Peroxisome Proliferator-Activated Receptor (PPAR)

High-Dose Treatment With Telmisartan Induces Monocytic Peroxisome Proliferator-Activated Receptor-γ Target Genes in Patients With the Metabolic Syndrome

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Abstract—The present study aimed to explore the anti-inflammatory effects and peroxisome proliferator-activated receptor-γ (PPARγ)–activating properties of the angiotensin type 1 receptor blocker telmisartan by analysis of serum interleukin 6 levels and monocytic PPARγ target gene expression in drug-naïve patients with the metabolic syndrome. This was a 14-week, randomized, double-blind, placebo-controlled 2-center study with telmisartan 80 mg/d and telmisartan 160 mg/d in 54 patients with the metabolic syndrome. In addition to clinical laboratory measurements, peripheral monocytes were extracted by negative isolation using a DynaMonocyte kit to evaluate ligand-activated PPARγ target gene expression (CD36 and CD163) at baseline and study end using quantitative real-time RT-PCR. In this low-risk patient population, telmisartan (80 and 160 mg) treatment did not significantly affect serum interleukin 6 levels. Expression of the PPARγ target gene CD36 in monocytes was markedly induced by telmisartan from baseline to study end (telmisartan 80 mg: 2.3±1.5-fold change versus placebo [P value not significant]; telmisartan 160 mg: 3.5±0.9-fold change versus placebo [P<0.05]). The recently reported PPARγ target gene CD163 was slightly induced by telmisartan (telmisartan 80 mg: 1.1±0.3-fold change versus placebo [P value not significant]; telmisartan 160 mg: 1.4±0.4-fold change versus placebo [P value not significant]), which did not reach statistical significance. This is the first clinical description of monocytic PPARγ target gene regulation with high-dose telmisartan treatment. These data implicate that the angiotensin type 1 receptor blocker telmisartan activates PPARγ in circulating monocytes of patients with the metabolic syndrome. (Hypertension. 2011;58:725-732.)

Key Words: PPAR ▪ angiotensin receptor blocker ▪ metabolic syndrome ▪ monocytes ▪ inflammation

Telmisartan is an orally active, long-acting, nonpeptide angiotensin type 1 (AT1) receptor blocker (ARB) with high selectivity for the AT1 receptor.1 Telmisartan is a potent antihypertensive drug and clinically used in patients with arterial hypertension.1 Based on recently published data from the Ongoing Telmisartan Alone and in Combination With Ramipril Global Endpoint Trial and Telmisartan Randomized Assessment Study in ACE-Intolerant Subjects With Cardiovascular Disease, telmisartan is indicated for the reduction of cardiovascular morbidity in patients with manifest atherothrombotic cardiovascular disease or type 2 diabetes mellitus with documented target organ damage.2,3

In addition to its AT1 receptor–blocking properties, telmisartan has been identified previously as a partial agonist/selective modulator of the nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ).4–6 PPARγ acts as a ligand-activated transcription factor and mediates improvement of whole-body insulin sensitivity.7 In addition, ligand-activated PPARγ mediates direct antiatherosclerotic actions resulting in marked reduction of cardiovascular morbidity and mortality.8–10 PPARγ is abundantly expressed in adipose tissue but also in vascular and nonvascular cells in the vessel wall (monocytes/macrophages, endothelial cells, and vascular smooth muscle cells).11 Compared with glitazones, as full PPARγ agonist, telmisartan binds to the receptor in a different manner, resulting in distinct pharmacological actions. Preclinical and clinical studies with telmisartan demonstrated positive actions on lipid and glucose metabolism, including prevention of new-onset diabetes mellitus in large clinical end point studies.12

Whether telmisartan-induced PPARγ-activity plays a significant role in the observed beneficial actions of telmisartan

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Parts of this study will be used in the doctoral thesis of I.-N.B. and P.T.

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in patients is currently unknown. The present study aimed to examine the actions of telmisartan in 2 different doses (80 and 160 mg/d) on inflammatory parameters and monocytic PPARγ activity in drug-naïve patients with the metabolic syndrome. Furthermore, selected lipid and glucose parameters were evaluated as secondary outcome measures. In parallel, human peripheral blood monocytes from drug-naïve healthy volunteers were stimulated ex vivo with telmisartan or the full PPARγ agonist pioglitazone, and PPARγ target gene expression was analyzed.

Materials and Methods

Patients
Drug-naïve patients between the age of 19 to 60 years with the metabolic syndrome defined by the International Diabetes Federation criteria13 with abdominal obesity (body mass index >25 kg/m² and waist circumference ≥94 cm [men] and ≥80 cm [women]), a blood pressure ≥130 mm Hg (systolic), and/or ≥85 mm Hg (diastolic), and triglycerides between 150 and 400 mg were eligible for inclusion in this study. Normal stress ECG, normal carotid ultrasound, and normal fundoscopy were required for inclusion. Patients were excluded from the study if they had any of the following: diabetes mellitus, secondary cause for insulin resistance, low-density lipoprotein (LDL) cholesterol >190 mg/dL, atherosclerotic disease, blood pressure >160 mm Hg (systolic) and/or 100 mm Hg (diastolic), regular alcohol consumption (>30 g/d), contraindication against telmisartan, previous antihypertensive medication or lipid-lowering therapy, malignancy, pregnancy or lactation, or women without adequate contraception. Written informed consent was obtained from all of the participants.

Study Design
This was an investigator-initiated, prospective 14-week, double-blind, placebo-controlled, randomized 2-center study with 80 and 160 mg of telmisartan in patients with the metabolic syndrome. Patients were initially randomized to telmisartan 80 mg, telmisartan 160 mg, or placebo. The initial telmisartan dose of 80 mg was increased to 160 mg after 2 weeks until study end at 14 weeks. The primary objective of the study was to evaluate the anti-inflammatory effect of telmisartan by analyzing the reduction of the inflammatory marker interleukin 6 (IL-6) in serum after 14 weeks of treatment. Predefined secondary objectives were the effects on monocytic PPARγ target gene expression and parameters of lipid and glucose metabolism. Blood pressure (systolic and diastolic) was measured in a sitting-position after 5 minutes of rest in the same arm. Mean values of 3 consecutive measures were taken for further analysis. The study protocol complies with the Declaration of Helsinki, as well as with local institutional guidelines, and was approved by the local ethics committees. The study was conducted from November 2007 to September 2009 and was registered at www.clinicaltrials.gov (identifier NCT00560430).

Glucose Metabolism
Glucose metabolism parameters were evaluated by fasting plasma glucose, an oral glucose tolerance test, and an intravenous glucose tolerance test. Please see the online Data Supplement for expanded materials and methods.

Lipid Metabolism
Fasting concentrations of triglyceride and cholesterol concentrations were measured using a commercial kit (Boehringer Mannheim, Mannheim, Germany). Preparative ultracentrifugation was performed to isolate very LDL, and very LDL cholesterol and very LDL triglyceride concentrations were determined. In the infranatant, LDL and high-density lipoprotein cholesterol were determined. In addi-
Table 1. Comparison of Primary (IL-6)/Secondary Outcomes at Baseline and After 14 wk

<table>
<thead>
<tr>
<th>Primary/Secondary Outcomes</th>
<th>Baseline</th>
<th>Study End</th>
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<tbody>
<tr>
<td></td>
<td>Placebo (n=18)</td>
<td>Placebo (n=18)</td>
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<tr>
<td></td>
<td>80 mg Telmisartan (n=17)</td>
<td>80 mg Telmisartan (n=17)</td>
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<td>160 mg Telmisartan (n=19)</td>
<td>160 mg Telmisartan (n=19)</td>
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<td><strong>P</strong></td>
<td></td>
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<tr>
<td>IL-6, ng/L</td>
<td>2.1±1.7</td>
<td>2.2±1.7</td>
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<tr>
<td></td>
<td>2.9±2.4</td>
<td>2.8±2.7</td>
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<tr>
<td></td>
<td>2.5±1.9</td>
<td>2.7±2.1</td>
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<tr>
<td>Total cholesterol, mg/L</td>
<td>195.4±40.3</td>
<td>201.1±35.6</td>
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<tr>
<td></td>
<td>211.9±38.4</td>
<td>217.9±31.6</td>
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<td></td>
<td>207.8±41.6</td>
<td>231.4±47.1</td>
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<tr>
<td>Fasting LDL cholesterol, mg/dL</td>
<td>117.8±32.2</td>
<td>113.3±33.3</td>
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<tr>
<td></td>
<td>129.8±34.1</td>
<td>128.5±24.7</td>
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<tr>
<td></td>
<td>127.0±34.5</td>
<td>143.5±40.7</td>
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<tr>
<td>Fasting HDL cholesterol, mg/dL</td>
<td>50.7±12.3</td>
<td>52.0±15.1</td>
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<tr>
<td></td>
<td>49.3±8.2</td>
<td>46.7±6.9</td>
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<tr>
<td></td>
<td>50.9±13.4</td>
<td>52.3±15.2</td>
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<tr>
<td>Fasting triglycerides, mg/dL</td>
<td>191.3±46.4</td>
<td>183.7±57.4</td>
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<td></td>
<td>221.1±95.2</td>
<td>228.7±144.4</td>
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<td></td>
<td>224.8±103.0</td>
<td>179.8±56.0</td>
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<tr>
<td>Fasting VLDL cholesterol, mg/dL</td>
<td>38.7±15.6</td>
<td>35.8±14.0</td>
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<td></td>
<td>44.8±21.2</td>
<td>43.1±27.7</td>
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<td></td>
<td>44.4±23.9</td>
<td>37.1±19.0</td>
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<tr>
<td>Fasting VLDL triglycerides, mg/dL</td>
<td>164.9±51.2</td>
<td>158.6±60.1</td>
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<td></td>
<td>191.7±95.4</td>
<td>198.9±138.6</td>
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<tr>
<td></td>
<td>202.7±96.3</td>
<td>154.0±58.6</td>
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<tr>
<td>Fasting glucose, mg/dL</td>
<td>84.7±17.1</td>
<td>88.1±11.9</td>
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<td></td>
<td>96.1±16.0</td>
<td>94.6±12.7</td>
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<tr>
<td></td>
<td>93.0±19.5</td>
<td>96.5±15.4</td>
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<tr>
<td>Fasting insulin, μIU/mL</td>
<td>8.8±5.8</td>
<td>9.2±7.2</td>
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<tr>
<td></td>
<td>8.0±7.8</td>
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<td>9.1±5.6</td>
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<td>DBP, mm Hg</td>
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<td>84.6±7.6</td>
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<td>83.7±4.7</td>
<td>76.2±6.3</td>
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<td>SBP, mm Hg</td>
<td>140.0±9.5</td>
<td>130.2±11.9</td>
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<td>138.8±8.8</td>
<td>125.1±7.0</td>
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<td></td>
<td>138.9±10.8</td>
<td>121.1±10.8</td>
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<tr>
<td>HbA1c, %</td>
<td>5.4±0.4</td>
<td>5.4±0.4</td>
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<td></td>
<td>5.5±0.5</td>
<td>5.5±0.7</td>
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<td>5.6±0.6</td>
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</table>

Data are mean±SD. P value (∆ baseline vs study end) is for between-group difference (160 mg telmisartan vs placebo).

160 mg: 50±10.4), sex (female/male: placebo: 6/12; telmisartan 80 mg: 7/10; telmisartan 160 mg: 6/13), body mass index (mean body mass index±SD [kg/m²]: placebo: 32.7±7.1; telmisartan 80 mg: 32.1±7.2; telmisartan 160 mg: 34.6±6.5), and waist circumference (mean±SD [cm] female/male: placebo: 98±13/119±17; telmisartan 80 mg: 100±12/111±17; telmisartan 160 mg: 115±16/116±17). Patients mainly fulfilled the International Diabetes Federation criteria for metabolic syndrome by increased waist circumference plus hypertriglyceridemia and increased blood pressure (≥130/85 mm Hg). According to current guidelines, 32 patients (59.3%) had the diagnosis of arterial hypertension (blood pressure ≥140/90 mm Hg; placebo: n=13 [72.2%]; telmisartan 80 mg: n=10 [58.8%]; telmisartan 160 mg: n=9 [47.4%]). Baseline characteristics for IL-6, lipid and glucose parameters, and blood pressure are outlined in Table 1. To exclude distinct cardiovascular end organ damage, such as coronary artery disease, intima-media thickening, or hypertensive retinopathy, a stress ECG, carotid ultrasound, and funduscopic examination were performed before inclusion. All of the patients had regular test results.

Inflammatory Parameters and Monocytic PPARγ Activation

To assess the anti-inflammatory actions of telmisartan, serum levels of IL-6 were determined at baseline and study end. Baseline values did not significantly differ among the groups (Table 1). Compared with previously published studies in different patient populations, IL-6 serum levels exhibited rather low baseline values, indicating a low inflammatory load in the present study population. After 14 weeks of treatment, IL-6 was not significantly reduced by telmisartan 80 mg or telmisartan 160 mg (Table 1).

To assess the PPARγ activating potential of different doses of telmisartan, peripheral blood monocytes were isolated from patients at baseline and study end, as described in the method section. Expression of 2 PPARγ target genes, CD36 and CD163, was determined by quantitative real-time PCR. Both genes are usually not regulated by AT1 receptor blockade. As depicted in Figure 1A and 1B, telmisartan treatment resulted in an induction of monocytic CD36 expression whereby only the telmisartan 160 mg group reached statistical significance (P<0.05 versus placebo). CD163 expression also increased under telmisartan therapy; however, the induction was not statistical significant (Figure 1B). An additional receptor from the LDL receptor superfamily, named CD91, was not significantly regulated by PPARγ ligands (Figure 1C).

Induction of Monocytic PPARγ Target Gene Expression by Telmisartan Ex Vivo

To confirm telmisartan’s PPARγ activating properties, freshly isolated monocytes from healthy individuals were stimulated with telmisartan or the full PPARγ agonist pioglitazone ex vivo followed by expression analysis of CD36. Telmisartan and pioglitazone significantly induced CD36 mRNA and protein expression in monocytes (Figure 2A and 2B), corroborating the results seen in telmisartan-treated patients. Finally, we assessed PPARγ transcriptional activation by telmisartan and pioglitazone in human THP-1 monocytes using an ELISA-based kit for analysis of PPARγ-DNA binding. As depicted in Figure 2C, both telmisartan and pioglitazone augmented the binding of PPARγ to a corresponding PPAR response element implicating an induction of transcriptional activity.

Metabolic Parameters and Blood Pressure

As outlined in Table 1, telmisartan treatment did not significantly regulate total cholesterol, high-density lipoprotein cholesterol, triglycerides, very LDL cholesterol/triglycerides,
fasting glucose/insulin, or hemoglobin A1c. Surprisingly, we detected an increase in LDL cholesterol with telmisartan 160 mg when compared with placebo (Table 1). In the present patient population with mild hypertension, telmisartan significantly reduced systolic blood pressure, whereas diastolic blood pressure was not significantly affected (Table 1). Postprandial lipid metabolism was studied by a standardized oral fat tolerance test. Postprandial lipid parameters did not change under therapy when compared with placebo (Table 2). Finally, glucose metabolism was investigated by oral glucose

**Figure 1.** Peroxisome proliferator-activated receptor-γ (PPARγ) target gene and CD91 expression in monocytes. Quantitative real-time PCR of the PPARγ target genes (A) CD36, (B) CD163, and CD91 (C) in isolated monocytes from patients treated with placebo (CD36 n=11, CD163 n=12, CD91 n=11), telmisartan 80 mg/d (CD36 n=12, CD163 n=14, CD91 n=12), or telmisartan 160 mg/d (CD36 n=18, CD163 n=18, CD91 n=15). Levels of placebo patients were arbitrarily set to 1. Shown are differences of transcript levels between baseline and end of study. Data are expressed as mean relative expression ± SEM of CD36/PO, CD163/PO, and CD91/PO mRNA ratios (x-fold induction over placebo baseline/study end). *P<0.05 vs placebo.

**Figure 2.** Peroxisome proliferator-activated receptor-γ (PPARγ) target gene expression in primary monocytes ex vivo. A, Human primary monocytes were isolated from healthy volunteers. Monocytes were cultured in the presence of vehicle, telmisartan (20 µmol/L), and pioglitazone (20 µmol/L) for 24 hours. Total RNA isolated from ex vivo stimulated monocytes was transcribed to cDNA, and quantitative real-time RT-PCR analysis of the PPARγ target gene CD36 was performed. Data are expressed as mean relative expression ± SEM of CD36/18S mRNA ratios (x-fold induction over vehicle-treated cells). **P<0.01 vs vehicle (n=4). B, Additionally, 20 µg of protein isolated from ex vivo stimulated monocytes (telmisartan 2 and 20 µmol/L, pioglitazone 2 and 20 µmol/L) were analyzed in Western blot by using CD36-specific antibody. Shown (top) are results from 1 representative experiment of n=3 individual experiments and corresponding densitometry analysis (bottom). *P<0.05 vs vehicle. C, ELISA-based analysis of PPARγ-DNA binding. THP-1 monocytes were stimulated with telmisartan (20 µmol/L) or pioglitazone (20 µmol/L) for 24 hours. Nuclear extracts were bound to PPAR response elements, and PPARγ-specific binding was quantified with anti-PPARγ antibody, secondary horseradish peroxidase–conjugated antibody, and spectrophotometric analysis of colorimetric signals. *P<0.05 vs vehicle.
important role in carbohydrate and lipid metabolism.20 The LDL on monocytes, adipocytic or muscular CD36 plays an
doses.

Here we first show that telmisartan is able to activate PPAR
activation. However, direct cellular PPARγ activation by
telmisartan has never been investigated in clinical studies. We
first show that telmisartan is able to activate PPARγ pathways in isolated monocytes from patients with the metabolic syndrome treated for 14 weeks. The PPARγ target
gene CD36 was upregulated in monocytes from telmisartan-
treated patients reaching statistical significance at 160 mg of
telmisartan. These data suggest a dose dependency of
telmisartan-mediated PPARγ activation and show that further
induction of monocyctic PPARγ can be achieved with higher
doses.

In addition to its role as a scavenger receptor for oxidized
LDL on monocytes, adipocytic or muscular CD36 plays an
important role in carbohydrate and lipid metabolism.20 The
importance of CD36 as a target for telmisartan has been
supported previously in an animal study showing that telmis-
artan’s beneficial effects on lipid and glucose metabolism are
markedly attenuated in spontaneously hypertensive rats har-
boring a deletion mutation in CD36 compared with wild-type
spontaneously hypertensive rats.21 Furthermore, CD36 has
been recently identified as a gene sensitive to mutations of the
CDK5 site (Ser273) in PPARγ involved in phosphorylation of
the receptor.22 Because PPARγ phosphorylation seems to play a crucial role for the metabolic actions of its ligands, in particular for partial agonists such as telmisartan, future
studies on telmisartan’s impact on PPARγ phosphorylation
might be of high interest to further determine the pharmaco-
logical profile of this compound.

Our results are consistent with a previously published
study using another PPARγ-activating ARB, losartan. EXP-
3179, the PPARγ-activating metabolite of losartan, was
shown to reach adequate plasma concentrations to induce
PPARγ target genes in monocytes from patients chronically
treated with 100 mg/d of losartan.23 Furthermore, Matsumura
et al24 showed recently that telmisartan activates PPARγ in
macrophages exerting antiatherosclerotic actions. The present
study investigated PPARγ activation in blood monocytes.
Therefore, it remains unanswered whether activation occurs
in other tissues. Because telmisartan has a high lipophilicity,
activation of PPARγ in lipid-rich tissues, such as fat tissue,
might be stronger than in circulating monocytes. However,
this requires additional studies with analysis of PPARγ target
genes in adipose tissue biopsy samples.

Telmisartan did not reduce IL-6 serum levels. These data are
in contrast to a previously published study by Fliser et al.17
In this study, the ARB olmesartan exhibited potent
anti-inflammatory actions by reducing C-reactive protein,
tumor necrosis factor-α, IL-6, and monocyte chemoattractant
protein 1 serum levels already after 6 weeks of treatment
when compared with placebo. Patients with essential hyper-
tension and any diagnosed atherosclerotic disease, type 2
diabetes mellitus, and/or elevated LDL cholesterol levels
(European Trial on Olmesartan and Pravastatin in Inflamma-
tion and Atherosclerosis) were included.17 An additional
inclusion criterion was an elevated C-reactive protein serum
concentration (>3 mg/L), preselecting patients with a high
inflammatory load. Patients in our study had rather low IL-6
serum levels at baseline (mean 2.5 ng/L) compared with

<table>
<thead>
<tr>
<th>Lipid/Glucose Parameters</th>
<th>Placebo (n=18)</th>
<th>80 mg Telmisartan (n=17)</th>
<th>160 mg Telmisartan (n=19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides AUC fat tolerance, mg·h/dL</td>
<td>1971.6±470.1</td>
<td>2138.1±738.2</td>
<td>2465.3±929.4</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL triglycerides AUC fat tolerance, mg·h/dL</td>
<td>1747.0±518.9</td>
<td>2096.5±786.7</td>
<td>2269.2±911.5</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose AUC intravenous GTT, mg·min/dL</td>
<td>1664±362</td>
<td>1874±307</td>
<td>1782±414</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose AUC oral GTT, mg·h/dL</td>
<td>255.6±69.3</td>
<td>291.4±72.8</td>
<td>281.3±66.9</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin AUC oral GTT, μU·h/mL</td>
<td>88.3±57.6</td>
<td>79.6±78.3</td>
<td>90.5±56.3</td>
<td>NS</td>
</tr>
<tr>
<td>Insulinogenic index, μU/mL</td>
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<td>0.6±0.4</td>
<td>1.2±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA index*</td>
<td>3.1±2.1</td>
<td>3.0±2.0</td>
<td>3.7±2.0</td>
<td>NS</td>
</tr>
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</table>

Data are mean±SD. P value (Δ baseline vs study end) is for between-group difference. AUC, area under the curve; GTT, glucose tolerance test; HOMA, homeostasis model assessment; NS, not significant; VLDL, very low-density lipoprotein.

*Δ insulin_{oral GTT}/Δ glucose_{oral GTT}
European Trial on Olmesartan and Pravastatin in Inflammation and Atherosclerosis (3.6 ng/L), which could, at least in part, provide an explanation for the discrepant results. It appears that a defined level of enhanced inflammation is required for a significant beneficial effect by ARBs. This is further supported by the Irbesartan and Lipico Acid in Endothelial Dysfunction Study, in which baseline IL-6 concentrations were also elevated, and the ARB irbesartan resulted in a significant reduction after 4 weeks of therapy.

Predefined secondary objectives of the present study were the analysis of metabolic parameters involved in glucose/lipid metabolism. We were not able to detect any significant beneficial action of telmisartan on fasting or postprandial glucose/lipid metabolism. Previous studies on metabolic actions of telmisartan in patients with the metabolic syndrome have shown conflicting results. Vitale et al demonstrated in patients with the metabolic syndrome that telmisartan 80 mg/d for 3 months significantly improved fasting glucose, insulin, HOMA indices, and glucose tolerance when compared with losartan 50 mg/d. Patients in this study had higher baseline values for fasting glucose, fasting insulin, and the HOMA index compared with our study indicating a more severe state of insulin resistance. In another study, we could show that telmisartan 40 mg/d given for 12 weeks to nondiabetic, insulin-resistant patients only improves glucose tolerance in an intravenous glucose tolerance test, whereas no improvement was detected in an oral glucose tolerance test or in HOMA indices. When assessed by intravenous glucose tolerance test, patients were more glucose intolerant at baseline than patients in the present study. Finally, Bahadir et al. found no regulation of the HOMA index with telmisartan 80 mg/d for 8 weeks in patients with the metabolic syndrome. Together, it appears that telmisartan induces an overall mild regulation of metabolic parameters in patients with the metabolic syndrome. Depending on disease severity, treatment period, and clinical methods for analysis of glucose/lipid metabolism, significant positive metabolic effects can be detected. Furthermore, increasing the dose of telmisartan to 160 mg does not appear to have a major impact on glucose/insulin metabolism in these patient populations.

The metabolic efficacy of telmisartan also seems to differ depending on the absence or presence of increased arterial blood pressure. This point is supported by studies comparing the metabolic actions of telmisartan in patients without or with arterial hypertension. Oral administration of telmisartan in overweight hypertensive patients was found to increase insulin sensitivity as determined by the gold-standard insulin clamp technique. In contrast, in overweight normotensive patients with normal insulin sensitivity at baseline, administration of telmisartan failed to increase insulin sensitivity, as measured by the insulin clamp technique. In our study, 59% of the patients were diagnosed with arterial hypertension. Because the presence of arterial hypertension seems to affect telmisartan’s metabolic efficacy, different metabolic results might have been obtained if we had focused only on hypertensive patients.

In the high-dose telmisartan group (160 mg/d), we observed a small but significant increase in LDL cholesterol (+12%), whereas there were no significant changes in the placebo or 80-mg telmisartan groups. Whether this increase is related to the CD36 and CD163 regulation mediated by telmisartan remains to be determined. Oxidized LDL has been shown to induce CD36 expression by PPARγ activation. Thus, one may speculate that, in addition to the direct activation of PPARγ by telmisartan, increased LDL levels may have contributed to CD36 upregulation. Because a positive correlation between monocytes CD163 and LDL cholesterol levels has been observed, this mechanism may also be involved in CD163 regulation. The relevance of the CD36-CD163-LDL cholesterol interactions for LDL clearance and atherogenesis under telmisartan would require a new set of experiments. However, as seen in clinical trials, telmisartan ultimately exhibits an antiatherosclerotic action in the vascular wall. With regard to PPARγ activation in macrophages, CD36 upregulation is paralleled by cholesterol efflux via induction of ABC transporters (eg, ABCA1). Recently, telmisartan has been shown to increase ABCA1 and ABCG1 in macrophages. Thus, induction of CD36 by high-dose telmisartan associated with higher LDL cholesterol levels may be counteracted by a parallel regulation of cholesterol efflux from lesion macrophages resulting in the observed antiatherosclerotic actions of this drug.

Furthermore, the results regarding LDL cholesterol levels are somewhat surprising, because previous studies have shown that ARBs either have no effect or decrease LDL cholesterol. In fact, all of the studies using telmisartan did show a positive effect. The discrepancy between our results and the published data may relate to the doses used. In the studies in which an improvement in LDL cholesterol was observed, doses of 40 mg or 80 mg of telmisartan per day were used. In our study, 80 mg of telmisartan per day had no effect on LDL cholesterol, but 160 mg per day resulted in a significant increase. Thus, it seems that low doses of telmisartan have either no or a beneficial effect on LDL cholesterol, whereas higher doses may result in an increase and may not be a recommendable antidyslipidemic approach.

**Perspectives**

This is the first clinical description of monocytic PPARγ target gene regulation with high-dose telmisartan treatment. These data implicate that the AT1 receptor blocker telmisartan activates PPARγ in circulating monocytes of patients with the metabolic syndrome. PPARγ activation in monocytes by telmisartan does not translate into lowering of the proinflammatory cytokine IL-6 and metabolic parameters. Previously, telmisartan was extensively studied regarding its antidiabetogenic actions. The impact of telmisartan’s bimodal mechanism of action (AT1 receptor blockade + PPARγ modulation) on glucose metabolism is still under clinical investigation. Here we report that telmisartan’s distinct pharmacological profile may also play a role in monocytes, a cell type crucial for atherogenesis. Ligand-activated PPARγ has been shown to be an important antiatherosclerotic mediator in monocytes. Thus, local activation of PPARγ in monocytes may contribute to the documented antiatherosclerotic actions of telmisartan. Significant PPARγ activation was only seen in patients treated with 160 mg of telmisartan. This observation may support the use of high-dose AT1 receptor blocker.
therapy to induce additional antiatherosclerotic actions and efficient cardiovascular risk reduction. However, the overall clinical relevance of cellular PPARγ activation for telmisartan’s cardioprotective actions, in particular, in high doses, remains to be determined.

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High-Dose Treatment with the AT1-Receptor Blocker Telmisartan Induces Monocytic PPARγ Target Genes in Patients with the Metabolic Syndrome

Ilse-Nirmala Bähr; Patrizia Tretter; Janine Krüger; Renee G. Stark, Julia Schimkus; Thomas Unger; Kai Kappert; Jürgen Scholze; Klaus G. Parhofer; Ulrich Kintscher
Expanded Materials and Methods

Glucose metabolism
Glucose metabolism parameters were evaluated by fasting plasma glucose (FPG), an oral glucose tolerance test (OGGT), and an intravenous glucose tolerance test (IVGTT). Fasting values included glucose and insulin concentrations. Insulin resistance was measured using the homeostasis model assessment (HOMA), defined as HOMA = FPG (mmol/L) x fasting plasma insulin (mU/L)/22.5. OGGT was performed using 75g glucose after a 12-hour fast and was evaluated concerning the area under the curve (AUC) defined by glucose concentrations determined at 0, 30, 60, 90, 120 minutes. In addition insulin concentrations were determined at 0 and 30 minutes to calculate the insulinogenic index. This is defined as: Insulinogenic Index = Δinsulin / Δglucose. To calculate the area under the insulin curve during OGGT, insulin was also measured at 60, 90, and 120 minutes. The IVGTT was performed using 25g glucose. Plasma glucose and insulin were measured in parallel at 0, 2, 4, 6, 8, and 10 minutes after glucose bolus application. Finally the AUC for plasma glucose and insulin was analyzed. Glycosylated hemoglobin (HbA1c) was determined by standard laboratory methods.

Lipid metabolism
Fasting concentrations of triglyceride and cholesterol concentrations were measured using a commercial kit (Boehringer Mannheim, Mannheim Germany). Preparative ultracentrifugation was performed to isolate very low density lipoprotein (VLDL), and VLDL-cholesterol and VLDL-triglyceride concentrations were determined. In the infranatant, LDL- and HDL cholesterol was determined. In addition, postprandial lipoprotein metabolism was evaluated using a standardized oral fat tolerance test as described previously. In brief, after fasting for 12 hours all subjects ingested a fatty meal consisting for 100mL milk (3.5% fat), 150mL cream (30% fat), 70mL corn oil, 90g egg, 10g sugar, and 3.5g coffee flavor. This standard meal yields 5460.12 kJ, 87% from fat, 7% from carbohydrates, and 6% from proteins. After the fat load, samples were taken every 2 hours for 10 hours. Total triglycerides, and triglycerides in the density of less than 1.006 g/mL fraction, containing chylomicrons, chylomicron remnants, and VLDL, were determined.

Monocyte Isolation
Human peripheral blood mononuclear cells (PBMCs) were isolated from venous citrated blood from various patients by using Vacutainer Cell Preparation Tubes (Becton Dickinson, Heidelberg, Germany), referred to as the Ficoll Hypaque method, employed a liquid density gradient medium of Ficoll 400® and sodium metrizoate or sodium diatrizoate solution. Monocytes were isolated from PBMCs by performing the Dynal monocyte negative isolation kit (Invitrogen GmbH, Karlsruhe, Germany), following the manufacturer’s instructions. Hereby, non-monocytes (e.g. T-, B-cells, NK cells) were depleted by magnetic Dynabeads® after preincubation with an antibody mix against non-monocytes. Monocyte cell viability was >98% as determined by trypan blue exclusion after isolation of peripheral blood monocytes. Finally, bead- and antibody-free pelleted monocytes were shock-frozen in liquid nitrogen and stored at −80°C until further processing. Quality of Dynabead-separated monocytes was controlled by flow cytometry (FACS). PBMCs and monocytes were resuspended in a density of 2 x 10⁶/ml in FACS-Buffer buffer supplemented with Beriglobulin and centrifuged 5min at 1200 rpm, 4°C. Afterwards, 50µl of eluted cells (1 x 10⁵ cells) were labelled for 20min at 4°C with fluorescence–conjugated-monoclonal antibodies CD14 (PE) and CD45 (FITC), BD Bioscience (Heidelberg, Germany) for 20min at 4°C. Cells were washed in 1ml of FACS-Buffer and centrifuged 5min at 1200 rpm, 4°C and fluorescence of cells was immediately measured using a FACScan instrument.
FACScalibur (BD Bioscience, Heidelberg, Germany). Regarding the analysis of the different subpopulations, the first selection was done on the basis of the SSC/FSC scatters, differentiating lymphocytes, monocytes and granulocytes/blast gates (quadrant analysis). FL1 and FL2 were compensated, the FITC (FL1) and the PE (FL2) were analyzed separately or in combination. The intensity of the given subpopulation, respectively CD14 and CD45 was analyzed in a frequency histogram. The purity of negative isolated monocyte-fractions showed >90% CD14 positive cells.

**RNA expression analysis**

Isolation of RNA from frozen monocyte pellets was performed by using the RNeasy Mini or Micro Kit from Qiagen (Hilden, Germany). The quantity and quality of extracted RNA was routinely analyzed using the RNA 6000 Nano LabChips Kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) according to the manufacturer’s instructions. 200-500ng extracted RNA was reversely transcribed to first-strand cDNA using random hexamer primers (Promega, Madison, WI, USA) in 20 µl reactions, ribonuclease inhibitor (0.5 U/µl) and SuperScript II reverse transcriptase (RT) provided in SuperScript First-Strand synthesis Kit (Invitrogen, Carlsbad, California) following the manufacturer’s protocol. 10ng cDNA as template in 25µl reactions were subjected to quantitative real-time polymerase chain reaction (qRT-PCR) using the Power SYBRGreen PCR Master Mix Reagent Kit (Applied Biosystems, Foster City, CA, USA). Amplification was carried out in the Mx3000P® QPCR System (Stratagene, La Jolla, CA, USA). The reaction was performed in duplicate – triplicate, whereas no-template controls (NTCs) and non-enzyme controls (NECs) were included. Gene specific amplicons were analyzed by inspection of dissociation curves. Quantitative real time PCR was performed using gene-specific primers, which were designed using the Primer 3 software, and sequences are provided in table S1. Expression of analyzed genes was normalized to the average expression of the housekeeping genes human 18S ribosomal RNA (18S) and human acidic ribosomal protein (PO).

**Analysis of monocytic gene expression in PBMCs after ex-vivo stimulation**

Human peripheral blood mononuclear cells (PBMCs) were isolated from drug naïve healthy volunteers following local institutional guidelines. After monocyte isolation, cells were cultured in 24well-plates in a density of 5 x 10^5 -1 x 10^6 cells/ml in RPMI 1640 (Gibco) without serum or antibiotics, and treated in the absence or presence of vehicle (DMSO), telmisartan or pioglitazone for 24 hours at concentrations indicated in the respective figure legend. Cells were grown in a humidified atmosphere of 95% air, 5% CO2 at 37°C. Thereafter adherent monocytes were rinsed twice with PBS, dissociated carefully in dissociation medium (RLT- Lysisbuffer, Qiagen, Hilden Germany) by using a cell scraper following total cellular RNA isolation procedure according to the manufacturer’s protocol including a DNase digest. cDNA was synthesized and subjected to quantitative real-time PCR procedures. Experiments were performed four times.

**Western Blotting**

Western Blot analysis was performed by standard techniques using 20µg cell lysate. Briefly, immunoblotting was performed with proteins being resolved on 10% reducing SDS-page. Membranes were incubated with primary antibody mouse-monoconal anti-CD36 (1:500, Santa Cruz, Heidelberg, Germany), mouse monoclonal anti-GAPDH (1:50000, Abcam, Cambridge, USA), rabbit-anti-mouse horseradish peroxidase (HRP) conjugated secondary antibody (1:1000, Dako, Hamburg, Germany; Denmark). Target proteins were detected by the Enhanced Chemiluminescence Detection System (GE Healthcare, USA).
Analysis of PPARγ DNA binding

To assess the level of PPARγ DNA binding, an indirect measure of transcriptional activation, by telmisartan an ELISA-based kit from TransAM™ (Active Motif, Rixensart, Belgium) was performed as previously described following the manufacturer’s instruction. In brief, THP-1 cells were cultivated in RPMI 1640 (10% FCS, 2mmol/L of L-glutamine, 100U/mL of penicillin, and 100µg/mL of streptomycin) and stimulated with telmisartan (20µmol/L) or pioglitazone (20µmol/L) for 24h. After stimulation nuclear extracts were isolated (Active Motif Nuclear Extract Kit), and added to 96-well plate to which oligonucleotides containing the PPAR-response element sequence have been immobilized. After 1h wells were washed, and incubated with an anti-PPARγ specific antibody for 1h. Finally, a horseradish peroxidase-conjugated secondary antibody was added and colorimetric signals were quantified by spectrophotometry.
<table>
<thead>
<tr>
<th>Table S1- Primer for qRT-PCR</th>
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<tr>
<td><strong>Gene-specific primers</strong></td>
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<tr>
<td>rthCD36-forward</td>
</tr>
<tr>
<td>rthCD36-reverse</td>
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<tr>
<td>rthCD163-forward</td>
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<td>rthCD163-reverse</td>
</tr>
<tr>
<td>rthCD91-forward</td>
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<td>rth18S-rRNA-forward</td>
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<tr>
<td>rth18S-rRNA-reverse</td>
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<tr>
<td>rthPO-forward</td>
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<td>rthPO-reverse</td>
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*Table S1, Bähr et al.*