Peroxisome proliferator-activated receptors (PPARs), including PPARα (NR1C1), PPARβ/δ (NR1C2) and PPARγ (NR1C3), belong to the nuclear receptor superfamily that functions as transcription factors to regulate cellular differentiation, development and metabolism (carbohydrate, lipid and protein). PPARα is predominantly expressed in liver, heart, kidney and skeletal muscle. PPARγ is mainly associated with adipose tissue, playing a role in various physiological and pathophysiological events, including adipocyte differentiation and insulin sensitivity. PPARβ/δ is abundantly and ubiquitously expressed at much higher levels than PPARγ and PPARα. Upon binding of its ligands, PPARs heterodimerize with the retinoid receptor (RXR) and bind to peroxisome proliferator hormone responsive elements in the promoter region of the target genes to activate/repress transcription. Enogenous ligands for the PPARs include free fatty acids and eicosanoids, and synthetic ligands for PPARγ and PPARδ have been developed, targeting diabetes and dyslipidemia.

The biological functions of PPARγ and PPARδ have been extensively studied in regard to metabolism. PPARγ activation enhances the lipid storage capacity of the adipose mass, and also increases the number of small, insulin-sensitive adipocytes, leading to improved insulin sensitivity. PPARγ is also implicated in the regulation of lipid metabolism, as well as the maturation of monocytes/macrophages and the control of inflammatory reactions. In both humans and mice, loss-of-function mutation of PPARγ is associated with insulin resistance and diabetes. In addition, targeted deletion of PPARδ in skeletal muscle, macrophages, and adipose tissue suggests that PPARδ in these tissues regulates whole-body glucose homeostasis.

The function of PPARδ was first examined in globally PPARδ-deficient mice and later studied using the recently developed synthetic PPARδ ligands, a high-affinity PPARδ ligand, GW501516, can reduce weight gain and reduce triglycerides, and increase high-density lipoprotein (HDL) - cholesterol in obese mice by increasing peripheral fatty acid catabolism. PPARδ also regulates glucose homeostasis. GW501516 also lowers the plasma insulin level and improves glucose tolerance and insulin sensitivity in diet-induced obese mice. Collectively, these findings suggest that PPARδ could be a potential therapeutic target for combating obesity and insulin resistance.

In addition to a regulatory function in glucose and lipid metabolism, PPARs are expressed in the cardiovascular system and their functions there have been shown to be most significant physiological effects on glucose and lipid metabolism. Experimental studies in animal models of metabolic disease have demonstrated their effects on improving lipid profile, insulin sensitivity, and reducing inflammatory responses. PPARγ and -δ are also expressed in the vasculature and their beneficial effects have been examined in various cardiovascular disease models such as atherosclerosis, hypertension, diabetic vascular complications, etc. using pharmacological ligands or genetic tools including viral vectors and transgenic mice. These studies suggest that PPARγ and -δ are antiinflammatory, antiatherogenic, antioxidant, and antifibrotic against vascular diseases. Several signaling pathways, effector molecules, as well as coactivators/repressors have been identified as responsible for the protective effects of PPARγ and -δ in the vasculature. We discuss the pleiotropic effect of PPARγ and -δ in vascular dysfunction, including atherosclerosis, hypertension, vascular remodeling, vascular injury, and diabetic vasculopathy, in various animal models, and the major underlying mechanisms. We also compare the phenotypes of several endothelial cell/vascular smooth muscle-specific PPARγ and -δ knockout and overexpressing transgenic mice in various disease models, and the implications underlying the functional importance of vascular PPARγ and -δ in regulating whole-body homeostasis.

Key Words: Atherosclerosis; Endothelial function; Peroxisome proliferator-activated receptors; Vasculature
likely independent of their metabolic actions. PPARγ and PPARδ are expressed in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). Here, we will focus on the role of PPARγ and PPARδ in vascular biology beyond their regulatory effects on glucose and lipid metabolism, and discuss their potential clinical implications.

**Role of PPARγ in ECs and VSMCs in Vitro**

**PPARγ Activation in ECs**

**Inflammation** PPARγ reduces the activation and inflammation of ECs by suppressing the expression of several proinflammatory cytokines and chemokines through inhibition of the nuclear factor kappa B (NF-κB), AP-1, protein kinase C (PKC) signaling pathways, and oxidative stress responses.19-21 Either PPARγ agonist thiazolidinediones (TZDs) or overexpression of the constitutively active PPARγ can inhibit the expression of proinflammatory adhesion molecules and chemokines, including intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin and monocyte chemotactic protein (MCP-1) in human ECs.24γ PPARγ agonist treatment increases NO release in human umbilical vein ECs by enhancing the interaction of Hsp90 and endothelial NO synthase (eNOS), resulting in increased eNOS phosphorylation and activation, and the subsequent production of NO.27 In addition, PPARγ activation also enhances NO bioavailability by reducing endothelial oxidative stress.24 Conversely, NO can activate PPARγ in ECs through a p38-MAPK dependent mechanism, suggesting a positive feedback loop between PPARγ and NO.28

Apart from enhancing vasodilation directly, PPARγ also modulates vascular tone through antagonizing the production or activation of vasoconstrictors, such as angiotensin II and endothelin-1, while promoting vasodilation, mainly by increasing the production of vasodilating nitric oxide (NO).26 PPARγ agonist treatment increases NO release in human umbilical vein ECs by enhancing the interaction of Hsp90 and endothelial NO synthase (eNOS), resulting in increased eNOS phosphorylation and activation, and the subsequent production of NO.27 In addition, PPARγ activation also enhances NO bioavailability by reducing endothelial oxidative stress.24

**Vasoreactivity** Apart from antiinflammatory effects, PPARγ also antagonizes potent vasoconstrictor endothelin-1 (ET-1). PPARγ agonists also suppress the expression and secretion of ET-1 in ECs by inhibiting the AP-1 transcriptional pathway.29 Apart from inducing vasoconstriction through binding to the ET-A receptor on VSMCs, ET-1 can also induce vasodilation by binding to the ET-B receptor (ETBR) on ECs and activating eNOS, an effect more pronounced in resistance arteries. Impor-

**In Vivo Effect of PPARγ Activation in Vascular Diseases (Figure 1)**

**Phenotype of EC/VSMC-Specific PPARγ Knockout and Mutation Mice**

Disruption of endothelial PPARγ accelerates diet-induced atherogenesis in low-density lipoprotein receptor (LDLr/-) mice.49 EC-specific PPARγ knockout (KO) mice also have exacerbated lipopolysaccharide (LPS) -induced pulmonary inflammation and injury as shown by increased infiltration of inflammatory cells, edema, ROS and proinflammatory cytokine production.40 Interestingly, mice with Tie2-Cre mediated EC-specific PPARγ deletion show significant dyslipidemia and lack of response to the free fatty acid (FFA) and triglyceride (TG) lowering effects of the PPARγ agonist rosiglitazone, which indicates endothelial PPARγ integrates metabolic and vascular responses and may contribute to the in vivo effects of PPARγ agonists on lipid metabolism, possibly because metabolic organs, including muscle, fat and liver, are highly vascularized and microvascular ECs control fatty acid transport and uptake, TG metabolism, and LDL function as well.51 These lines of evidence concur with the in vitro data supporting antiinflammatory and
cytes, causing impaired intravascular thermoregulation, endothelial dysfunction, and exaggerated atherosclerosis.

Effect of PPARγ Activation in Diabetic Vascular Function

Evidence suggests that treatment with TZDs improves vascular function in diabetic mice and humans. A 12-week treatment with rosiglitazone reduced plasma levels of C-reactive protein (CRP) and increased the median coronary flow velocity reserve, which is an indicator of coronary endothelial function, in patients with diabetes and coronary artery disease.

Pioglitazone treatment improved eNOS activity and enhanced the vasodilatory response to acetylcholine in diabetic mice. Telmisartan, a partial PPARγ agonist, also protected against diabetic vasculopathy in a mouse model of obesity and type 2 diabetes, through normalizing of vascular PPARγ downregulation in the diabetic mice.

However, the in vivo effect of PPARγ activation by TZDs on endothelial function may be an indirect effect of improved glucose tolerance, insulin sensitivity, and increased vasoprotective adiponectin production in diabetic mice.

Rosiglitazone was withdrawn from the market because of increased risks of myocardial infarction or other cardiovascular events based on several meta-analyses from randomized trials and Medicare databases. Such effects may be related to some unknown genes that might be induced or repressed by either PPARγ or its coactivators/repressors in the transcriptional complex, which is yet to be discovered.

anti-atherogenic functions of endothelial PPARγ, and a potential contributory role in lipid metabolism. Together with the evidence from in vitro studies of VSMC, PPARγ also regulates vasomotor and inflammatory responses in the vasculature in vivo. Contraction of mouse aorta is reduced and vasodilatation is enhanced in response to β-adrenergic agonists in the aorta from VSMC-specific PPARγ KO mice, inducing a phenotype of biphasic response in blood pressure to norepinephrine. However, overexpressing DN-PPARγ also caused increased Rho kinase activity in another study. It is thus possible that PPARγ interacts with different proteins in conduit and resistance arteries in response to vasoactive stimuli to maintain vascular tone and blood pressure. In addition, PPARγ has a clear role against vascular remodeling. Inducible PPARγ KO in VSMCs exacerbated AngII-induced vascular remodeling. Endothelium-dependent relaxation to acetylcholine was reduced in VSMC-specific PPARγ KO mice and further impaired by AngII infusion, revealing the protective role of VSMC PPARγ in AngII-induced vascular injury. Loss of PPARγ in VSMC promotes transplantation-associated vascular lesion formation through increased VCAM-1 expression. Deletion of PPARγ in VSMCs also accelerates vascular calcification in LDLr−/− mice, mediated through the secreted frizzled-related protein-2, which functions as a Wnt5a antagonist. Interestingly, VSMC-specific PPARγ KO mice (SM22α-Cre) show an unexpected phenotype of loss of perivascular adipocytes, causing impaired intravascular thermoregulation, endothelial dysfunction, and exaggerated atherosclerosis.
Moreover, in experimental settings, the effects of PPARγ on the vascular system are only observed over a short period in rodent studies aiming at understanding the biology of PPARγ in vasculature, so we cannot exclude the possibility of any unknown causes that are responsible for the adverse effect of PPARγ on the cardiovascular outcomes in type 2 diabetic patients.

**Role of PPARγ in Atherosclerosis**

The antiatherogenic effects of PPARγ agonists have been demonstrated in LDLR−/− and ApoE−/− mouse models, and humans.  

Li et al demonstrated that rosiglitazone and GW7845 reduced the development of atherosclerosis in male LDLR−/− mice. The protective effect of troglitazone was observed in ApoE−/− mice, and also in high fructose- or high fat-fed mice. Adenoviral overexpression of PPARγ1 can induce caveolin-1 expression and enhance macrophage cholesterol efflux, thus attenuate atherosclerosis in ApoE−/− mice. Likewise, inhibition of PPARγ exaggerates atherosclerosis. Bone marrow transplantation from PPARγ null mice to LDLR−/− mice resulted in an increase in atheroma formation. Bone marrow reconstitution with macrophage-specific KO of PPARγ also exacerbated atherogenesis, by increasing macrophage recruitment. In addition to its ability to reduce plaque formation, PPARγ also promotes plaque stability. Rosiglitazone can modify plaque composition and decrease the number of buried fibrous caps to stabilize vulnerable plaques in ApoE−/− mice. Pioglitazone reduces MMP expression and macrophage activity in carotid plaques from ApoE−/− mice, confirming the plaque-stabilizing effect of PPARγ activation. Such effects are also verified using adenosine-mediated overexpression of PPARγ in ApoE−/− mice, including reduced lipid deposition, and reduced macrophage number, increased collagen and SMCs in the plaques, accompanied by decreased MMP-9, tissue factor, MCP-1. Therefore, PPARγ is atheroprotective during different stages of plaque formation by enhancing cholesterol efflux, reducing macrophage number, reducing extracellular matrix reaction, and stabilizing vulnerable plaques.

**Effect of PPARγ Against Hypertension**

Genetic analysis shows that 2 dominant negative mutations in PPARγ are associated with severe hypertension in humans, indicating an important role of PPARγ in blood pressure regulation. PPARγ agonists lower blood pressure in diabetic mice. We showed that rosiglitazone attenuated ET-1-induced vasoconstriction and enhanced the ET-1-mediated vasodilating effect through upregulation of ETBR expression in mouse aortas and mesenteric arteries, which may contribute to the regulation of vascular tone in hypertension by PPARγ agonists. We also showed that PPARγ ameliorates hyperreactivity of the pulmonary artery and thus inhibits vascular remodeling in rat models of pulmonary arterial hypertension. PPARγ activation inhibits 5-HT 2B receptor expression by inhibiting 5-HT-induced AP-1/c-jun binding to AP-1 responsive elements in the 5-HT2BR promoter, thus suppressing the effect of 5-HT on the pulmonary vasculature. Pioglitazone also attenuates vascular fibrosis and improves vascular function in spontaneously hypertensive rats through increasing COX-2-derived prostaglandin I2 (PGI2) production, reducing oxidative stress, leading to increased NO bioavailability and thus to improved vasodilation in resistance arteries.

**Role of PPARδ in ECs and VSMCs in Vitro**

**PPARδ in ECs**

PPARδ is abundantly and ubiquitously expressed in all types of cells, including ECs and VSMCs. In ECs, PPARδ activation inhibits inflammation, oxidative stress, and apoptosis; while stimulating angiogenesis. In human ECs, the PPARδ ligands, L-165041, GW0742 and GW501516, attenuate inflammatory responses by reducing the translocation of NF-κB and the subsequent expression of VCAM-1, ICAM-1, E-selectin, MCP-1 and growth-regulated oncogene alpha (GROα). Apart from its ability to suppress proinflammatory adhesion molecules and chemokines, GW0742 also protects ECs against oxidative stress by upregulation of antioxidant enzymes, including superoxide dismutase-1, catalase and thioredoxin reductase. Importantly, an endogenous ligand for PPARδ is prostacyclin (PGI2), which can be released by the endothelium. Besides its vasodilatory and antiaggregating functions, prostacyclin also enhances the proangiogenic function of human and mouse endothelial progenitor cells through PPARδ. Prostacyclin and L-165041 both can prevent H2O2-induced apoptosis in human ECs by inducing the binding of PPARδ to the 14–3–3α promoter, which augments proapoptotic Bcl-2 protein Bad sequestration and reduces Bad translocation to mitochondria to prevent initiation of the death signal.

**PPARδ in VSMCs**

Similar to PPARγ, PPARδ also exerts an antiproliferative effect on VSMCs. PPARδ activation blocks cell cycle progression and reduces VSMC proliferation. L-165041 inhibits PDGF-induced VSMC proliferation and neointimal formation after carotid injury in rats by inhibiting cyclin D1 and cyclin-dependent kinase 4 (CDK4) expression and decreasing the phosphorylation of the retinoblastoma tumor suppressor protein, while GW501516 reduces IL-1β-induced proliferation and migration of rat aortic VSMCs through increase expression of p53 and p21. PPARδ activation also induces cell cycle inhibitors p21 and p27 in rat aortic VSMCs. In addition, GW501516 exerts antiinflammatory and antiapoptotic effects in murine aortic VSMCs, through its direct target gene TGFβ1.

**In Vivo Effect of PPARδ Activation in Vascular Diseases (Figure 2)**

**Effect of PPARδ in Metabolic Disease**

Existing evidence indicates that PPARδ activation exerts beneficial effects on lipid and glucose metabolism in mouse models of obesity and diabetes. GW501516 or overexpression of PPARδ reduces weight gain and circulating TG level, while increasing HDL in high fat diet-induced obese mice, db/db, and ob/ob mice through stimulating peripheral fatty acid catabolism. In obese mice, administration of GW501516 lowers the plasma insulin level and improves insulin sensitivity by enhancing glucose consumption and fatty acid synthesis in the liver. Both GW0742 treatment and hepatic overexpression of PPARδ rescue hepatic steatosis by inhibiting lipogenesis in obese diabetic mice.

**PPARδ Against Endothelial Dysfunction**

GW0742 and L-165041 at higher concentrations (>1 μmol/L) are reported to directly induce endothelium-dependent relaxation, eNOS phosphorylation and NO production in rat aorta, through activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Administration of GW0742 in vivo restores endothelial function in streptozotocin-induced type 1 diabetic rats by increasing NO bioavailability, as well as decreasing NADPH oxidase-driven superoxide production. Moreover, our recent study demonstrated that another PPARδ agonist, GW501516, restores endothelial dysfunction in the aorta and...
mesenteric resistance arteries of diabetic db/db mice and diet-induced obese mice through enhancing PI3K/Akt signaling with subsequent increases in eNOS activity and NO production.98

**PPARδ Against Hypertension**

The effect of PPARδ ligands in hypertension has also been examined. In hypertensive rats, administration of GW0742 decreases systolic blood pressure, mesenteric vascular hypertrophy, and endothelial dysfunction, accompanied by an increase in eNOS activity and decreased NADPH oxidase activity and expression of proinflammatory and proatherogenic genes, which is similar to the effect of PPARγ.99 Additionally, GW501516 and L-165041 enhance the recovery of blood flow in mice after hind limb ischemia and stimulate the proliferation of endothelial progenitor cells by acting on the PI3K/Akt pathway.100 In general, PPARδ activation is beneficial for vascular function.

**PPARδ Against Atherosclerosis**

Various lines of evidence show that PPARδ may retard the development and progression of atherosclerosis through modulating lipid metabolism in different tissues and exerting an antiinflammatory effect through macrophages. PPARδ agonists normalize the levels of HDL, LDL and TG in obese and diabetic animal models as well as in obese men.14,16,94,101 Importantly, PPARδ acts as sensor for very low-density lipoprotein (VLDL), regulating TG homeostasis locally in peripheral tissues such as macrophages and vessel walls.102 The activation of PPARδ ameliorates the dyslipidemia that is recognized as the major contributor to atherosclerosis susceptibility. In line with the evidence from in vitro studies of ECs and VSMCs, PPARδ agonists were found to reduce atherosclerotic lesion area through antiinflammatory mechanisms in both ApoE–/– and LDLr–/– mice.103–105 PPARδ activation suppresses the expression of ICAM-1 and MCP-1 in the mouse aorta and TNFα expression in macrophages, thus reducing atherosclerosis in LDLr–/– mice.105 Notably, selective deletion of PPARδ in macrophages attenuates atherosclerosis in LDLr–/– mice.106 This unexpected observation can be explained by the physical interaction between PPARδ and Bcl-6. Bcl-6 is not sequestered in the absence of PPARδ and thus suppresses the transcription of proatherogenic genes such as MCP-1.

Recently, the effects of several PPARδ or dual PPARα/δ agonists have been examined in small-scale clinical trials. PPARδ agonist, MBX-8025, improved the lipid profile and reduced the CRP level in overweight dyslipidemia subjects.107,108 Similar effects were also observed with GW501516 in subjects with metabolic syndrome.101,109,110 Dual PPARα/δ agonist, GFT505, improves both lipid and glucose metabolism in patients with central obesity, dyslipidemia, and impaired glucose metabolism.101,111 Although no adverse effect has been found in these clinical trials, GW501516 may have increased the incidence of adenoma in a mouse model of colorectal cancer.112,113 The safety of PPARδ needs to be further evaluated before it can be used to treat metabolic disease.
Conclusions

The antiinflammatory and antiatherogenic effects of PPARα and PPARδ have been clearly demonstrated in vivo and in vitro. Mechanistic studies have also revealed important intermediate transcription factors and target genes that are responsible for the vasoprotective effects. However, there are still missing steps to be identified, such as coactivators and repressors of PPARα and PPARδ, and epigenetic modifiers, as well as intermediate metabolites that may activate certain enzymes or induce post-translational modification. Exploring the interaction of these novel targets with PPARα and PPARδ, especially in vivo, in the context of diabetes and atherosclerosis may provide more insights. In addition, more detailed analysis of the metabolism of the whole organism under the conditions of EC-specific loss-of-function and gain-of-function will be important in revealing the potential role of endothelial PPARα and PPARδ in metabolic disease. These data may provide us with more clues on how to safely regulate PPARα and PPARδ activity in vivo to treat cardiovascular diseases.

References


