Telmisartan But Not Valsartan Increases Caloric Expenditure and Protects Against Weight Gain and Hepatic Steatosis

Ken Sugimoto, Nathan R. Qi, Ludmila Kazdova, Michal Pravenec, Tosho Ogihara, Theodore W. Kurtz

Abstract—The potential effects of angiotensin II receptor blockers (ARBs) on adipose tissue biology and body weight are of considerable interest, because these agents are frequently used to treat hypertension in patients who are prone to visceral obesity, the metabolic syndrome, and diabetes. In rats fed a high-fat, high-carbohydrate diet, we compared the effects of 2 ARBs, telmisartan and valsartan, on body weight, food intake, energy expenditure, fat accumulation, fat cell size, and hepatic triglyceride levels. Telmisartan, but not valsartan, promoted increases in caloric expenditure and protected against dietary-induced weight gain. In the telmisartan-treated rats, absolute food intake, but not food intake adjusted for body weight, was lower than in valsartan-treated rats or controls. Telmisartan reduced the accumulation of visceral fat and decreased adipocyte size to a much greater extent than valsartan and was also associated with a significant reduction in hepatic triglyceride levels. Moreover, telmisartan, but not valsartan, increased the expression of both nuclear-encoded and mitochondrial-encoded genes in skeletal muscle known to play important roles in mitochondrial energy metabolism. Thus, in addition to a class effect of ARBs in modulating adipocyte size, these findings raise the possibility that certain molecules, like telmisartan, may have a particularly strong impact on fat cell volume and fat accumulation, as well as distinctive effects on energy metabolism, that may help protect against dietary-induced visceral obesity and weight gain. (Hypertension. 2006;47:1003-1009.)

Key Words: liver ■ hypertension, obesity ■ receptors, angiotensin II

In patients with the metabolic syndrome, diabetes, or both, increased visceral fat is believed to play an important role in the pathogenesis of insulin resistance and dyslipidemia. Although surgical removal of subcutaneous abdominal fat may have little or no effect on glucose or lipid metabolism, surgical removal of intra-abdominal visceral fat can improve insulin sensitivity. Moreover, pharmaceutical treatments that redistribute fat from visceral to subcutaneous depots can also improve insulin resistance and dyslipidemia. For example, thiazolidinedione ligands of the peroxisome proliferator–activated receptor γ (PPARγ) are thought to improve carbohydrate and lipid metabolism at least in part by promoting the differentiation of small adipocytes that buffer against inappropriate deposition of fat in muscle and visceral tissues and that are more metabolically efficient than large, hypertrophied adipocytes. Given that abdominal obesity and the metabolic syndrome are very common in patients with hypertension, the availability of antihypertensive agents that retard the accumulation of visceral fat, promote the formation of small, metabolically active adipocytes, and attenuate weight gain could be of considerable clinical value.

Recently, we and others have discovered that telmisartan, an angiotensin II receptor blocker (ARB) approved for the treatment of hypertension, is also a partial agonist of PPARγ. Whereas full agonists of PPARγ, such as rosiglitazone and pioglitazone, promote weight gain while altering fat distribution and adipocyte differentiation, partial agonists (mixed agonists/antagonists) of PPARγ may have the capacity to retard weight gain while promoting adipocyte differentiation. For example, we have found that telmisartan can promote adipocyte differentiation but also attenuate weight gain while improving glucose and lipid metabolism in rats fed a high-fat, high-carbohydrate diet. Sharma et al have reported that blockade of the angiotensin II type 1 receptor, per se, can promote adipocyte differentiation and have proposed that this may contribute to the antidiabetic effects of angiotensin II receptor antagonists. It is unknown whether bifunctional molecules like telmisartan that both activate PPARγ and block the angiotensin II receptor exert different effects on adipocyte size and on the primary determinants of body weight than ordinary angiotensin receptor blockers, such as valsartan, that lack the capacity to activate PPARγ.
In the current studies, we investigated the effects of telmisartan and valsartan on energy intake, energy expenditure, motor activity, body weight, fat accumulation, and fat cell size in rats fed a high-fat, high-carbohydrate diet. Telmisartan, but not valsartan, attenuated weight gain and promoted increases in energy expenditure in the absence of changes in motor activity. Absolute food intake, but not food intake adjusted for body weight, was lower in the telmisartan-treated rats. Moreover, previous studies in pair-fed animals have shown that telmisartan can attenuate weight gain in the absence of any effects on absolute food intake. Thus, the telmisartan-induced attenuation of weight gain may be related to an intrinsic increase in caloric expenditure and not only to decreases in appetite or food absorption or to increases in energy expenditure related to changes in motor activity. Telmisartan reduced the accumulation of visceral and subcutaneous fat and decreased adipocyte size to a much greater extent than valsartan and also significantly reduced hepatic triglyceride levels. These findings suggest that the effects of telmisartan on energy expenditure, adipocyte size, and visceral fat stores may not only involve blockade of the type 1 angiotensin II receptor but perhaps other mechanisms as well and should motivate future studies of its impact on molecular mechanisms regulating dietary-induced weight gain and fat metabolism.

Methods
Male Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, MA) and fed tap water and a high-fat, high-carbohydrate diet (Teklad TD03293, containing 60% fructose, 10% lard, and 0.06% magnesium) starting at 6 weeks of age for 3 months. The rats were individually housed and randomized to 3 groups (10 rats in each group) to receive either telmisartan in drinking water (9.7 μmol/kg body weight [BW] per day), valsartan, or vehicle for 10 days via gavage at the same dose. All rats were fed a high-fat, high-carbohydrate diet (except when tested under fasting conditions). The calorimeter was routinely calibrated each time before use, and the motor activity was counted every second. The air flow rate through the chambers was fixed between 1.95 and 2.75 L/min as needed to ensure that the oxygen differential between inflow and outflow was ≤0.3% at resting conditions as recommended in the technical specifications of the equipment manufacturer. Energy expenditure was calculated as 3.91 VO2 + 1.10 VCO2 −1.93 N (urinary nitrogen), where N is assumed negligible. Respiratory quotient was calculated as a ratio of VCO2 to VO2. The results for both food intake and energy expenditure were scaled according to body weight or three-quarter power of body weight. The group trends and findings were similar with or without three-quarter power scaling, and, for the sake of simplicity, results are presented using the body weight correction without three-quarter power scaling.

Adipocyte Number and Volume
Adipocyte number and volume were determined based on the method of Fine and DiGiorlamo. A portion of adipose tissue (~200 to 300 mg) dissected from the distal end of the right epididymal fat pad was minced and digested in Krebs Ringer bicarbonate buffer (pH 7.4) that contains 4% serum albumin and 1 mg/mL of type I collagenase (Sigma) at 37°C for 30 to 45 minutes. The digested tissue suspension was centrifuged and washed, and the adipocytes were collected. Six aliquots of adipocytes evenly suspended in Krebs Ringer bicarbonate 4% BSA without collagenase were aspirated into 6 hemocytor capillary tubes, respectively, and centrifuged in a hemocytor centrifuge (Adams MHCT II). Lipocrit of each tube was calculated by dividing the volume of packed adipocytes into the total volume of suspension buffer in the tube; lipocrit of each rat was taken from the average readings of 6 tubes. The remaining adipocytes were stained in 1% methylene blue, and 5 aliquots of evenly suspended cells were examined under a microscope equipped with a stage micrometer. The mean diameter of adipocytes was calculated from ≥600 cells using digital images taken at randomly chosen fields in which all of the cells were accounted for in the field measurement. Adipocyte number was determined as the adipocyte volume estimated from the lipocrit divided by the mean adipocyte volume calculated from the average diameter of adipocytes.

Mitochondria-Related Gene Expression Levels
Given the central role of mitochondria in energy metabolism, real-time polymerase chain reaction (PCR) analysis was used to quantify expression levels of several key genes related to mitochondria function in muscle: (1) mitochondrial transcription factor A (TFAM), (2) the transcription factor of mitochondrial DNA expression levels, mitochondria copy number, and mitochondria function, and (2) cytochrome C oxidase subunit 1 (MTCO1), a mitochondrial-encoded enzyme involved in oxidative phosphorylation. The primers for the MTCP1 gene were designed to avoid amplifying mitochondrial DNA–like sequences in genomic DNA as recommended by Garnier et al using an annealing temperature of 60°C. The upstream primer for MTCP1 gene expression analysis was agg agg aat aat agg gac gcg, and the downstream primer was tga gag aag tag tag gac gcc. For amplification of the gene encoding TFAM at an annealing temperature of 53°C, the upstream and downstream primer sequence was cgg ggg aaa tgt gag ctt gc, and the downstream primer sequence was cat tgg ctc ttc cca aga ct. Gene expression levels were assessed in the 10-week-old rats that had been fed a high-fructose and high-fat diet and treated with telmisartan, valsartan, or...
vehicle for 10 days. Total RNA was extracted using Trizol reagent (Invitrogen), and cDNA was prepared and analyzed by real-time PCR testing using QuantiTect SYBR Green reagents (Qiagen, Inc) on an Opticon continuous fluorescence detector (MJ Research) as described previously. Gene expression levels were normalized relative to the expression of \( \beta \)-actin, which served as the internal control, with results being determined in triplicate. The results in the telmisartan-treated rats and valsartan-treated rats were expressed as fold increases relative to the mean normalized expression level of the control rats that was arbitrarily defined as 1.

Biochemical Measurements in Serum and Liver Tissue

Serum samples for glucose, insulin, triglycerides, leptin, and adiponectin were obtained in the nonfasting state and measured as described previously. Frozen liver tissues were powdered under liquid \( \text{N}_2 \) and extracted in chloroform-methanol (2:1 v/v). After adding 2% KH\( \text{PO}_4 \), the solution was centrifuged, and the organic phase was removed and evaporated to dryness under \( \text{N}_2 \). The residue was then dissolved in isopropanol alcohol, and triglyceride content was determined by enzymatic assay (Pliva-Lachema).

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis of metabolic data were performed using ANOVA and the Student–Newman–Keuls test for multiple group comparisons or with equivalent nonparametric tests as required. Statistical analysis of the gene expression data were performed using the REST XL program, which tests for significance by a randomization procedure.

Results

Effects on Body Weight Gain and Organ Fat Accumulation

All of the rats gained weight during the course of the study. However, the weight gain in rats treated with telmisartan was significantly less than in rats treated with valsartan or in controls. Although absolute food intake was lower in the telmisartan-treated rats, food intake adjusted for body weight was similar in all 3 of the groups (Figure 1). Telmisartan-treated rats and, to lesser extent, valsartan-treated rats accumulated significantly less inguinal subcutaneous fat and epididymal visceral fat compared with control, untreated rats (Figure 2). Serum leptin levels reflected the degree of fat accumulation and were reduced to a greater extent in the telmisartan-treated rats than in the valsartan-treated rats (Figure 2). Despite significantly lower fat mass in the telmisartan-treated rats, no differences were detected in serum adiponectin levels among the 3 groups (data not shown). Serum glucose levels were lower in the telmisartan-treated rats, 200 ± 8 mg/dL, compared with the valsartan-treated rats, 236 ± 14 mg/dL (\( P < 0.05 \)), and control rats, 238 ± 11 mg/dL (\( P < 0.05 \)). Insulin levels in the telmisartan-treated rats, 7.9 ± 1 ng/mL, were lower than in the controls, 12.8 ± 1.5 ng/mL (\( P < 0.05 \)) and also tended to be lower than in the valsartan-treated rats, 10.6 ± 1 ng/mL, although the telmisartan versus valsartan comparison did not
achieve statistical significance ($P=0.07$ in single $t$ test). Triglyceride levels in the telmisartan-treated rats, $340\pm55$ mg/dL, and the valsartan-treated rats, $370\pm51$ mg/dL, appeared to trend lower than in controls, $513\pm142$ mg/dL, but the differences did not achieve statistical significance.

In telmisartan-treated rats but not in valsartan-treated rats, liver weight and hepatic triglyceride levels were significantly decreased compared with control rats (Figure 3). Effects on heart weight, kidney weight, and weight of soleus and extensor digitorum longus muscles are presented in Table 1. In the telmisartan-treated rats, heart weights were slightly decreased, whereas kidney weights and muscle weights were increased compared with the valsartan-treated rats and controls. Thus, the telmisartan-induced reductions in liver weight and fat weight are not simply because of a nonspecific impairment in organ growth, because other organ weights (eg, kidney and skeletal muscle) were actually somewhat greater in the telmisartan-treated rats.

Effects on Adipocyte Size and Number

Although epididymal fat pad weight was lower in telmisartan-treated rats compared with the valsartan-treated rats and controls, the number of adipocytes per gram of fat tissue was significantly greater in the telmisartan-treated rats compared with the other groups (Figure 4). The average adipocyte volume was markedly reduced in rats treated with telmisartan compared with controls (Figure 4). Adipocyte volume in valsartan-treated rats was also reduced compared with controls (Figure 4). However, the reduction in adipocyte volume in rats treated with telmisartan was greater than in rats treated with valsartan (62.0% versus 20.7%). Thus, telmisartan appeared to promote the formation of small adipocytes while attenuating adipocyte hypertrophy, fat mass accumulation, and weight gain. Valsartan had less pronounced effects on adipocyte volume and fat mass accumulation and did not attenuate weight gain.

Effects on Total Body Energy Expenditure

We used indirect calorimetry to investigate whether telmisartan might be attenuating weight gain by increasing energy expenditure. Under all of the testing conditions, diurnal patterns of energy expenditure and total activity showed higher levels during the nighttime, when the rats were awake and active, and lower levels during the daytime, when the rats were at rest. Rats treated with telmisartan, but not valsartan, showed significant increases in total body energy expenditure both during the nighttime and daytime compared with controls under all of the dietary conditions. For example, Figure 5 shows the pattern of increased energy expenditure in telmisartan-treated rats compared with valsartan-treated rats and controls under nonfasting conditions at week 4 of the study. Table 2 shows increased 24-hour energy expenditure results in the telmisartan-treated rats during other feeding conditions and at different time points in the study. Increased energy expenditure was observed in the telmisartan-treated rats regardless of whether the results were scaled according to body weight or three-quarter power of body weight. Total activity levels were similar among the 3 groups, suggesting that the telmisartan-induced increases in energy expenditure involve more than just increases in energy metabolism second-

### TABLE 1. Additional Organ Weights (g/100 g BW)

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart</th>
<th>Left Kidney</th>
<th>Right Kidney</th>
<th>Soleus Muscle</th>
<th>EDL Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan</td>
<td>0.25±0.004*</td>
<td>0.39±0.012*</td>
<td>0.40±0.014*</td>
<td>0.023±0.001*</td>
<td>0.025±0.001*</td>
</tr>
<tr>
<td>Valsartan</td>
<td>0.26±0.008</td>
<td>0.34±0.011</td>
<td>0.35±0.012</td>
<td>0.020±0.001</td>
<td>0.020±0.001</td>
</tr>
<tr>
<td>Control</td>
<td>0.27±0.005</td>
<td>0.33±0.008</td>
<td>0.35±0.010</td>
<td>0.021±0.001</td>
<td>0.020±0.001</td>
</tr>
</tbody>
</table>

EDL indicates extensor digitorum longus.
*Significantly different from valsartan and control groups, $P<0.05$. 
ary to increased physical activity (Figure 5). Respiratory quotients were similar among the 3 experimental groups (data not shown) suggesting that the telmisartan-induced increases in energy expenditure may be related to increases in both fatty acid and carbohydrate metabolism.

Effects of Mitochondrial Gene Expression Levels
Telmisartan, but not valsartan, was associated with significant increases in the expression of genes encoding TFAM and MTCO1 in soleus muscle (Figure 6). Thus, telmisartan affected the expression of both nuclear and mitochondrial genes known to play important roles in mitochondrial function.

Discussion
In the current study, we have found that telmisartan, but not valsartan, significantly attenuates weight gain in rats fed a high-fat, high-carbohydrate diet. Food intake adjusted for body weight was similar in all of the groups, although absolute food intake unadjusted for body weight was lower in the telmisartan-treated rats. Previous studies in rats and mice fed high-fat diets have shown that telmisartan may attenuate weight gain in the absence of detectable changes in absolute food intake including when animals have been pair fed to insure identical food consumption. These observations suggest that increases in energy expenditure, and not simply reductions in appetite, are contributing to the ability of telmisartan to attenuate weight gain. Of course, because of the difficulties in measuring food intake with 100% accuracy, the possibility that group differences in energy intake might be contributing to the group differences in body weight cannot be completely ruled out. Measurements of caloric expenditure using indirect calorimetry indicated that administration of telmisartan, but not valsartan, was associated with increases in energy expenditure. In addition, telmisartan was observed to induce increases in energy expenditure and attenuate weight gain in the absence of detectable increases in total motor activity. These observations indicate that the increases in energy expenditure induced by telmisartan may involve more than just activity-induced increases in metabolic rate. Telmisartan’s weak activity on PPARα might also merit further metabolic investigation.

Given that telmisartan, but not valsartan, attenuated weight gain and increased energy expenditure, the current findings raise the possibility that the effects of telmisartan on energy metabolism may go beyond just blockade of the type 1 angiotensin II receptor. Telmisartan and valsartan have sim-
Telmisartan is a partial agonist (mixed agonist/antagonist) of angiotensin II receptor blockade. The greater metabolic impact of telmisartan compared with valsartan might also be related to greater in vivo blockade of the type 1 angiotensin II receptor by telmisartan than valsartan at the doses we administered.

In rats treated with either telmisartan or valsartan and fed the high-fat, high-carbohydrate diet, adipocyte size and fat accumulation were reduced compared with untreated control rats. These observations are consistent with the hypothesis of Sharma et al.10 that angiotensin II receptor blockade per se may affect adipocyte biology and promote the formation of small, metabolically efficient adipocytes. In the current study, however, the effects of telmisartan on adipocyte size and visceral fat stores were much greater than those of valsartan. In previous studies, telmisartan was shown to have greater effects than valsartan on adipocyte differentiation and on adipocyte glucose transport, although both drugs were tested at concentrations clearly exceeding those required to block angiotensin II receptors.19 These findings raise the possibility that, in addition to a class effect of ARBs in modulating adipocyte size, certain molecules, like telmisartan, may have particularly robust effects on fat cell size and metabolism that involve more than just angiotensin II receptor blockade.

Recently, we and others have observed that the ARB telmisartan is a partial agonist (mixed agonist/antagonist) of PPARγ, an intracellular receptor that is a major regulator of fat cell differentiation.7,8 The other clinically approved ARBs appear to have relatively little or no effect on PPARγ activity, with the exception of irbesartan and a metabolite of losartan, both of which are much less potent activators of PPARγ than telmisartan.7,8,20 In contrast to full agonists of PPARγ, such as rosiglitazone or pioglitazone, which promote weight gain, recent studies have suggested that partial agonists (mixed agonists/antagonists) of PPARγ may have the capacity to improve glucose and lipid metabolism while attenuating weight gain.9,18 Whereas full activation of PPARγ may promote adipocyte hypertrophy and weight gain, as well as adipocyte differentiation, partial activation of PPARγ may protect against dietary-induced adipocyte hypertrophy and weight gain while promoting formation of small, metabolically efficient adipocytes.9,21 This raises the possibility that the effects of telmisartan on weight gain and adipocyte size might be related, at least in part, to its ability to function as a partial agonist of PPARγ.

In the current studies, we also found that telmisartan, but not valsartan, increases the expression of genes for both a nuclear-encoded transcription factor (TFAM) that regulates mitochondrial function and for a mitochondrial-encoded protein (MTCO1) involved in oxidative phosphorylation. In comparison to conventional full agonists of PPARγ, such as the thiazolidinediones, partial agonists of PPARγ, like telmisartan, may have the ability to preferentially recruit certain transcriptional coactivators that are particularly important in regulating genes that control mitochondrial function and energy metabolism.18,22 For example, partial agonists appear to preferentially recruit PPARγ coactivator 1-α, a transcriptional coactivator known to stimulate expression of TFAM, which, in turn, can increase mitochondrial gene expression (eg, MTCO1) and, ultimately, mitochondrial biogenesis.22,23 Although the precise cellular and molecular mechanisms that mediate the robust effects of telmisartan on body weight, energy expenditure, and fat metabolism remain to be determined, studies on PPARγ coactivator recruitment and target gene

**TABLE 2. Twenty-Four Hour Energy Expenditure (Kcal/kg BW/h)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Regular Feeding (Week 1)</th>
<th>Fasting (Week 7)</th>
<th>Refeeding (Week 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan</td>
<td>11.36±0.20*</td>
<td>7.35±0.21*</td>
<td>8.06±0.19*</td>
</tr>
<tr>
<td>Valsartan</td>
<td>10.34±0.18</td>
<td>6.53±0.24</td>
<td>7.23±0.22</td>
</tr>
<tr>
<td>Control</td>
<td>10.07±0.17</td>
<td>6.26±0.14</td>
<td>6.83±0.09</td>
</tr>
</tbody>
</table>

*Significantly different from valsartan and control groups, P<0.05.

**Figure 6.** Soleus muscle expression of the genes encoding TFAM and MTCO1. (A) Real-time PCR analysis showing fold expression of TFAM in rats treated with telmisartan or valsartan for 10 days relative to untreated controls. (B) Fold expression of MTCO1 in rats treated with telmisartan or valsartan for 10 days relative to untreated controls. *P<0.05 vs control group and valsartan group by the randomization test of Pfaffl et al.15
expression, as well as on mitochondrial number, structure, and function, could represent potentially fruitful areas of investigation in the future.

In addition to attenuating weight gain, telmisartan significantly attenuated the development of hepatic steatosis and hepatomegaly, whereas valsartan did not. The hepatic effects of telmisartan could not be attributed to nonspecific effects on organ weight, because telmisartan did not attenuate the growth of kidney or skeletal muscle. Additional studies will be required to assess the relative contributions of hepatic and extrahepatic mechanisms that could mediate the ability of telmisartan to attenuate dietary-induced accumulation of fat in liver. Given the frequent occurrence of fatty liver in patients with the metabolic syndrome, the current findings may point to new opportunities for limiting the hepatic accumulation of fat in hypertensive patients at increased risk for developing hepatic steatosis.24

Perspectives

The potential effects of ARBs on adipose tissue biology are of considerable interest, because these agents are frequently used to treat hypertension in patients who are prone to visceral obesity, the metabolic syndrome, and diabetes. As proposed by Sharma et al,10 the ability to promote the differentiation of small, metabolically efficient adipocytes may represent a class effect of ARBs that could contribute to the lower rates of new onset diabetes in patients treated with these agents compared with other antihypertensive drugs. The current findings suggest that a subset of ARBs, like telmisartan, may have particularly strong effects on adipocyte size that might go beyond just angiotensin II receptor blockade and may also have a special capacity to increase energy expenditure and protect against dietary-induced increases in weight gain and visceral fat accumulation. Thus, these findings should motivate future studies on the ability of molecules like telmisartan to attenuate weight gain and reduce the risk for visceral obesity in patients with hypertension.

Acknowledgments

We acknowledge the support by National Institutes of Health grants HL35018, HL63709, and TW01236 (to T.W.K.); grant 1M683705002 from the Ministry of Education of the Czech Republic (to M.P.); grant AV0Z 50110509 to the Institute of Physiology; and grant 8495-3 from the Ministry of Education of the Czech Republic (to M.P.); grant 54:3442–3452. Sharma AM, Janke J, Gorzelniak K, Engel G, Luft FC. Angiotensin blockade prevents type 2 diabetes by formation of fat cells. Hypertension. 2002;40:609–611.


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Hypertension. 2006;47:1003-1009; originally published online March 27, 2006; doi: 10.1161/01.HYP.0000215181.60228.f7

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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