcription level is regulated by the above transcription factors (Fig. 4D-F), and mRNAs of downstream proteins associated with the glucose-insulin pathway (Fig. 4G-I).

The expression of PPARα mRNA did not significantly change in either tissue of the Tel and Veh animals. The PPARγ mRNA decreased nonsignificantly in fat and significantly in liver in the Veh group, and treatment with telmisartan resulted in its elevation above nondiabetic control levels (Fig. 4A-C). The PPARδ mRNA increased in diabetic animals, albeit insignificantly, and was further elevated in all 3 tissues to significant levels by telmisartan treatment. The PGC1α mRNA was elevated in all tissues in diabetic animals

Table 1 – The $\delta C_T + 20$ values of the mRNA expression in fat, liver, and muscle tissues of control nondiabetic animals (n = 8)

	Adip	AdpR1	AdpR2	APPL1	AMPK	Glut4	PPARα	PPARγ	PPARδ	PGC1α
Fat	15.17 ± 0.24	22.51 ± 0.3	22.71 ± 0.48	23.76 ± 0.63	25.34 ± 0.52	24.91 ± 0.40	27.69 ± 0.13	22.28 ± 0.22	25.30 ± 0.62	29.76 ± 0.45
Liver	27.01 ± 1.16	23.47 ± 0.26	22.53 ± 0.25	27.27 ± 0.4	23.50 ± 0.43	31.86 ± 0.29	25.74 ± 0.89	27.81 ± 0.37	27.20 ± 0.28	28.31 ± 0.35
Muscle	23.79 ± 0.67	24.69 ± 0.30	25.49 ± 0.53	28.73 ± 0.41	25.13 ± 0.35	24.15 ± 0.32	27.21 ± 0.83	28.66 ± 0.63	28.27 ± 0.45	28.62 ± 0.10

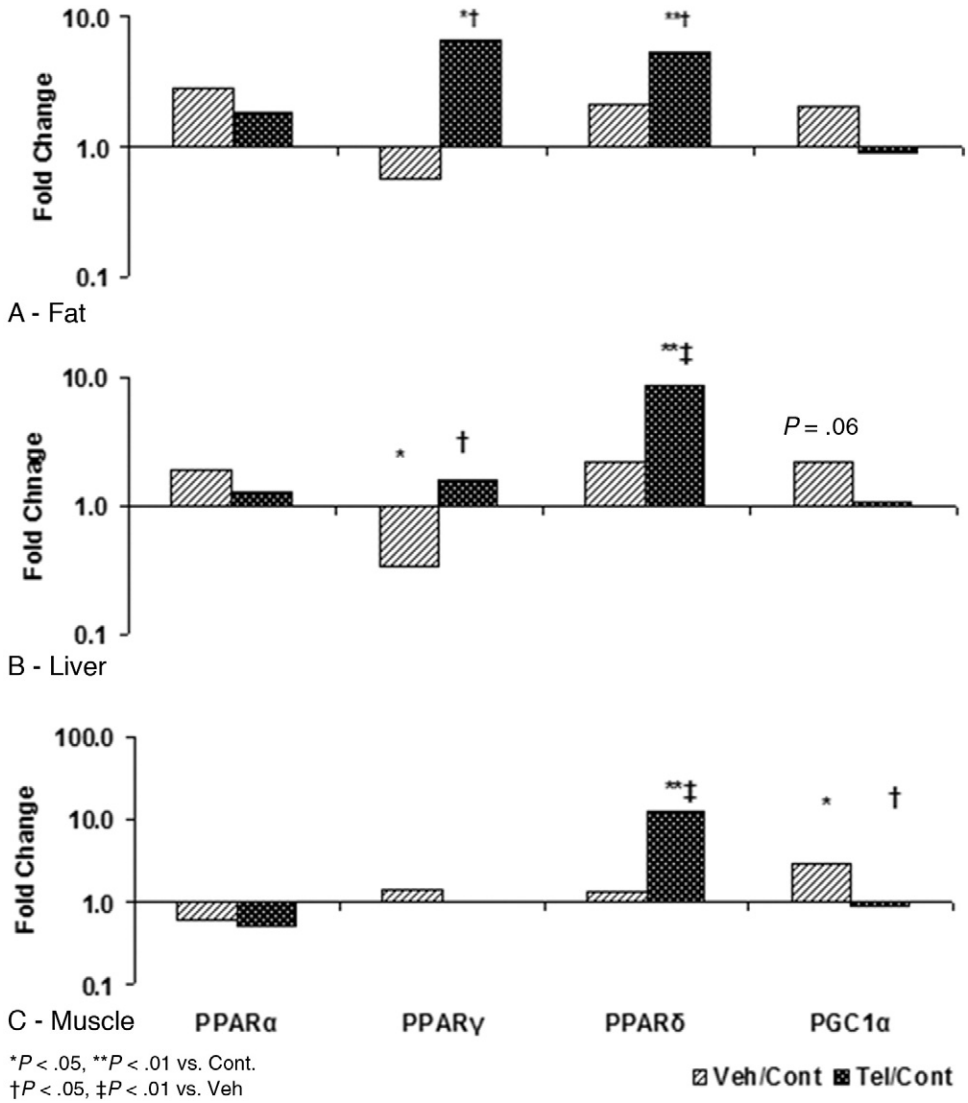

Fig. 4 – Fold change of mRNA expression of the different genes determined by the ratio of $2^{(-\Delta CTtreat)}/2^{(-\Delta CTcont)}$ post normalizing to the endogenous gene GAPDH, giving a net fold change relative to control animals. A to C, The expression of PPARs family and PGC1α in fat, liver, and muscle tissue. D to F, Gene modulation of adiponectin, AdipR1, AdipR2, and APPL1 in fat, liver, and muscle, respectively. G to I, The expression of 2 effector molecules, AMPK and GLUT4, in fat, liver, and muscle tissue, respectively. Significance was calculated vs control as well as the Veh group. One-way ANOVA analysis was used, and P < .05 was considered significant.

(significantly only in muscle), whereas in the Tel group, it remained equivalent to control values.

In the second category (Fig. 4D-F), the most notable changes were seen in the adiponectin mRNA levels, which decreased significantly in fat and liver, but increased in muscle tissue in the diabetic Veh group. Telmisartan treatment resulted in the restoration of adiponectin mRNAs levels to corresponding tissues' basal values of control nondiabetic animals. The change induced in AdpR1 mRNA in the Veh group was limited to a significant decrease in fat, whereas in the Tel group, it was restored to control levels. Diabetes in the Veh group did not induce changes in AdpR2 mRNA in any of the tissues. The AdipR2 mRNA was significantly higher in liver in the Tel group than in the Veh group, whereas it was significantly decreased in the muscle. The APPL1 mRNA levels were significantly decreased in liver but not altered in fat and muscle of diabetic animals. In contrast, the APPL1 mRNA exhibited significant increase in fat and muscle of the Tel group compared with control animals.

In the group of downstream genes (Fig. 4G-I), AMPK mRNA was not significantly affected by diabetes, whereas it was significantly increased in all 3 tissues in the Tel group. The Glut4 mRNA was significantly decreased in fat in the Veh group and significantly increased in fat and muscle in the Tel group compared with control levels.

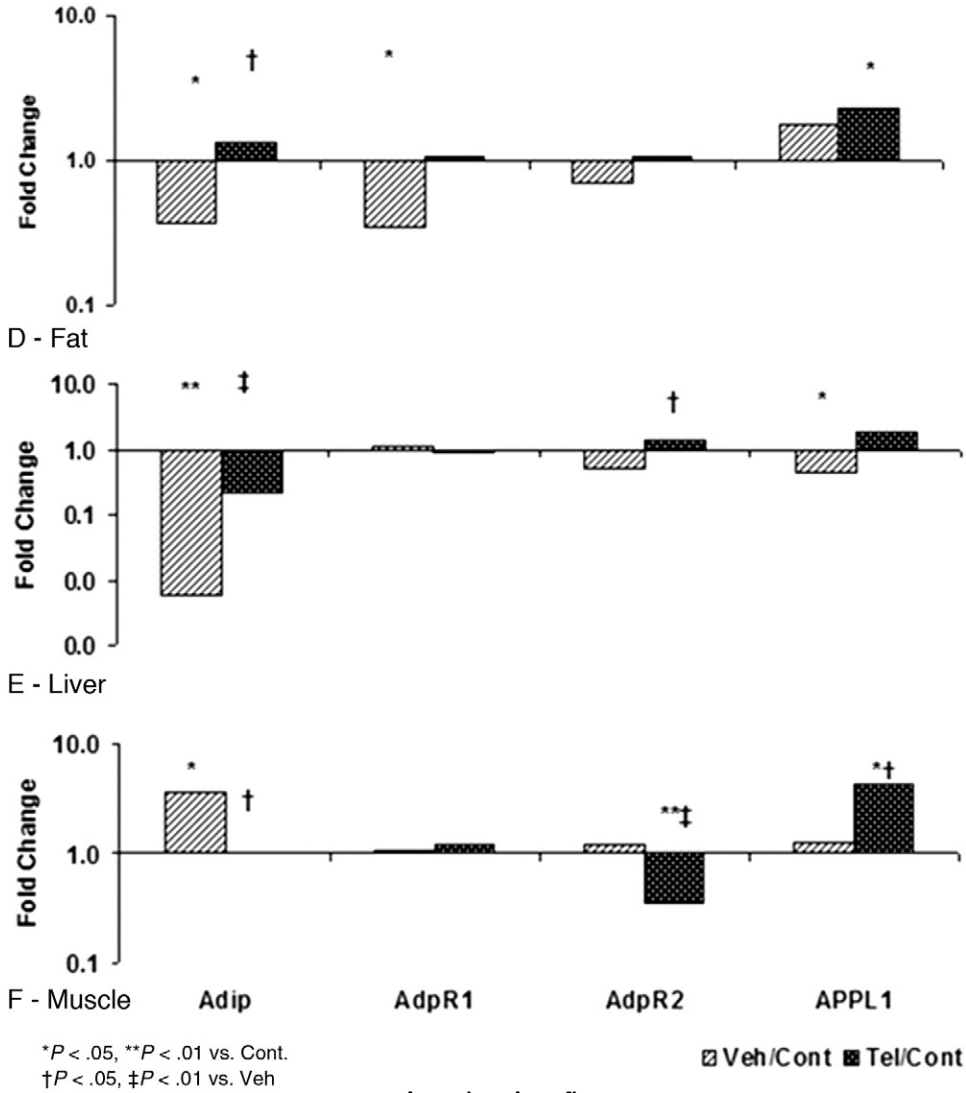

Fig. 4 *(continued)*

4. Discussion

In the present study, prophylactic treatment with telmisartan significantly blunted the development of hypertension and diabetes, evidenced by glucose tolerance and insulin sensitivity levels nearing those of nonhypertensive, nondiabetic controls. Telmisartan markedly lowered the development of insulin resistance, as evidenced by decreased fasting insulin, BGL, and HOMA index; improved tolerance to glucose; and increased insulin response. The improved insulin-glucose homeostasis in telmisartan-treated CRDH rats was accompanied by elevated serum adiponectin. The expression of mRNAs of selected genes—PPARα, PPARγ, PPARδ, APPL1, adiponectin, AdipR1 and AdipR2, AMPK, and Glut4—exhibited tissue-specific changes upon developing hypertension-diabetes comorbidity. These changes demonstrate the involvement of telmisartan-induced PPARγ activation and adiponectin-associated increased insulin sensitivity in fat. In the liver and muscle, the mechanism of glucose regulation may involve other key molecules including PPARδ.

Despite the finding that Ang II infusion increased hypertension and lowered serum adiponectin levels [22], we have shown in a previous study an increase in adiponectin levels in diabetic animals treated with telmisartan or rosiglitazone, but not with valsartan, an Ang II receptor blocker lacking PPARγ agonist properties [16]. In view of PPARγ transcriptional control of adiponectin expression [23,24] and the decrease in adiponectin mRNA in fat and liver, our findings support the proposed mechanism of antihyperglycemic effect of telmisartan as a partial agonist of PPARγ. However, unlike the weight gain associated with treatment with full PPARγ agonist, telmisartan has been reported to increase energy expenditure and lower body weight [25,26]. In this study, the weights of the Tel and the Veh groups were significantly lower than those of the nondiabetic control animals, which received regular chow. However, in comparison to the Veh group, the Tel group showed a slight improvement in weight gain, which could be

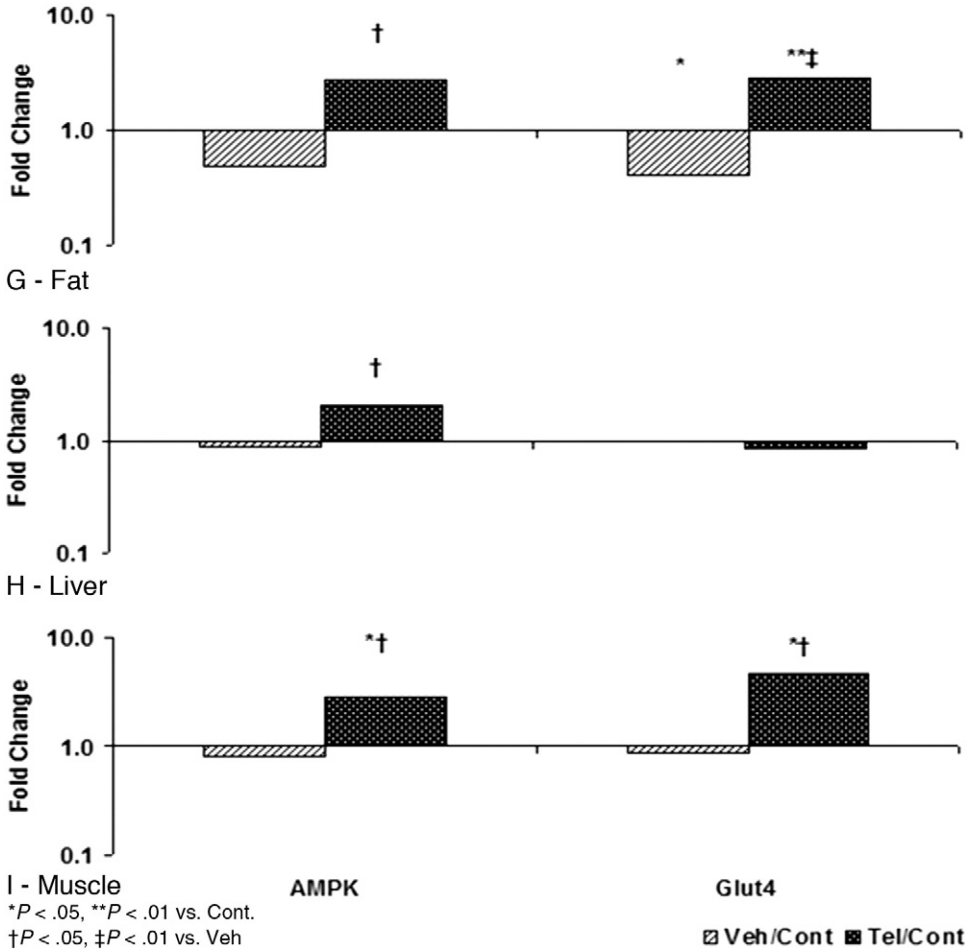

Fig. 4 (*continued*)

attributed to improved well-being of the animals. In a preventive study in diabetic Torii rats, telmisartan prevented hyperglycemia by lowering the transcription of renin-angiotensin system components associated with Ang II–induced oxidative stress [12], suggesting that telmisartan can affect hypoglycemia via different, model-dependent mechanisms. In addition, treated Torri animals showed a significant weight gain over diabetic animals, an indication of the link between weight gain and improved glycemic control.

It has been reported that PPARα and PPARδ are also key players in the regulation of genes involved in glucose, FA, and cholesterol metabolism [27]. In the present study, PPARγ and PPARδ mRNAs expression was enhanced by telmisartan in fat and liver, whereas only PPARδ mRNA was upregulated in muscle. Although telmisartan has been shown to increase hepatic PPARα protein and to act as a PPARα partial agonist [28], the expression of PPARα mRNA was not significantly affected by either diabetes or telmisartan in our study.

Messenger RNA of PGC1α, a PPARγ cofactor [29], increased in muscle and to a lesser extent in fat and liver in diabetic animals—an effect that was fully prevented by telmisartan treatment. PPARγ coactivator 1α integrates metabolic pathways promoting hepatic gluconeogenesis, β-oxidation, and mitochondrial function, increasing insulin-independent glucose uptake and metabolism [30,31], and activates the transcription of Glut4 [32]. Thus, in diabetic CRDH rats, the upregulation of PGC1α could counteract insulin resistance and hyperglycemia; and when these conditions were prevented by telmisartan, PGC1α expression diminished to normal levels. The decreased glucose transport in diabetic animals could result in PGC1α-promoted increased gluconeogenesis, the need for which (including increased PGC1α transcription) is absent in telmisartan-treated normoglycemic animals. This is in agreement with induced hepatic and pancreatic PGC1α expression and gluconeogenesis in mouse models of insulin resistance and T2DM [32,33].

Phosphotyrosine binding domain and a pleckstrin homology domain-containing adaptor protein, which interacts with the cytoplasmic domains of AdipoRs, has been shown to double adiponectin binding and its effects [34], including AMPK activation, glucose uptake, and β-oxidation [35]. Knockdown and overexpression of APPL1 demonstrated APPL1's central role in AdipoRs signaling to their effectors genes, including AMPK and p38MAPK [36]. Our findings of increased APPL1 mRNA expression in fat of diabetic animals suggest compensation for adiponectin and AdpR1 mRNAs downregulation. Interestingly, the increase persisted in telmisartan-treated rats

despite the return of adiponectin and AdpR1 mRNAs to control levels. In liver and muscle, telmisartan caused elevation of APPL1 mRNA compared with diabetic animals, suggesting that telmisartan improves adiponectin in these tissues.

Adenosine monophosphate kinase is one of the probable targets of the major antidiabetic drugs, biguanides and thiazolidinediones, and of insulin-sensitizing adipokines [37]. Adenosine monophosphate kinase enhances insulin-independent uptake of glucose, controlled by the cellular ratio of AMP to adenosine triphosphate [5]. In our study, the expression of AMPK mRNA was upregulated by telmisartan compared with both control and diabetic animals. Similarly, Glut4 mRNA was downregulated in fat of diabetic animals and elevated in fat and muscle of telmisartan-treated animals. These findings complement previous reports on telmisartan as well as an adiponectin-induced increase in the expression of Glut4 at the transcriptional and protein levels and its translocation to basement membrane [24,34,38].

4.1. *AdipoR1 and AdipoR2*

The expression of adiponectin, which decreases in obesity, lowers hepatic glucose output and enhances glucose uptake and utilization in fat and muscle [39], whereas targeted disruption of AdipRs interferes with adiponectin signaling [40,41]. In our study, the most pronounced effect of telmisartan was the increase in serum adiponectin, matched by the abrogation of the diabetes-associated decrease in its mRNA in fat and liver, and further prevented the diabetes-associated decrease in AdpR1 mRNA in fat, leading to improved fat metabolism. Treatment of hypertensive patients with telmisartan induced an insulin sensitization effect together with an increase in adiponectin [15,42], whereas only a trend of increase was seen in normotensive patients [43,44]. Interestingly, muscle adiponectin mRNA increased in diabetes and returned to control levels upon telmisartan treatment, whereas AdpR2 mRNA level decreased. A possible explanation could be a concordant regulation of AdpR2 in muscle by adiponectin.

4.2. *Adiponectin-mediated insulin sensitivity*

4.2.1. *Fat*

Telmisartan was shown to enhance insulin sensitivity through PPARγ-mediated expression of adiponectin [45,46]. In the present study, the telmisartan-induced modulation of mRNAs of selected target genes in fat is compatible with the direct activation of PPARγ or direct activation via adiponectin [47,48]. Telmisartan-induced restoration of adiponectin and AdpRs mRNAs and upregulation of APPL1 mRNA indicate a causal role of adiponectin; the regain of insulin sensitivity proceeds via the PPARγ-adiponectin pathway. However, the increase of PPARδ mRNA in fat tissue should not be ignored in view of the recent findings that PPARδ agonists reduce adiposity and improve glucose tolerance and insulin sensitivity in different mouse models of obesity [49,50].

4.2.2. *Liver*

In liver, AdipoR1 increases AMPK activity, thereby affecting gluconeogenesis, whereas AdipoR2 is involved in the activation of the nuclear receptor PPARα and its downstream target genes, mediating β-oxidation and reactive oxygen species scavengers [51]. Adiponectin mRNA, which is expressed in very low amounts in the liver [5], was significantly reduced by diabetes but was restored to baseline levels by telmisartan. This was accompanied by a modest increase in AMPK mRNA, leading to improved glucose metabolism. The effect of adiponectin, or globular adiponectin, was shown to proceed via AMPK/Glut4 activation/translocation: it reduced glucose output in the liver [52] and stimulated glucose uptake in rat adipocytes [53], whereas the absence of AMPKα2 subunit inhibited adiponectin-induced hepatic glucose output [5].

Telmisartan was recently identified as a partial PPARα agonist, at least in the liver, suggesting that its effects were not restricted to PPARγ activation alone [28,54]. Peroxisome proliferator activator receptor α agonists enhanced insulin sensitivity due to increased FA oxidation in liver, muscle, and pancreas in rodent models of insulin resistance [55]. In our study, telmisartan caused no significant change in the expression of PPARα mRNA, but did prevent the increase in serum triglycerides in diabetic animals. Telmisartan also prevented the accumulation of lipid droplets (ie, lipotoxicity) in the liver. These findings are in agreement with those of telmisartan-treated mice with obesity induced by a high-fat diet [18,28]. Although little is known about the liver function of PPARδ, hepatic PPARδ mRNA was also upregulated in telmisartan-treated CRDH rats in our study. Peroxisome proliferator activator receptor δ has been shown to reduce hepatic glucose output and enhance FA synthesis in mice [56].

4.2.3. *Muscle*

Telmisartan, unlike losartan, enhanced glucose utilization and improved insulin sensitivity through PPARγ in skeletal muscle [57]. These effects could be attributed to the action of adiponectin; telmisartan normalized adiponectin and AdipoR1 to control levels, but induced a decrease in AdipoR2 levels. This may suggest that adiponectin-mediated effects proceed via AdpR1, which is preferentially expressed in muscle over AdipoR2. The role of AdipoR1, but not of AdipoR2, in AMPK activation, glucose uptake, and β-oxidation was demonstrated in vitro by AdipoR1 knockdown [41], whereas muscle-AdipR1 knockout mice further confirmed AdipR1's role in mitochondrial dysfunction [51]. A concomitant increase in APPL1, AMPK, and GLUT4 mRNAs implies that adiponectin is an important mediator in the determination of muscle insulin sensitivity, despite its normal levels of mRNA. In this context, the Tel-induced decrease in AdipoR2 mRNA remains to be clarified. It is possible that the observed changes in muscle mRNAs expression of selected genes in telmisartan-treated CRDH rats reflect increased transcription of PPARδ rather than PPARγ mRNA. Long-term administration of telmisartan enhanced persistent postexercise oxygen consumption and promoted the production of slow-twitch skeletal muscle fibers in wild-type but not in PPAR-δ–deficient mice, suggesting a PPAR-δ/AMPK pathway [58] as a novel antidiabetes therapeutic target.

The strength of the present study is in 3 key conclusions: Firstly, in the clinically relevant model of diabetes/hypertension comorbidity, prophylactic telmisartan caused significant changes in the expression of selected genes involved in glucose-insulin homeostasis, most of which were in the

opposite direction to those found in untreated animals. It is likely, therefore, that the antidiabetic effect of telmisartan is linked to its effect on gene expression. Secondly, in telmisartan's antidiabetes activities, fat appears to be the target tissue, with only minor effects in liver and muscle. Finally, we observed a significant rise of PPARδ in all examined tissues, implying its involvement in the regulation, at least in part, of tissue glucose homeostasis.

The limitation of this study is that the findings relevant to gene expression cannot be further studied by knockout technology in rats. To examine the specific role of each of the genes, knockout should be further pursued in mice.

In conclusion, we have shown that prophylactic treatment with telmisartan prevents the onset of diabetes through gene expression modifications that promote enhanced insulin sensitivity in energy-demanding organs: fat, liver, and muscle. The modulation of gene expression supports a PPARγ/adiponectin-mediated pathway, predominantly in fat but also in liver and muscle, with the added benefit of telmisartan-induced expression of PPARδ. Telmisartan has been shown here to have a pleiotropic effect on various tissues, preventing lipotoxicity and glucotoxicity and providing protection against the end-organ damage that invariably accompanies the combination of diabetes and hypertension.

Supplementary materials related to this article can be found online at doi:10.1016/j.metabol.2011.06.007.

Acknowledgment

This study was funded and supported by Boehringer-Ingelheim Micardis Product Pipeline Scientific Support Grant. Supported in part by the Andy Lebach Chair of Clinical Pharmacology and Toxicology at Tel Aviv University (Y.O. – Incumbent).

Conflict of Interest

The authors confirm that there are no conflicts of interest.

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