PPARγ Activation in Human Endothelial Cells Increases Plasminogen Activator Inhibitor Type-1 Expression
PPARγ as a Potential Mediator in Vascular Disease

Nikolaus Marx, Todd Bourcier, Galina K. Sukhova, Peter Libby, Jorge Plutzky

Abstract—Plasminogen activator inhibitor type-1 (PAI-1) is a major physiological inhibitor of fibrinolysis, with its plasma levels correlating with the risk for myocardial infarction and venous thrombosis. The regulation of PAI-1 transcription by endothelial cells (ECs), a major source of PAI-1, remains incompletely understood. Adipocytes also produce PAI-1, suggesting possible common regulatory pathways between adipocytes and ECs. Peroxisomal proliferator-activated receptor-γ (PPARγ) is a ligand-activated transcription factor that regulates gene expression in response to various mediators such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) and oxidized linoleic acid (9- and 13-HODE). The present study tested the hypotheses that human ECs express PPARγ and that this transcriptional activator regulates PAI-1 expression in this cell type. We found that human ECs contain both PPARγ mRNA and protein. Immunohistochemistry of human carotid arteries also revealed the presence of PPARγ in ECs. Bovine ECs transfected with a PPAR response element (PPRE)–luciferase construct responded to stimulation by the PPARγ agonist 15d-PGJ2 in a concentration-dependent manner, suggesting a functional PPARγ in ECs. Treatment of human ECs with 15d-PGJ2, 9(S)-HODE, or 13(S)-HODE augmented PAI-1 mRNA and protein expression, whereas multiple PPARα activators did not change PAI-1 levels. Introduction of increasing amounts of a PPARγ expression construct in human fibroblasts enhanced PAI-1 secretion from these cells in proportion to the amount of transfected DNA. Thus, ECs express functionally active PPARγ that regulates PAI-1 expression in ECs. Our results establish a role for PPARγ in the regulation of EC gene expression, with important implications for the clinical links between obesity and atherosclerosis. (Arterioscler Thromb Vasc Biol. 1999;19:546-551.)

Key Words: atherosclerosis ■ endothelium ■ peroxisomal proliferator-activated receptor ■ plasminogen activator inhibitor-1 ■ 15-deoxy-Δ12,14-prostaglandin J2

Endothelial cells (ECs) are an important source of plasminogen activator inhibitor type-1 (PAI-1) plasma activity.1 PAI-1, a member of the serine protease inhibitor (serpin) family, is the major physiological inhibitor of tissue plasminogen activator and urokinase and thus, limits fibrinolysis.2 Considerable evidence links PAI-1 to myocardial infarction and deep venous thrombosis3–5; endothelial production of PAI-1 likely influences these events. As such, the regulation of PAI-1 expression in ECs has received focused attention.6–8 Cytokines such as transforming growth factor-β and tumor necrosis factor-α increase PAI-1 expression.9,10 Circulating lipids,11 some lipid-lowering therapies,12 and the clinical condition of obesity itself12 all affect PAI-1 expression. This response to lipids, as well as the evidence that adipocytes themselves can express PAI-1,13 raises the possibility that transcriptional mediators important in adipogenesis and adipocyte signaling may play similar roles in ECs.

Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily, are ligand-activated transcription factors that play an important role in lipid metabolism.14–16 One of these PPARs, PPARγ, has been implicated in the transcriptional regulation of several genes involved in lipid metabolism and appears to promote the differentiation of cells toward a more adipocyte-like phenotype.17–19 Both synthetic and natural ligands for PPARγ have been described. Among the synthetic ligands, thiazolidinediones, a group of compounds that includes troglitazone, increase insulin sensitivity.20 Naturally-occurring PPARγ ligands include fatty acids, eicosanoid derivatives,21 and 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2).19 Recent work has established that 9-hydroxy-(S)-10,12-octadecadienoic acid [9(S)-HODE] and 13(S)-HODE, known components of oxidized LDL, are PPARγ activators, with concomitant evidence invoking PPARγ signaling in monocytes and macrophages.22,23 Clinical observations suggest that obese patients have elevated adipose tissue PPARγ levels compared with those in lean controls.24 Once activated, PPARγ binds to the PPAR response elements (PPRE) in the promoter region of target genes.25–27 Although PPARγ has been extensively studied in adipocytes, monocytes/macrophages, and vascular smooth muscle cells,22,23,28–30a essentially nothing is known about PPARγ in EC biology and gene expression.

The present study investigated whether PPARγ was expressed and active in human ECs, and if so, whether PPARγ...
might regulate PAI-1 expression in this cell type, abundant in adipose tissue. In addition to focusing attention on the possible role of PPARγ signaling in ECs, such findings offer a novel molecular link between the clinical associations between obesity, coagulation status, and vascular events.

Methods

Cell Culture

Human saphenous vein ECs were isolated from outgrowths of explants from unused portions of saphenous veins harvested at coronary artery bypass surgery. Cells were cultured in medium 199 (BioWhittaker) containing 25 mmol/L HEPES, 1% heparin, 50 mg/L EC growth factor, 1% glutamine, 1% penicillin-streptomycin, and 5% FCS on low-serum medium (0.1% FCS). ECs were cultured as described before. 30

NIH3T3-L1 preadipocytes, originally obtained from American Type Culture Collection (Manassas, Va) and generously provided to us by Dr Bruce Spiegelman, Dana Farber Cancer Institute, Boston, Mass, were cultured in DMEM, 10% bovine calf serum, and 1% penicillin-streptomycin. Differentiation of preadipocytes into adipocytes was induced as described by others. 17 Monocyte-derived macrophages were cultured as described before. 30

RNA Extraction and Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

Total RNA from 10 7 cells was isolated by the single-step guanidinium thiocyanate–phenol-chloroform method with the use of RNAzol from Tel-Test. Two microgram of total RNA was reverse-transcribed into cDNA with 1 U/mL reverse transcriptase (Superscript, Gibco-BRL) at 37°C for 1 hour in standard buffer. Amplification of PPARγ cDNA used 2 oligonucleotide primers from nucleotides +384 to +705 (a 321-bp fragment): sense-primer, 5’-GCCGGGATCTGGTGGAGATT-3’ (Gibco-BRL) with 200 ng of each primer (IDT), 33 mmol/L MgCl2, and 0.5 U Taq polymerase (Gibco-BRL) for 30 cycles. PCR products (10 μL/25 μL) were analyzed on a 2% agarose gel.

Northern Blot Analysis

Five microgram of total RNA from unstimulated or 15d-PGJ2–stimulated ECs was used for standard Northern blot analysis. After electro-

Figure 1. Human ECs express PPARγ mRNA and protein. A, RT-PCR of PPARγ mRNA in human ECs reveals cDNA of the expected size. A 100-bp DNA ladder (MW), positive controls of PPARγ in NIH3T3-L1 preadipocytes (PAC), differentiated preadipocytes (AC), monocyte-derived macrophages (Mø), and negative controls without cDNA (Co) are also shown (top). RT-PCR of GAPDH (bottom) from these same samples served as a normalization control. Four independent experiments showed similar results. B, Western blot (bottom) from these same samples served as a normalization control. Controls without cDNA (Co) are also shown (top). RT-PCR of GAPDH (bottom) from these same served as a normalization control. Figure 2. Expression of PPARγ in ECs of human carotid arteries. A, Immunostaining with CD31 identified the EC layer at the luminal surface of the artery (red; magnification ×40). B, Parallel sections revealed PPARγ expression in the nuclei of ECs (positive nuclei, stained blue, are indicated by arrowheads). C, Parallel sections stained with PPARγ antibodies preabsorbed with the immunizing peptide (Pre-abs PPARγ) showed no signal, demonstrating that staining for PPARγ in B was specific. Analysis of 4 separate carotid sections revealed similar results. (B and C, magnification ×100).

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phoresis, RNA was transferred to nylon membranes (ICN) in 20x SSC by using a capillary blotting technique. Blots were UV cross-linked, prehybridized (50% formamide, 5x Denhardt’s solution, 5x SSC, 0.5% SDS, and 20 mmol/L salmon sperm DNA), and hybridized in the same buffer with a radiolabeled (5'32P]dATP) PPAR-1 oligonucleotide (Calbiochem). The membranes were washed at 60°C in 1% SDS–2x SSC and autoradiographed with Kodak X-Omat film at -70°C with an intensifying screen.

Preparation of Nuclear and Cytosolic Extracts and Western Blot Analysis

For Western blotting, nuclear and cytosolic extracts of 10³ cells were prepared. Cells were lysed in 10 mmol/L HEPES, pH 7.9, 1.5 mmol/L MgCl₂, 10 mmol/l KCl, and 0.5% NP-40. Nuclei were pelleted at 13 000 g for 5 minutes, and the resulting supernatant was used as the cytosolic fraction. Nuclei were lyzed in 20 mmol/L HEPES, pH 7.9, 1.5 mmol/L MgCl₂, 420 mmol/L NaCl, and 0.2 mmol/L EDTA. After centrifugation at 13 000 g for 5 minutes, the supematant was diluted in an equal volume of 20 mmol/L HEPES, pH 7.9, 100 mmol/L KCl, 0.2 mmol/L EDTA, and 20% glycerol and used as the nuclear extract. Protein concentration of nuclear and cytosolic extracts was determined colorimetrically (Pierce). Processed samples were applied to 10% SDS–polyacrylamide gel electrophoresis (PAGE) gels and transferred to nitrocellulose membranes (Milli-pore) by semi-dry blotting, as described previously.

Membranes were treated overnight with Tris-buffered saline–TWEEN containing 5% dry milk and incubated with goat anti-human PPAR-γ antibodies (mAbs; Santa Cruz, San Diego, Calif) followed by avidin-biotin-peroxidase complex (Vectastain ABC kit). Antibody binding was visualized with 3-amino-9-ethylcarbazole (Tropix). With similar techniques, human skin fibroblasts were transfected with PPAR-γ-transfected human skin fibroblasts served as a positive control. For the detection of secreted PAI-1 in supernatants from unstimulated or stimulated ECs, 50 µL from 500 µL total supernatant was subjected to 10% SDS-PAGE and processed as indicated above. For the detection of PAI-1, membranes were stained with a mouse anti-human PAI-1 mAb (American Diagnostica Inc, Greenwich, Conn). Quantification was performed using the NIH-Image densitometry software.

Immunohistochemistry of Human Carotid Artery Specimens

Surgical specimens of human carotid arteries were obtained by protocols approved by the Human Investigation Review Committee at the Brigham and Women’s Hospital, Boston, Mass. Serial cryostat sections (5 mm) were cut, air dried onto microscopic slides, and fixed in acetone at -20°C for 5 minutes. Staining for PPAR-γ was performed with a polyclonal rabbit anti-human PPAR-γ peptide antibody (a generous gift from Dr Mitchell Lazar, University of Pennsylvania, Philadelphia) ECs were identified by staining with anti-CD31 antibody (Dako, Carpinteria, Calif). Sections were preincubated with PBS containing 0.3% hydrogen peroxidase activity and stained for 1 hour with primary antibody diluted in PBS supplemented with 5% appropriate serum. Negative control was performed by preabsorbing the anti-PPAR-γ antibodies with the peptide from which the antibody had been derived, subsequently using these “preabsorbed PPAR-γ antibodies” at similar concentrations as in experimental conditions. Finally, sections were incubated with the respective biotinylated secondary antibody (Vector Laboratories, Burlingame, Calif) followed by avidin-biotin-peroxidase complex (Vectastain ABC kit). Antibody binding was visualized with 3-amino-9-ethylcarbazole (Vector Laboratories) or with true blue peroxidase substrate (Kirkegaard & Perry Laboratories). Sections were counterstained with Gill’s hematoxylin or contrast red (Kirkegaard & Perry Laboratories).

Transient Transfection Assay

Bovine ECs were transiently transfected with PPRE-TK-luciferase (LUC)7 (generously provided by Dr Bruce Spiegelman, DFCI) and pCMV–β-galactosidase (β-gal), by using lipofectamine, according to the manufacturer’s protocol (Gibco-BRL). After incubation for 5 hours liposomes were removed, and after 12 hours of culture in DMEM with 10% FCS, cells were stimulated in DMEM containing 0.1% FCS with 15d-PGJ2 at the indicated concentrations. Cells were harvested after 24 hours, and luciferase and β-gal activity was measured using the dual-light assay (Tropix).

With similar techniques, human skin fibroblasts were transfected with the PPAR-γ expression construct (pCMX-PPAR-γ; generously provided by Dr Bruce Spiegelman, DFCI) at different concentrations (100 ng/5x10^5 cells or 250 ng/5x10^5 cells). To verify similar transfection

Figure 3. PPAR-γ activator 15d-PGJ₂ activates PPAR-γ in bovine aortic ECs. A reporter construct containing 3 copies of a consensus PPRE placed upstream from the TK-luciferase reporter (PPRE₃-TK-LUC) was transiently transfected into bovine EC along with the internal control pCMV–β-gal. Cells were treated for 24 hours with vehicle alone (control, Co) or with 15d-PGJ₂ at the doses indicated. Luciferase activity normalized to β-gal activity is expressed as fold activation relative to control. Error bars reveal mean of 3 experiments performed in triplicate; error bars indicate SEM. *P<0.05 compared with control.

Figure 4. PPAR-γ activators increase PAI-1 mRNA and protein expression in human ECs. A, PAI-1 mRNA expression in human ECs was increased in response to PPAR-γ activators 15d-PGJ₂ (10 µmol/L), 9(S)-HOE (20 µg/L), or 13(S)-HOE (20 µg/L) compared with cells treated with vehicle alone (Co), as shown by Northern blot analysis (left). Ethidium bromide staining (right) demonstrated equal loading of intact RNA. B, Western blot analysis of supernatants from human ECs treated with these same PPAR-γ activators at similar doses showed increased PAI-1 secretion in response to these agents compared with control. C, 15d-PGJ₂ increased PAI-1 protein in EC supernatants in a concentration-dependent manner (maximal 6.0±1.7-fold increase at 10 µmol/L 15d-PGJ₂). All experiments were performed 3 independent times with similar results. D, None of 6 different PPAR-α activators [DHA 25 µmol/L, EPA 25 µmol/L, ETYA 25 µmol/L, WY14643 100 µmol/L, clolfibrate (clo) 100 µmol/L, or fenofibrate (fen) 100 µmol/L] increased PAI-1 protein in supernatants of human ECs compared with control (Co). Treatment with 15d-PGJ₂ (10 µmol/L) served as a positive control.
efficiency under all conditions tested, we cotransfected cells with pCMV–β-gal (500 ng/5 x 10^6 cells). Cells were stimulated for 24 hours in serum-free medium with or without 5 μmol/L 15d-PGJ_2, harvested, and processed as described above.

**Statistical Analysis**

Results of the experimental studies are reported as mean±SEM. Differences were analyzed by Student’s paired t test. A value of P<0.05 in the 2-tailed test was regarded as significant.

**Results**

**Human ECs Express PPARγ mRNA and Protein**

Cultured human ECs express PPARγ mRNA as determined by RT-PCR (Figure 1A). Western blot analysis revealed PPARγ protein expression in the nuclear fraction but not in the cytosolic preparation (Figure 1B, top). The identity of the detected band was confirmed by its size and comigration with a signal from the nuclei of PPARγ-transfected human fibroblasts. Nuclei from untransfected, unstimulated cells (control). Data are shown as mean of 4 independent experiments; error bars indicate SEM. *P=0.01 compared with untransfected, stimulated cells.

**Comparison of PPARγ expression in ECs with other known PPARγ-expressing cells (preadipocytes, adipocytes, and monocyte-derived macrophages) by both RT-PCR and Western blotting suggests that PPARγ is present at levels slightly less than in preadipocytes and monocyte-derived macrophages and substantially less than in differentiated adipocytes (Figure 1A, top, and 1B, bottom). Northern blotting revealed barely detectable PPARγ in ECs (data not shown).

**ECs in Human Carotid Arteries Express PPARγ**

Immunohistochemistry of human carotid arteries revealed PPARγ staining in the nuclei of ECs (Figure 2B). ECs were identified by immunoreactive CD31 (PECAM-1) in parallel sections (Figure 2A) No immunostaining was observed when parallel sections were stained with anti-PPARγ antibodies preabsorbed with peptide (Figure 2C), indicating the specificity of the detected signals.

**Treatment of PPRE-Luciferase–Transfected Bovine ECs With the PPARγ Activator 15d-PGJ_2 Increases Luciferase Activity**

To assess the presence of functional endogenous PPARγ in ECs, we transiently transfected bovine ECs with a PPRE-luciferase construct and stimulated these cells with increasing amounts of the PPARγ activator 15d-PGJ_2. Luciferase activity was assayed and normalized to the β-gal activity of a cotransfected pCMV–β-gal construct. Stimulation with 15d-PGJ_2 increased normalized luciferase activity in a concentration-dependent manner, with a maximal 5.9±1.2-fold increase (P<0.05, n=3) at 10 μmol/L 15d-PGJ_2 (Figure 3). These results suggest the presence of inducible PPARγ activity in these cells.

**PPARγ, but Not PPARα, Activators Increase PAI-1 mRNA and Protein Expression in Human ECs**

To investigate the effect of PPARγ activation on PAI-1 mRNA expression in human ECs, they were stimulated with the PPARγ activators 15d-PGJ_2 (10 μmol/L), 9(S)-HODE (20g/L), or 13(S)-HODE (20g/L) for 18 hours, and Northern blot analysis was then performed. Unstimulated cells showed low PAI-1 mRNA expression, whereas stimulation with all tested PPARγ activators increased PAI-1 mRNA levels, with a maximum response seen with 15d-PGJ_2 (Figure 4A).

Western blotting of EC supernatants collected after 24 hours of treatment with the same PPARγ activators as above revealed an increase in PAI-1 protein (Figure 4B) in a pattern consistent with the Northern blot data. Using the most potent PPARγ activator, 15d-PGJ_2, we found a concentration-dependent increase in PAI-1 secretion from human ECs with a maximal 6.0±1.7-fold rise at 10 μmol/L 15d-PGJ_2 compared with unstimulated cells (P=0.03, n=3; Figure 4C). In contrast, none of 6 different PPARα activators increased PAI-1 protein levels in human ECs (Figure 4D).

**Overexpression of PPARγ in Human Fibroblasts Increases PAI-1 Expression in Response to 15d-PGJ_2 in a Concentration-Dependent Manner**

To investigate whether PPARγ can indeed increase PAI-1 levels, we turned to an artificial approach that would permit a demonstration of PPARγ’s influence on PAI-1 expression. To do so, we overexpressed PPARγ in human fibroblasts and measured PAI-1 protein levels in supernatants of cells incubated with or without 15d-PGJ_2. This strategy allowed studies to be done in readily transfectable cells that endogenously express PAI-1 but that have only low levels of PPARγ. Untransfected fibroblasts, expressing PPARγ at negligible levels (Figure 1B), secrete PAI-1 under basal conditions at very low levels. Introduction of increasing amounts of the PPARγ expression construct enhanced PAI-1 secretion from these cells in proportion to the amount of transfected DNA. Consistent with our finding in ECs, treatment with 15d-PGJ_2 further augmented PAI-1 in the supernatants of these transfected fibroblasts compared with unstimulated cells (Figure 5A, top). Densitometry of 15d-PGJ_2
(5 μmol/L)–stimulated cells indicated a 2.6±0.6-fold increase in secreted PAI-1 in the supernatants of cells transfected with 250 ng pCMX-PPARγ DNA compared with untransfected, stimulated cells (Figure 5B; P=0.01, n=4). Analysis of cotransfected β-gal activity indicated comparable transfection efficiency among the groups (Figure 5A, bottom).

Discussion

We hypothesized that PPARγ might be expressed and active in regulating gene transcription in human ECs. If so, associations between triglyceride levels, obesity, and coagulation suggested that PAI-1 might be a PPARγ target gene. Our findings support both of these hypotheses.

Human ECs express PPARγ mRNA, as demonstrated by RT-PCR. Western blots showing a band co-migrating with PPARγ-transfected fibroblasts established that human ECs express PPARγ protein. ECs contain slightly less but comparable amounts of PPARγ protein relative to preadipocytes and monocyte-derived macrophages. The lack of a strong PPARγ signal in Northern blot analysis agrees with other PPAR reports.27,32 The functional relevance of a given protein does not depend solely on its mRNA levels but also on its in vivo translation and protein half-life.33,34 Importantly, ECs in vivo clearly contain PPARγ protein, as shown by immunohistochemistry of human carotid arteries. Furthermore, the ability to activate a canonical PPRE transfected into bovine ECs with 15d-PGJ2 strongly supports the presence of a functional PPARγ. We found that PPARγ activity in ECs, with either 15d-PGJ2 or the HODEs, increased PAI-1 expression.

15d-PGJ2, a metabolite of PGD2, potently stimulates PPARγ, exhibits much less activity toward PPARα or PPARδ,19,21,35 and lacks a known role in other transcriptional signaling pathways. PPARα activation is an unlikely mechanism for PAI-1 induction in ECs, given the lack of an effect of multiple PPARα activators on PAI-1 levels. Furthermore, the stronger induction of PAI-1 by 15d-PGJ2 reported here, compared with that elicited by the HODEs, agrees with previous studies suggesting that 15d-PGJ2 is a more potent PPARγ ligand.35 Finally, the proportionate induction of endogenous PAI-1 expression through increasing heterologous expression of PPARγ in fibroblasts bolsters the hypothesis that PAI-1 expression can be influenced by PPARγ.

The PPAR family thus far is known to consist of 3 members, α, δ, and γ. Like all PPARs, PPARγ on activation forms heterodimeric complexes with the retinoic X receptor and associates with a PPRE site in the promoter of target genes.25,27,36 PPARγ, strongly implicated in adipogenesis, is induced early in adipocyte differentiation, after which it remains expressed at high levels.37,38 Little is known about PPARγ in nonadipocytes. Recent work suggests that PPARγ inhibits macrophage activation, thereby reducing cytokine production and macrophage gene expression.28,29 Similarly, we have localized PPARγ in macrophages in human atheromas and demonstrated a functional role for PPARγ in inhibiting matrix metalloproteinase-9 gelatinolytic activity elaborated by human monocyte–derived macrophages.30 In contrast, recent reports, in addition to suggesting that components of oxidized LDL act as PPARγ ligands, also found that activation of PPARγ induced macrophage differentiation toward foam cells by increasing scavenger receptor expression.22,23 The net effect of PPARγ stimulation in atherogenesis thus remains unresolved but could well be influenced by PPARγ in the endothelium.

PPARγ signaling in ECs is quite plausible. Adipose tissue is highly vascularized and, as such, rich in endothelium. ECs localize strategically at the interface between circulating lipid components and tissues. Some of these lipid components, eg, 9- and 13-HODE, long known to activate ECs, have recently been found to act as PPARγ ligands.21 Hence, ECs may well encounter at least 3 naturally occurring PPARγ ligands: 15d-PGJ2, 9(S)-HODE, and 13(S)-HODE.

PPARγ regulation of PAI-1 expression presents intriguing possibilities for insight into the known links between obesity and deep venous thrombosis, insulin resistance, non–insulin-dependent diabetes mellitus, myocardial infarction, and accelerated atherosclerosis.2 PPAR γ has been implicated in both mouse models and human forms of obesity.24 Abundant laboratory and epidemiological evidence suggests the dysregulation of various metabolic and circulatory factors in obesity.2 PAI-1 is one such example. PAI-1 levels correlate with serum triglycerides, increase with obesity, and fall with weight reduction,2 findings that may reflect high adipocyte PAI-1 message levels.40 In fact, adipose tissue, in addition to ECs and hepatocytes, may be an important source of PAI-1.13 Elevated PAI-1 levels may explain in part findings such as those from the Nurses’ Health Study, demonstrating obesity as an independent risk factor for pulmonary embolism.41

Recent work reported a VLDL response element in the promotor region of the PAI-1 gene, located at residues −672 to −657.42 Of note, this site has some characteristics of a PPAR binding site, although no data supporting an interaction with any PPAR were reported. It remains unclear where in the PAI-1 promoter PPARγ is acting. PPARγ-dependent regulation of PAI-1 in ECs suggests the need for promoter studies in this cell type. It will also be of interest to investigate PPARγ regulation of PAI-1 in other cells such as adipocytes.

The present data implicate PPARγ as a novel regulator of gene expression in vascular cells, suggesting that PPARγ positively controls gene expression of PAI-1 in ECs, thus potentially promoting thrombosis. Of note, the PPAR ligands used here were naturally-occurring activators. It remains unclear if the synthetic thiazolidinediones such as troglitazone would have similar effects. In fact, clinical studies suggest that troglitazone decreases serum PAI-1 levels in some groups of patients with insulin resistance.43 This PPARγ effect, like the induction of foam cells, might promote atherogenesis; in contrast, inhibition of macrophage activation and matrix metalloproteinase-9 activity through PPARγ might limit it. PPARγ, as a highly regulated central transcriptional pathway present in various cell types, might well have varying effects on a complex pathological process like atherosclerosis. The data presented here suggest that PPARγ, as a novel mediator in EC signaling, must be considered in attempting to understand atherogenic mechanisms.

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