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Endothelium-Specific Interference With Peroxisome Proliferator Activated Receptor Gamma Causes Cerebral Vascular Dysfunction in Response to a High-Fat Diet

Andreas M. Beyer, Willem J. de Lange, Carmen M. Halabi, Mary L. Modrick, Henry L. Keen, Frank M. Faraci, Curt D. Sigmund, PhD

Abstract—The ligand-activated transcription factor PPAR γ is expressed in vascular endothelium where it exerts anti-inflammatory and antioxidant effects. However, its role in regulating vascular function remains undefined. We examined endothelial function in transgenic mice expressing dominant-negative mutants of peroxisome proliferator activated receptor gamma (PPAR γ) under the control of an endothelial-specific promoter to test the hypothesis that endothelial PPAR γ plays a protective role in the vasculature. Under baseline conditions, responses to the endothelium-dependent agonist acetylcholine were not affected in either aorta or the basilar artery in vitro. In response to feeding a high-fat diet for 12 weeks, acetylcholine produced dilation that was markedly impaired in the basilar artery of mice expressing dominant-negative mutants, but not in mice expressing wild-type PPAR γ controlled by the same promoter. Unlike basilar artery, 12 weeks of a high-fat diet was not sufficient to cause endothelial dysfunction in the aorta of mice expressing dominant-negative PPAR γ , although it became evident after 25 weeks. The responses to acetylcholine in basilar artery were restored to normal after treatment with a scavenger of superoxide. Baseline blood pressure was only slightly elevated in the transgenic mice, but the pressor response to angiotensin II was augmented. Thus, interference with PPAR γ in the endothelium produces endothelial dysfunction in the cerebral circulation through a mechanism involving oxidative stress. Consistent with its role as a fatty acid sensor, these findings provide genetic evidence that endothelial PPAR γ plays a critical role in protecting a range of blood vessels in response to a high-fat diet. (*Circ Res.* 2008;103:0-0.)

Key Words: endothelium ■ oxidative stress ■ transcription ■ transgenic animals ■ vascular

Peroxisome proliferator activated receptor gamma (PPAR γ) is a ligand-activated transcription factor targeted by the thiazolidinedione (TZD) class of antidiabetes medications. Activation of PPAR γ by TZDs improves insulin sensitivity and lowers blood pressure in type II diabetes, whereas individuals with dominant-negative mutations in PPAR γ present with severe insulin resistance, type II diabetes, and early-onset hypertension.¹ Studies in humans and animals suggest that TZDs are generally cardioprotective, although their clinical safety has been recently challenged.² That TZDs can lower blood pressure in the face of weight gain and water and salt retention by the kidneys suggests that the antihypertensive effects may be particularly profound.

Heterozygous mice carrying one normal PPAR γ allele and one dominant-negative allele (L/+ mice) exhibit a moderate increase in blood pressure.^{3,4} We have recently reported that L/+ mice exhibit endothelial dysfunction and hypertrophy and inward remodeling in the cerebral vasculature.⁴ Oxidative

stress was the basis of endothelial dysfunction in the model as superoxide was increased and vascular function was restored to normal by a free radical scavenger. These data provided genetic evidence suggesting a functional role for PPAR γ in the vascular wall. However, because this knockin mouse exhibited defective PPAR γ signaling in all cells, we were not able to determine whether the abnormalities were due to systemic or vascular-specific interference with PPAR γ function.

PPAR γ is expressed in both vascular muscle and endothelium. We recently reported that PPAR γ plays a critical role in vascular muscle, where it is required to mediate cGMP-dependent signaling from nitric oxide released from the endothelium and is required to inhibit vasoconstriction to endothelin-1.⁵ Mice with vascular muscle-specific interference with PPAR γ also exhibited mild hypertension. Despite many reports showing that TZDs improve endothelial function in diabetes and hypertension, the importance of PPAR γ

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in the endothelium, independent of ligand-mediated activation, which may have off target effects, has yet to be defined.⁶ Only one study has used an animal model that can avoid the use of exogenous ligand treatment to directly implicate endothelial PPAR γ .⁷ In that model, PPAR γ was knocked down in endothelial cells by breeding PPAR γ -flox mice with mice expressing cre-recombinase controlled by the Tie-2 promoter. Endothelial PPAR γ -null mice exhibited an increase in blood pressure only after feeding a high-fat diet. In addition, recent reports suggest that endothelial-specific PPAR γ knockout mice also exhibit osteopetrosis⁸ and production of a toxic milk in pregnancy⁹ suggesting complete loss of PPAR γ has pleiotropic effects, which may affect multiple organ systems. In the current study, we used endothelium-directed expression of 2 different clinically relevant, dominant-negative mutants of PPAR γ (P467L and V290M) to test the hypothesis that endothelial PPAR γ is a critical mediator of vascular function and its loss through dominant-negative interference causes endothelial dysfunction. The use of these mutants prevents problems associated with off-target effects of TZD drugs and the complete loss of PPAR γ function, which is clearly deleterious.

Methods

Generation of Transgenic Animal Models

The transgene consisted of the VeCad promoter subcloned into a modified form of pStec-1.¹⁰ Human PPAR γ cDNA was cloned downstream of the promoter with EcoRV. The P467L and V290M mutations were generated using a site-directed mutagenesis kit (Stratagene) using the primers: GATCTCCTGCACAGCCTCCATGGAGCGAAACTGGCAGCC, GCTGCCAGTTTCGCTCCATGGAGCTGTGCAGGAGATC for V290M, and ACAGACATGAGTCTTCACTGCTCCTGCAGGAGATCTAC, GTAGATCTCCTGCAGGAGCAGGTGAAGACTCATGTCTGT for P467L. The transgene was excised by ClaI, purified, and microinjected into one cell-fertilized mouse (C57BL/6J \times SJL/J F2) embryos. All transgenic mice were maintained by backcross breeding with C57BL/6J mice for at least 4 generations. Genotyping was performed using the primers CAGCTCACAAAGGAACAATAACAG and CTCATAGTGAATCCAGAAG.

All mice were fed standard mouse chow (LM-485; Teklad Premier Laboratory Diets) and water ad libitum. For the high-fat diet treatment, male animals were fed D12451 (45 kcal% fat) from Research Diets for 12 to 25 weeks beginning at 8 to 10 weeks of age. Experimental animals were 4 to 8 months of age. Care of the mice used in the experiments met the standards set forth by the National Institutes of Health in their guidelines for the care and use of experimental animals. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

Molecular Assays

Spleen DNA from transgenic and control mice was isolated as described.¹¹ Genomic DNA (10 μ g) was digested with BamHI and Southern blotted. The transgene was digested with EcoRI to generate a 776-bp fragment to label with Rediprime II Random Primer Labeling System (Amersham Biosciences). For restriction fragment length polymorphism analysis, genomic DNA was amplified using transgene-specific primers, GACTTCTCCAGCATTCTACTCCA and CACTGCATTCTAGTTGTGG, to generate a 1408-bp product. The amplified product was digested with BsrBI, NcoI, or BamHI. To assay transgene expression, RNA was isolated using Tri-Reagent (Molecular Research Center Inc, Cincinnati, Ohio) and subjected to RNase protection using the RPAIII Kit (Ambion, Austin, Texas) as described previously.⁵ Protected fragments were 200 for mouse

PPAR γ , 280 bases for human PPAR γ , 105 nt for cyclophilin, and 125 nt for 28S as described previously.⁵

To assay for endothelial specificity, mice were given a lethal dose of pentobarbital, and the aorta was removed and cleaned of fat and connective tissue. The vessel was divided into 2 segments and one was denuded of endothelium by rolling on a steel wire. Total RNA was extracted from endothelium-intact and endothelium-denuded samples using TriReagent. RNA samples were treated with DNase-I (Fermentas), cDNA was generated by reverse transcriptase using Superscript III (Invitrogen), and polymerase chain reaction was performed using the Hi-Fidelity Platinum Taq system (Invitrogen). Primer sequences were: smooth muscle myosin heavy chain (CGAAGTTGATGCGAATGAA, TGGAGCCGAAAGACAGA), VeCAD (CTAGATGTGGATGAGCCCCCTGTC, AATTGGCCTCTGTCACTGGTCTTG), E-PPAR γ (TGCAGAAGTTG-GTCGTGAGG, AGAAGTCAACAGTAGTGAAGG), and GAPDH (TGACCACCAACTGCTTAG, GATGCAGGGATGATGTTC).

Aortic Ring Preparation

Male and female mice were given a lethal dose of pentobarbital and the thoracic aorta was quickly removed and prepared for measurements of contraction and relaxation as described in detail.^{12,13}

Drugs and Reagents

Acetylcholine (Ach), sodium nitroprusside, A23187, papaverine, PE, KCl, 5-HT, U-46619, Tempol, lucigenin, and NADPH were obtained from Sigma-Aldrich Biochemical and dissolved in physiological saline solution. PGF_{2 α} (Lutylase Pfizer Pharmaceutical) was from the University of Iowa pharmacy. Endothelin-1 was from Peninsula Laboratories Inc and dissolved in water.

Studies of Cerebral Arteries In Vitro

Male and female mice were given a lethal dose of pentobarbital, the brain removed, and the basilar artery isolated and prepared for measurements of vessel diameter in vitro as described.¹⁴ At the end of each experiment, papaverine (100 μ mol/L) was used to produce maximal vasodilation. Vasodilator responses are expressed as percent dilation (percent of induced tone) with 100% representing the difference between the resting value and the constricted value with U46619.

Measurement of Oxidative Stress

Age-matched mice were either fed a normal diet or a high-fat diet for 12 weeks as described previously. Superoxide was measured by the lucigenin assay in the aorta and a pooled sample of cerebral arteries (basilar artery, middle cerebral arteries, and circle of Willis) as previously described.^{13,15}

Microarray Analysis

Mouse aortic endothelial cells were cultured by Dominion Pharmakine. Each culture (3 from NT and 3 from E-V290M) was from aortic samples pooled from 2 mice. Each culture was grown in RPMI media supplemented with 50 U/mL penicillin, 50 g/mL streptomycin, 10% fetal calf serum, 10 U/mL heparin, 1 μ g/mL dexamethasone, and 0.1 mg/mL endothelial cell growth supplement (Sigma-Aldrich) on human fibronectin-coated plates to the third passage and then frozen. Cultures were considered to not be contaminated with smooth muscle based on α -actin staining (generally less than 10% smooth muscle cell per culture). RNA was generated using the Trizol method. For the microarray hybridizations, 3 independent biological replicates from each experimental group were used. All the microarray procedures were conducted at the University of Iowa DNA Core facility using standard Affymetrix protocols. In brief, approximately 3 μ g of total RNA was used as input to a one-step amplification procedure to generate biotin-labeled RNA fragments for hybridization to the Affymetrix GeneChip Mouse Genome 430 2.0 array. This array contains 45 101 probe sets interrogating 22 485 distinct genes. Data from the microarray studies, including CEL files, have been submitted to the Gene Expression Omnibus at the National Center for Biotechnology Information (Accession No: GSE11870).

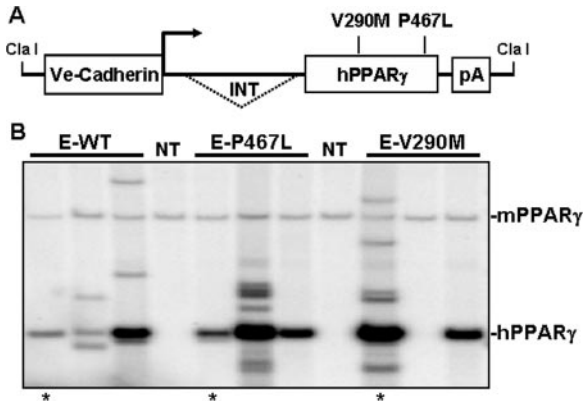


Figure 1. Generation of E-PPAR γ transgenic mice. A, A schematic representation of the transgenic construct. B, Southern blot analysis of E-WT, E-P467L, and E-V290M lines used in this report. The position of the endogenous mouse PPAR γ (mPPAR γ) and transgene (hPPAR γ) is indicated. The lines marked by the asterisks are those used in this report. There was no correlation between transgene copy number and expression.

Blood Pressure Analysis

Blood pressure was measured by radiotelemetry as described previously.⁵ Mice were given 7 to 10 days to recover, after which time heart rate and arterial pressure were continuously recorded (sampling every 5 minutes for 20-second intervals) for 1 week as a baseline measure of blood pressure. Data were collected and stored using Dataquest ART. Angiotensin II was infused through a minipump (Alzet) at a dose of 1000 ng/kg/min for 5 days as previously described and blood pressure was measured by tail cuff using the Visitech 2000 system.¹⁶

Statistical Analysis

All data are expressed as mean \pm SEM. Comparisons were made with 2-way repeated-measures analysis of variance using a Tukey post hoc test or *t* test where appropriate. *P* < 0.05 was considered significant. Data were analyzed by use of SigmaStat (Systat Software).

Results

We generated transgenic mice for 3 constructs all expressing PPAR γ under the control of the endothelial-specific vascular cadherin (VeCad) promoter (Figure 1A). The constructs expressed either wild-type (E-WT) or one of 2 dominant-negative mutations (V290M or P467L) previously shown to cause severe hypertension clinically.¹ Multiple founders were identified and validated by both Southern blot (Figure 1B)

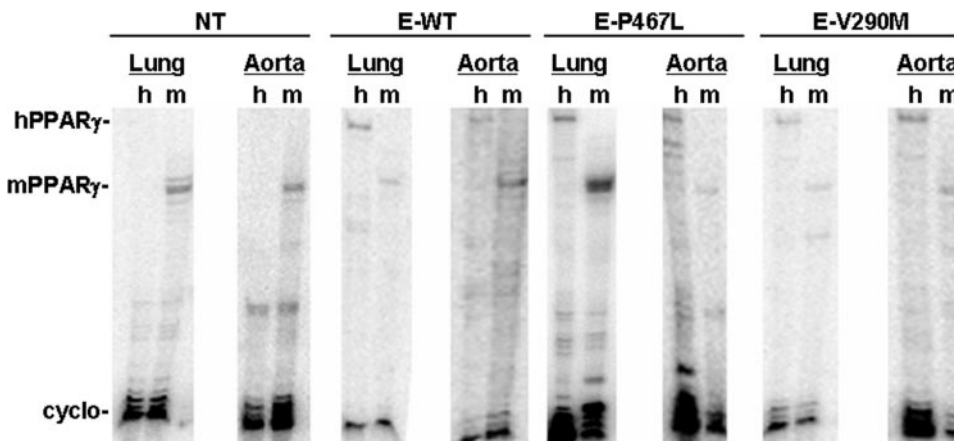


Figure 2. Expression of PPAR γ in the blood vessel wall. Expression of endogenous mPPAR γ with hPPAR γ transgene in the lung and aorta assayed by RNase protection. The aorta RNA is actually a combination of aorta and carotid artery pooled from several transgenic mice of the indicated line and construct. Cyclophilin expression is the internal control. h, human; m, mouse.

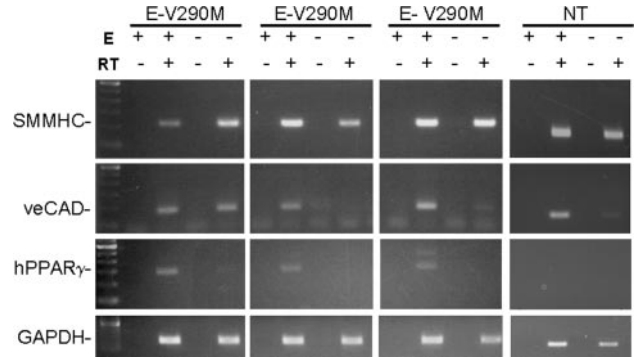


Figure 3. Cell-specific expression of E-V290M transgene. Cell specificity of transgene expression was analyzed through reverse transcription-polymerase chain reaction. Smooth muscle myosin heavy chain and VeCad were used as markers for smooth muscle and endothelial cells, respectively. GAPDH was used as an internal control. PPAR γ primers were specific for the human PPAR γ transgene. The presence of reverse transcription and endothelium (E) is indicated by + and -.

and the presence of the correct mutation by restriction fragment length polymorphism analysis (Supplemental Figure I). Expression of PPAR γ was assessed in each line of mice by RNase protection, and lines were selected on the basis of overexpression compared to endogenous mouse PPAR γ in the lung and aorta (Figure 2). Although the copy number of the transgene varied, we chose lines that expressed approximately equal amounts of the transgene. As expected, the transgene was expressed in all tissues because they all contain endothelial cells (Supplemental Figure II). Therefore, to demonstrate endothelial specificity, we assayed for expression of smooth muscle myosin heavy chain (a smooth muscle-specific marker), VeCad (an endothelial cell marker), human PPAR γ , and GAPDH in endothelium intact and endothelium-denuded aorta (Figure 3). The data show a preservation of smooth muscle myosin heavy chain and GAPDH expression in all samples but a depletion of VeCad and human PPAR γ after removal of the endothelium. Consistent with this, there was no change in body weight (26.5 ± 1.0 versus 27.7 ± 1.0 g), brown adipose ($0.37 \pm 0.1\%$ versus $0.30 \pm 0.03\%$), or white reproductive adipose tissue (2.2 ± 0.3 versus $2.0 \pm 0.2\%$) of E-V290M compared with nontransgenic littermates.

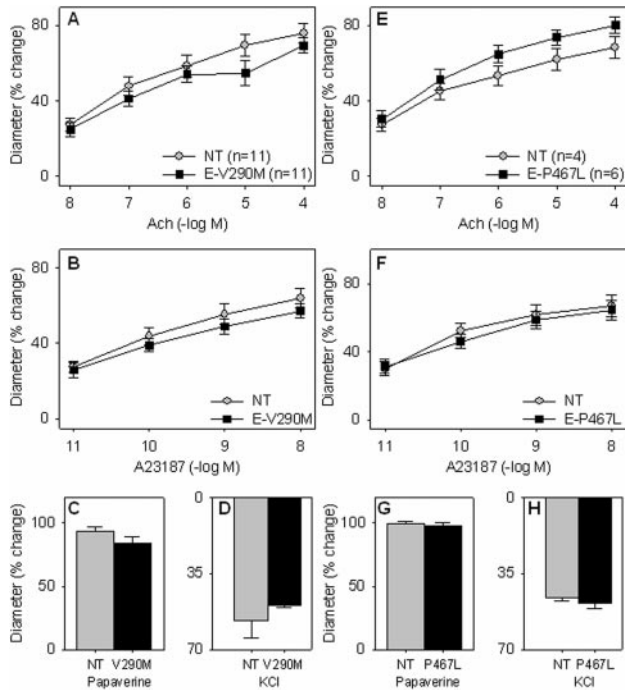


Figure 4. Endothelial function in the basilar artery under a normal diet. Vascular function was measured in E-V290M (A–D) and E-P467L (E–H) mice compared with nontransgenic littermates. Relaxation was measured in response to Ach (A, E), A23187 (B, F), and papaverine (C, G) and contraction was measured in response to KCl (D, H).

We next examined vascular function in the aorta *in vitro* by measuring relaxation to Ach and sodium nitroprusside after precontraction with PGF_{2α}. There was no difference in the response to either agonist in mice expressing either the V290M or P467L mutation in PPAR γ (Supplemental Figure III). There was also no difference in the contractile response to several receptor-dependent and receptor-independent vasoconstrictors (Supplemental Figure IV). To examine vascular function in a resistance vessel supplying a major organ, we studied the basilar artery *in vitro*. Under normal diet conditions, there were no differences in the dilator response to 2 endothelial-dependent agonists, Ach and the calcium ionophore A23187, in either E-V290M or E-P467L mice (Figure 4). There was also no difference in the dilator response to the endothelial-independent agonist papaverine nor the vasoconstrictor KCl.

Previous studies suggest that endothelial PPAR γ -null mice exhibit an increase in arterial pressure only in response to a high-fat diet.⁷ Consequently, we examined vascular function in mice fed a high-fat diet for 12 weeks. The increase in body weight due to the high-fat diet was similar in E-V290M (from 26.5 \pm 1.0 g to 34.1 \pm 2.2 g, Δ =7.6 g) and nontransgenic littermates (from 27.7 \pm 1.0 g to 34.7 \pm 1.9 g, Δ =7.0 g). Whereas a high-fat diet had no effect on endothelial function in the basilar artery of nontransgenic mice, it caused marked impairment in dilation to both Ach and A23187 in E-V290M or E-P467L mice (Figure 5). Endothelial-independent dilation was not altered nor was the contractile response to KCl. Importantly, the impairment observed in the E-V290M and E-P467L was not evident in high-fat diet-fed transgenic mice

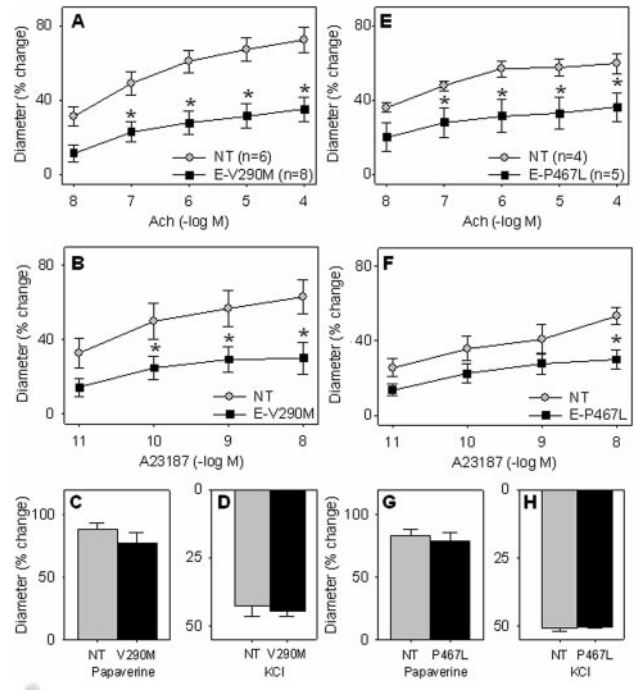


Figure 5. Endothelial function in E-V290M and E-P467L basilar artery after a high-fat diet. Vascular function was measured in E-V290M (A–D) and E-P467L (E–H) mice compared with nontransgenic littermates. Relaxation was measured in response to Ach (A, E), A23187 (B, F), and papaverine (C, G) and contraction was measured in response to KCl (D, H). **P*<0.05 versus nontransgenic.

expressing a wild-type copy of human PPAR γ (E-WT) suggesting the impairment was due to dominant-negative interference and not to mere overexpression of PPAR γ (Figure 6). A high-fat diet for 12 weeks did not cause vascular dysfunction in the aorta (Supplemental Figure VA–D), although a modest increase in contraction to endothelin-1 was noted at the highest concentrations tested (Supplemental Figure VI). Interestingly, increasing the duration of the high-fat diet to 25 weeks caused endothelial dysfunction in the aorta suggesting there is increased susceptibility to dysfunction even in conduit vessels caused by interference with PPAR γ in the endothelium (Supplemental Figure VE–F).

We previously reported that cerebral vascular dysfunction observed in mice systemically expressing the P465L mutation in PPAR γ was due to oxidative stress.⁴ To test if a similar mechanism is operant in E-V290M mice fed a high-fat diet, we examined the response to Ach before and after treatment with Tempol, a superoxide dismutase mimetic (Figure 7A). As previously stated, the response to Ach was impaired in the basilar artery from mice fed a high-fat diet. Tempol significantly improved the Ach response in mice fed a high-fat diet. We next measured superoxide in the aorta and cerebral arteries in age-matched NT and E-V290M mice fed a normal or high-fat diet using lucigenin (Supplemental Figure VII). Surprisingly, there was no difference in baseline lucigenin or the response to increasing doses of NADPH irrespective of diet or vessel. As expected, Tiron significantly attenuated the lucigenin signal in the presence of NADPH.

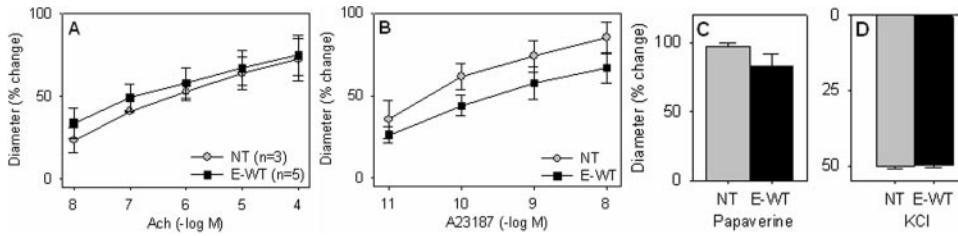


Figure 6. Endothelial function in E-WT basilar artery after a high-fat diet. Vascular function was measured in E-WT mice compared with nontransgenic littermates. Relaxation was measured in response to Ach (A), A23187 (B), and papaverine (C) and contraction was measured in response to KCl (D).

To obtain a potential molecular explanation for our observations, we performed gene expression profiling on aortic endothelial cells cultured from NT and E-V290M mice. Gene set enrichment analysis, which examines large sets of genes as a group, revealed significant ($P=0.019$) upregulation of genes considered to be pro-oxidant, including subunits of NADPH oxidase. Individually, there was a modest but significant increase in expression of p22phox, Nox2, and NoxA2, but not of Nox2 and Nox4 (Figure 7B). Interestingly, there was also a significant decrease in catalase and CuZnSOD (SOD1) expression. We also noted a significant increase in endothelial nitric oxide synthase (NOS3) and Gpx1 expression, which may represent compensatory changes.

We next measured arterial pressure using radiotelemetry. In mice fed a normal-fat diet, we observed a small increase in arterial pressure that was greater during the nighttime hours (9 mm Hg, $P=0.07$) than during the day (5 mm Hg; Figure 8A–B). Interestingly, although the increase in arterial pressure was only modest, the E-V290M mice were more susceptible to the pressor response caused by subcutaneous administration of angiotensin II through an osmotic minipump (Δ systolic blood pressure: 38.5 ± 3 versus 26.1 ± 3 mm Hg; Figure 8C–D). We did not observe a significant increase in arterial pressure after 12 weeks of a high-fat diet (109.5 ± 1.9 mm Hg from 105.2 ± 5.7 mm Hg in controls; 112.3 ± 2.7 mm Hg from 111.7 ± 3.9 mm Hg in E-V290M). For reasons that remain unclear, mice implanted with radiotelemeters during the high-fat diet feeding period only gained approximately 50% of the weight as mice lacking transmitters. Similarly, mice implanted with radiotelemeters after the 12-week high-fat diet period lost weight despite being maintained on the high-fat diet.

Discussion

The importance of PPAR γ in the regulation of adipogenesis and type II diabetes is widely accepted, but its role in the vascular wall is not well understood. Although the role of PPAR γ in resident macrophages in the vasculature during atherosclerosis has been extensively studied,¹⁷ much less is known about the function of PPAR γ in the endothelium in vivo. PPAR γ is expressed in the endothelium where it has been reported to modulate expression of genes involved in vasoconstriction and oxidative stress.^{18–21} This is the first study that uses dominant-negative mutations of clinical importance to study the role of PPAR γ specifically in the vascular endothelium. In humans, naturally occurring mutations interfering with PPAR γ function are associated with severe hypertension and metabolic abnormalities.¹ Our results provide genetic evidence supporting an important role for PPAR γ in the vascular endothelium. The major finding from our study is that interference with PPAR γ function specifically in the endothelium results in a significant impairment in endothelial-dependent relaxation in the basilar artery, a resistance vessel in the cerebral circulation. This impairment in basilar artery function was only observed when the mice were fed a high-fat diet and was not observed in the aorta (a conduit artery) of mice fed high fat for the same period of time. Like other recent studies, our data suggest that a high-fat diet has heterogeneous effects on different blood vessels.²² The mechanism accounting for the high-fat diet-induced dysfunction appeared to involve oxidative stress as the function of the vessel returned to normal after addition of a scavenger of superoxide. This is consistent with other data showing increased oxidative stress in the vasculature after a high-fat diet.²³

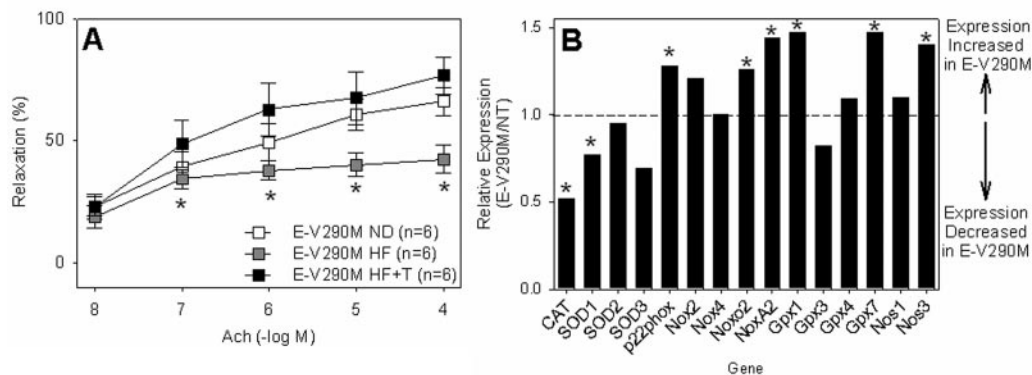


Figure 7. Oxidative stress. A, Vascular function was measured in E-V290M mice \pm high-fat diet and Tempol as indicated. $*P < 0.05$ versus high-fat diet plus Tempol. B, Expression of select genes in the oxidative stress pathway as measured by microarray analysis. Each bar reflects the change in gene expression in E-V290M versus NT as a composite of 3

arrays each from NT and E-V290M. In cases where multiple probe sets were available, they were averaged. $*P < 0.05$ after correction for multiple comparisons.

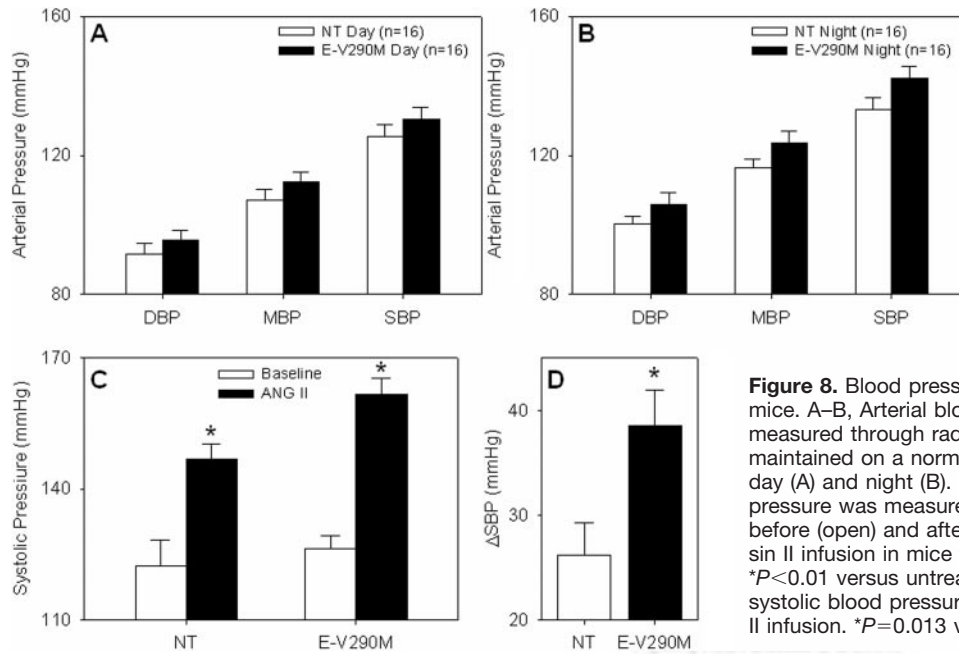


Figure 8. Blood pressure of E-V290M mice. A–B, Arterial blood pressure was measured through radiotelemetry in mice maintained on a normal diet during the day (A) and night (B). C, Systolic blood pressure was measured by tail cuff before (open) and after (closed) angiotensin II infusion in mice fed a normal diet. * $P < 0.01$ versus untreated. D, Increase in systolic blood pressure after angiotensin II infusion. * $P = 0.013$ versus NT.

Our data imply that resistance vessels may be more sensitive to the loss of endothelial PPAR γ than conduit vessels, because the basilar artery exhibited dysfunction after 12 weeks on a high-fat diet, whereas it took 25 weeks before the same effect was observed in the aorta. The mechanism for this difference is unclear. Nevertheless, these data are consistent with our recent finding that knockin mice carrying a dominant-negative allele of PPAR γ (P465L, L/+), expressed in all tissues, exhibit severely impaired cerebral vascular function but only modest aortic dysfunction.⁴ Interestingly, the knockin mice expressing the dominant-negative mutant in both endothelium and vascular muscle exhibit impaired cerebral artery and arteriolar function under baseline conditions without the requirement for high-fat diet-induced stress. Consequently, we hypothesize that a stressor such as a high-fat diet may be required to unmask a phenotype when the function of PPAR γ in the endothelium alone is impaired, but not when its function is impaired in both endothelium and vascular muscle. Recall, endothelium-specific PPAR γ knockout mice exhibit an increase in blood pressure only after being fed a high-fat diet.⁷ These data are consistent with the role of PPAR γ as a fatty acid sensor.²⁴ The link between PPAR γ and oxidative stress coupled with our finding that Tempol reverses the high-fat diet-induced cerebral vascular dysfunction suggests that free fatty acids or some other PPAR γ ligand(s) may increase in the endothelium in response to a high-fat diet. It is likely that an increase in PPAR γ activity in the endothelium may provide protective mechanisms by increasing synthesis of nitric oxide and antioxidants.^{18,19} The V290M or P467L mutations lie in the ligand-binding domain of PPAR γ and are thought to destabilize a region of the protein required for coactivator recruitment in response to ligand.²⁵ Thus, the expression of these mutant proteins interferes with this protective mechanism resulting in oxidative stress and impaired vessel function. Along these lines, it is particularly interesting that expression of p22phox

was increased and expression of catalase and CuZnSOD decreased in cultured endothelial cells from E-V290M mice. Catalase, CuZnSOD, and p22phox genes have been reported to be targets of PPAR γ ,^{18,26–28} and CuZnSOD-deficient mice exhibit oxidative stress and vascular dysfunction.¹³ Moreover, p22phox-overexpressing mice exhibit augmented Ang II-induced vascular hypertrophy.²⁹ The increase in endothelial nitric oxide synthase expression is interesting in light of its upregulation in mice overexpressing p22phox.³⁰ Consequently, the upregulation of antioxidant enzymes (CuZnSOD and catalase), which is thought to be part, or indicative, of the oxidative stress response, may be PPAR γ -dependent. This increase, which would normally be protective, is prevented due to interference with PPAR γ function.

Our gene expression data and our finding with Tempol are consistent with an increase in oxidative stress in E-V290M mice. We recognize that not being able to detect an increase in superoxide using chemiluminescence does not support a role for an oxidant-dependent mechanism of vascular dysfunction. Considering related work in this area,⁴ our new microarray findings, and other studies suggesting antioxidant effects of PPAR γ ,^{18,26,27} it still seems likely that a superoxide-related mechanism contributes to vascular dysfunction after the combination of endothelial-specific interference with PPAR γ and a high-fat diet. The lack of a detectable increase in superoxide using lucigenin in this model may reflect the sensitivity of that assay in relation to the subcellular localization of superoxide or other factors that may have influenced the results.

The necessity for a high-fat diet in the E-V290M and E-P467L mice described here, but not in the P465L (L/+) knockin mice,⁴ also suggests a potential contribution of PPAR γ in vascular muscle. Indeed, we recently demonstrated the importance of vascular muscle PPAR γ by reporting profound aortic dysfunction in mice expressing the same PPAR γ mutations (S-P467L and S-V290M) under the control

of a smooth muscle-specific promoter.⁵ Like these studies, the effects were only evident in mice expressing the dominant-negative mutant but not in mice expressing wild-type PPAR γ , strongly suggesting the resultant abnormalities were due to dominant-negative interference with PPAR γ function and not simple overexpression of PPAR γ . S-P467L and S-V290M mice exhibited a loss of responsiveness of the aorta to nitric oxide and hypercontraction to endothelin-1. It is unclear why aortic function in the L/+ was only modestly impaired, although the dominant-negative was expressed in vascular muscle. The most likely explanation is the level of dominant-negative activity. The L/+ mice express one wild-type and one dominant-negative allele (a 1:1 ratio), whereas we were able to achieve a higher level of mutant PPAR γ expression at both the mRNA and protein level in the aorta by using the smooth muscle myosin heavy chain promoter. Indeed, we also reported that the magnitude of the aortic dysfunction correlated with the level of dominant-negative PPAR γ . Therefore, the magnitude of impairment and the type of vessel affected is dependent on the level of interference garnered and the cell type expressing the mutation.

Male L/+ and male and female S-P467L mice exhibited an approximate 10-mm Hg increase in arterial pressure. Baseline blood pressure in the E-V290M mice was elevated by 9 mm Hg during the nighttime hours but did not achieve statistical significance. Interestingly, although not overtly hypertensive, these mice exhibited an increased pressor response to angiotensin II suggesting that, like a high-fat diet, they may be sensitive to additional stressors. The increase in angiotensin sensitivity is consistent with studies showing that activation of PPAR γ by TZDs prevents the angiotensin-induced pressor response and lowers blood pressure in mice overexpressing angiotensin II.^{12,31} Interestingly, there was no increase in angiotensin II-mediated contraction in the aorta of E-V290M mice (data not shown).

Endothelial-specific PPAR γ knockout mice exhibit normal blood pressure under baseline conditions but become hypertensive after being fed a high-fat diet.⁷ We therefore anticipated a similar finding in our mice. Although we put considerable effort into measuring blood pressure in these mice (both throughout the entire 12-week high-fat diet period or after completion of the high-fat diet), we observed significant weight loss (approximately 50% of the gain) after implantation of the radiotelemeters making interpretation of those results difficult. Consequently, further studies are needed to assess if the high-fat diet-induced vascular dysfunction translates to an increase in arterial pressure. Interestingly, since the original publication of the endothelial PPAR γ knockout,³² there have been other reports that have used the same methodology of breeding PPAR $\gamma^{\text{fllox/fllox}}$ mice with Tie2-Cre mice, which express Cre-recombinase in endothelial cells. One study showed that the promoter is active in hematopoietic cells and osteoclasts and resulted in osteopenia and increased bone mass.⁸ In another study, maternal deletion of PPAR γ resulted in growth retardation and other defects in nursing pups irrespective of their genotype caused by the production of milk containing elevated inflammatory lipids.⁹ Therefore, the loss of PPAR γ in cells in which the

Tie2 promoter is active (endothelial cells and other cell types) may have other unidentified consequences.

In summary, our results identify endothelial PPAR γ as a critical regulator of endothelial function in the cerebral circulation, especially under conditions of high fat-induced stress.

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Disclosures

None.

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