Obesity, Peroxisome Proliferator-Activated Receptor, and Atherosclerosis in Type 2 Diabetes

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Abstract—Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. The 3 PPAR isotypes, PPAR-α, PPAR-γ, and PPAR-δ, play a key role in the regulation of lipid and glucose metabolism. Obesity and the interrelated disorders of the metabolic syndrome have become a major worldwide health problem. In this review, we summarize the critical role of PPARs in regulating inflammation, lipoprotein metabolism, and glucose homeostasis and their potential implications for the treatment of obesity, diabetes, and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2006;26:28-40.)

Key Words: PPARs ■ atherosclerosis ■ obesity ■ diabetes

Type 2 diabetes is a complex metabolic disorder that affects between 6% and 20% of the population in Western industrialized societies. Around the globe, the prevalence of type 2 diabetes is expected to increase exponentially, especially among the young.1 Type 2 diabetes is characterized by hyperglycemia, insulin resistance, and progressive loss of β-cell function throughout the course of the disease and is associated with dyslipidemia, hypertension, and obesity (components of the metabolic syndrome).2 Both type 1 and type 2 diabetes are considered a coronary artery disease (CAD) risk equivalent.3 However, CAD often precedes the onset of diabetes, because 50% of patients with new onset type 2 diabetes already have a CAD diagnosis.

Components of the metabolic syndrome—insulin resistance, hypertension, low high-density lipoprotein (HDL), and hypertriglyceridemia—are themselves CAD risk factors, whereas hyperglycemia additionally contributes to vascular damage. Whether hyperinsulinemia and insulin resistance directly contribute to vascular damage is controversial and under active investigation.4 Although type 1 and 2 diabetes are associated with increased atherosclerosis, the pathogenesis of CAD in diabetes is multifactorial. Changes in metabolic factors, increased oxidative stress and glycoxidation, endothelial dysfunction, inflammation, and the prothrombotic state, observed in diabetics play a role in cardiovascular complications of diabetes.5

Initially, type 2 diabetes was referred to as disorder of carbohydrate metabolism. For the past decade, it was considered a disorder of fatty acid metabolism, because free fatty acids (FFAs) circulate in high levels in obesity and promote...
insulin resistance and hepatic glucose production. Recently, increasing evidence indicates that abnormalities in adipokine secretion from fat and in mitochondrial metabolism play a central role in the pathogenesis of this disease. Insulin resistance, defined as a defect in the ability of insulin to drive glucose into its major target tissue, skeletal muscle, is a key factor in the pathogenesis of type 2 diabetes and a cofactor in the development of dyslipidemia, hypertension, and atherosclerosis. Insulin resistance is present in >90% of people with type 2 diabetes and predate the development of hyperglycemia by many years. In the early states, insulin resistance is compensated by an increase in pancreatic insulin secretion.

The prevalence of obesity, defined as a body mass index (BMI) of ≥30 kg/m², has increased dramatically. Currently, 30.5% of the adult population in the United States is considered obese, whereas >60% fall into the overweight category (BMI ≥25 kg/m²). The epidemiologic relationship between obesity and insulin resistance is well established. Obesity-related insulin resistance involves the release of mediators, such as FFAs, tumor necrosis factor α (TNF-α), or resistin from adipocytes and decreased production of adiponectin, all of which impair insulin action in skeletal muscle. As body fat increases, the rate of lipolysis is elevated, leading to increased FFAs mobilization and elevated levels of circulating FFAs. The mechanism of FFA-mediated insulin resistance is currently not completely understood. More than 40 years ago, Randle et al proposed that FFAs compete with glucose as an energy substrate in the isolated rat heart and diaphragm muscle. Randle et al postulated that increased FFA oxidation results in an elevation of the intramitochondrial acetyl coenzyme A (CoA):CoA and reduced nicotinamide adenine dinucleotide:oxidized nicotinamide adenine dinucleotide ratios, with subsequent inhibition of pyruvate dehydrogenase. As a consequence, intracellular citrate levels increase, leading to the inhibition of phosphofructokinase, a key rate-controlling enzyme in glycolysis. Subsequent accumulation of glucose-6-phosphate inhibits hexokinase II activity, which leads to an accumulation of intracellular glucose and decreased glucose uptake. In contrast to the mechanism of FFA-induced insulin resistance as proposed by Randle et al, a study by Roden et al indicates that increased plasma FFA levels initially cause insulin resistance by inhibition of glucose transport and/or phosphorylation followed by a reduction in both the rate of glucose oxidation and muscle glycogen synthesis. However, because elevated plasma FFA levels were found to be associated with a decrease in intracellular glucose concentration, glucose transport activity appeared to be the rate-controlling step for FFA-induced insulin resistance. Rather than increasing intracellular glucose-6-phosphate concentrations in healthy human subjects, elevated FFA concentrations decreased intramuscular glucose-6-phosphate levels. Similar results were found during hyperglycemic-hyperinsulinemic clamps of type 2 diabetics and of normoglycemic insulin-resistant offspring of parents with type 2 diabetes. An emerging body of evidence suggests that FFA-mediated insulin resistance involves alterations in protein kinase C (PKC) signaling. After an acute increase in FFAs in healthy human subjects, an intracellular accumulation of diacylglycerol and, subsequently, activation of the PKC isoforms β and δ in skeletal muscle was observed. In addition, a decrease in IκB-α, an inhibitor of nuclear factor κB (NF-κB), was found, suggesting an involvement of the IκB kinase-β (IKK-β)/IκB-α/NF-κB pathway in the pathogenesis of FFA-induced insulin resistance in human muscle. The activation of PKC might cause insulin resistance by ultimately inducing serine/tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) sites, thereby inhibiting IRS-1 binding and activation of phosphoinositide 3-kinase. Other than members of the PKC family, inflammatory signaling intermediates, such as IKK-β, might mediate the serine phosphorylation of IRS-1.

In this model, inhibition of phosphatidylinositol 3-kinase activity leads to reduced insulin-stimulated glucose-transport by decreasing glucose transporter 4 translocation to the plasma membrane. In addition, a growing body of evidence indicates that chronic FFA elevation impairs the insulin secretory response to glucose and, thus, plays an important role in the pathogenesis of diabetes. Based on evidence in the male Zucker diabetic fatty rats, excessive lipid accumulation in islet cells may also have a “lipotoxic” effect in humans, leading to β-cell dysfunction and apoptosis.

Adipose tissue as an endocrine organ also releases proinflammatory mediators that promote vascular damage and atherosclerosis. TNF-α inhibits insulin signaling contributing to insulin resistance and activates multiple mechanisms of inflammation via NF-κB. Leptin can alter insulin action and has recently been recognized to be an important mediator of obesity-related hypertension. Angiotensinogen, the precursor of angiotensin II, a key mediator of vascular injury, can be produced and secreted by adipose tissue. Plasma activator inhibitor 1 (PAI-1) is typically increased in the obesity/insulin-resistance state and plays an important role in atherothrombosis. In contrast, excessive visceral adipose tissue has been shown to be associated with decreased adiponectin levels, an important hormone that exerts antiadipogenic and antiatherogenic functions. Adiponectin activates AMP-activated protein kinase, which promotes skeletal muscle glucose uptake and suppresses hepatic glucose production. Importantly, adiponectin also inhibits NF-κB activation, thus, attenuating inflammation. Visfatin, a growth factor with insulin mimetic action, was recently cloned from fat. Unlike adiponectin, plasma levels of visfatin increase in parallel with visceral fat in both mice and humans, so the role of visfatin in insulin resistance needs additional investigation. Taken together, these observations suggest that the adipocyte is an integral coordinator of the relationship among obesity, diabetes, and CAD.

Aging and insulin resistance are associated with progressive defects in mitochondrial oxidation, even in the absence of obesity. This mitochondrial alteration leads to increased intracellular fatty acid metabolites, fatty acetyl-CoA, and diacylglycerol, which can impair insulin signaling in skeletal muscle and other tissues resulting in insulin resistance. Recent studies suggest that insulin-resistant individuals have decreased expression of peroxisome proliferator-activated receptor (PPAR) coactivator 1α and 1β, contributing to decreased numbers of muscle mitochondria and a lower ratio.
of type 1 oxidative muscle fibers to type 2 fibers, which are more glycolytic and have less mitochondria.\textsuperscript{43,44} Such changes have been reported in both nondiabetic and diabetic whites and Mexican Americans.\textsuperscript{43,45} These studies suggest that decreased numbers and/or functions of mitochondria contribute to insulin resistance, possibly separate from the effects of obesity. The interaction of obesity and mitochondrial defects on insulin-mediated glucose uptake deserves additional investigation.

PPARs

PPARs are ligand-activated transcription factors and belong to the nuclear receptor superfamily. PPARs regulate transcription of target genes by forming heterodimers with the retinoid X receptor (RXR) and binding to specific PPAR response elements (PPREs) in the promoter region of target genes.\textsuperscript{46-47} In the absence of ligands, PPAR/RXR heterodimers can actively repress transcription through the recruitment of corepressor complexes that contain nuclear receptor corepressor and/or silencing mediator for retinoid and thyroid receptors.\textsuperscript{48,49} In the presence of ligands, PPAR/RXR heterodimers activate transcription through the recruitment of coactivator proteins. Moreover, PPARs can also repress gene expression by antagonizing the activities of other signal-dependent transcription factors, such as NF-κB and activator protein 1.\textsuperscript{50} Three isoforms, encoded by separate genes, have been identified: PPAR-γ, PPAR-α, and PPAR-β/δ (hereafter referred to as PPAR-δ), which share 60% to 80% homology in their ligand- and DNA-binding domains. Unsaturated long-chain fatty acids, as well as their eicosanoids derivatives are endogenous ligands for all 3 of the PPAR isotypes.\textsuperscript{51,52} Synthetic ligands for 2 forms of the receptor, PPAR-α and PPAR-γ, have been developed for clinical use; ligands for PPAR-δ are currently under clinical development. Each PPAR receptor subtype exhibits distinct patterns of expression and overlapping but distinct biological activities\textsuperscript{9} (Figure). Whereas PPAR-α and PPAR-γ are predominantly present in liver and adipose tissue, respectively, PPAR-δ is ubiquitously expressed.\textsuperscript{53,54}

**PPAR-γ**

By alternative promoter usage and splicing, three isoforms of PPAR-γ, PPAR-γ1, PPAR-γ2, and PPAR-γ3, have been identified. Proteins produced from PPAR-γ2 contain an additional NH\textsubscript{2}-terminal region, composed of 30 amino acids, whereas proteins derived from PPAR-γ1 and -γ3 mRNA are identical. PPAR-γ2 expression is primarily restricted to adipose tissue, whereas PPAR-γ1 is widely expressed.\textsuperscript{55} PPAR-γ plays a critical role in glucose homeostasis and is the molecular target of a class of insulin-sensitizing drugs referred to as thiazolidinediones (TZDs).\textsuperscript{56} Troglitazone has been the first synthetic PPAR-γ ligand but was withdrawn from use because of rare but serious hepatotoxicity.\textsuperscript{57} The presently clinically available PPAR-γ ligands, rosiglitazone and pioglitazone, are not associated with any apparent hepatotoxicity\textsuperscript{58} and are widely used for treatment of type 2 diabetes.\textsuperscript{56} TZDs reduce peripheral insulin resistance characteristic of patients with type 2 diabetes.\textsuperscript{59} This effect results in increased peripheral glucose use, reduced hepatic glucose output, and, consequently, improvement in overall glycemic control. Besides their effects on carbohydrate metabolism, PPAR-γ ligands also have beneficial effects on plasma lipids. Both pioglitazone and rosiglitazone increase serum levels of HDL,\textsuperscript{60,61} with pioglitazone also being associated with a marked reduction in plasma triglyceride levels.\textsuperscript{62} PPAR-γ ligands also inhibit the expression of a variety of proinflammatory genes in macrophages, including inducible nitric oxide synthase, matrix metalloproteinases, and several interleukins.\textsuperscript{63,64} These actions may also be relevant for obesity-related insulin resistance, because macrophage accumulation...

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![Diagram](http://atvb.ahajournals.org/content/30/1/30/F1.large.jpg)
in adipose tissue and gene expression has been shown to play an important role in the pathogenesis of obesity-induced insulin resistance.65

**Obesity, Lipid Metabolism, and Type 2 Diabetes**

PPAR-γ is expressed at high levels in adipose tissue and is a central regulator of adipocyte gene expression and differentiation.56,60 PPAR-γ is induced during adipocyte differentiation, and retroviral expression of PPAR-γ stimulates adipose differentiation of cultured fibroblasts.60 Several studies demonstrate that PPAR-γ is both necessary and sufficient to promote differentiation of fat cells both in vivo and in vitro.69,70 In contrast, PPAR-γ antagonists inhibit adipocyte differentiation.71 Consistent with these findings, humans with dominant-negative mutations in PPAR-γ manifest partial lipodystrophy and severe peripheral and hepatic insulin resistance because of increased triglyceride and fatty acid deposition into skeletal muscle and liver.72

The mechanisms underlying insulin-sensitizing effects of TZDs are complex and not completely understood. Activation of PPAR-γ in insulin-resistant animals or humans results in an increase in the sensitivity of both the liver to insulin-mediated suppression of hepatic glucose production and insulin-mediated skeletal muscle glucose uptake.73,74 These in vivo effects on insulin signaling are because of the combined actions of PPAR-γ ligands on the adipose tissue and on liver and skeletal muscles. Rosiglitazone was reported to fail to reduce glucose or insulin levels in mice, which lack white adipose tissue, suggesting that white adipose tissue is required for the antidiabetic effects of PPAR-γ ligands.75 PPAR-γ ligands profoundly alter gene expression in adipose tissue. Expression of resistin and TNF-α, which both induce insulin resistance, are reduced by PPAR-γ ligands, suggesting that the insulin-sensitizing effect of PPAR-γ agonists is related to its antiinflammatory properties.67,76 In addition, expression and secretion of adiponectin, a hormone exclusively produced by the adipocyte, is increased in the presence of PPAR-γ agonists both in vivo and in vitro.78 These data suggest that the adipose tissue is the primary target of PPAR-γ ligands, resulting in an improvement in insulin sensitivity in liver and muscle.79 However, in recent studies using different mouse models lacking adipose tissue, PPAR-γ ligands were found to improve insulin sensitivity, indicating a beneficial effect outside the adipose tissue.80,81 Consistent with these observations, selective deletion of PPAR-γ in the skeletal muscle and the liver in mice results in severe whole body insulin resistance.82,83 Hveayer et al82 postulated that selective deletion of PPAR-γ in skeletal muscle caused insulin resistance in muscle, followed by impaired insulin action in adipose tissue and liver. In contrast, Norris et al84 found that in mice with muscle-specific deletion of PPAR-γ, insulin sensitivity in skeletal muscle was normal but impaired in the liver. Much of the differences in the mouse studies may be dependent on strain differences.

An increasing body of evidence suggests that dysregulation of the AMP-activated protein kinase (AMPK) signaling pathway leads to alterations in cellular FFA metabolism, which, in turn, cause ectopic lipid accumulation, cellular dysfunction, and inflammation. Previous studies indicate that dysregulation of AMPK might be the common causal factor of components of the metabolic syndrome, including insulin resistance, hypertension, and endothelial and pancreatic β-cell dysfunction.85 AMPK activation was found to induce FFA oxidation, decrease FFA incorporation into glycerolipids, and increase insulin sensitivity.36–40 In addition, several studies have shown that TZDs activate AMPK activity both in vitro and in vivo, suggesting that AMPK might be a mediator of the insulin-sensitizing effects of TZDs.90–92 Increased plasma levels of adiponectin, which was found to increase AMPK activity in skeletal muscle, liver, and adipose tissue, might mediate the observed effect of TZDs on AMPK activity.36,93

A recent study performed by Winkler et al59 demonstrated a reduction in the number of small dense low-density lipoprotein (LDL) particles in pioglitazone-treated patients. Similar findings were reported in a study investigating the effect of rosiglitazone on the relative predominance of both small dense LDL particles and large buoyant LDL particles.93 This shift from small dense LDL particles toward a large buoyant phenotype may contribute to the prevention or delay of the atherogenic process.

As insulin resistance improves, components of the metabolic syndrome, including dyslipidemia and hypertension, also improve with TZD treatment.94–97 Taken together, PPAR-γ activation disconnects obesity from the metabolic syndrome. Ligands promote fat cell differentiation and fatty acid storage, primarily in subcutaneous tissue, but suppress inflammatory adipokine production while stimulating adiponectin production. These effects extend into PPAR-γ actions in the vasculature.

**Inflammation and Atherosclerosis**

PPAR-γ is expressed in vascular smooth muscle cells (VSMCs), endothelial cells, macrophages, and T-cells, where it plays an important role in the regulation of inflammatory responses.80,96–100 PPAR-γ-specific ligands inhibit the production of a host of inflammatory cytokines, such as TNF-α, interleukin (IL) 1β and IL-6 in monocytes,100 inducible nitric oxide synthase, matrix metalloproteinase 9, and scavenger receptor 1 in macrophages90 or endothelin-1 and IFN-inducible protein 10 in endothelial cells.101,102 Moreover, PPAR-γ agonists have been shown to decrease the expression of the adhesive, proinflammatory molecule osteopontin in macrophages.103 Previous studies suggest that the antiinflammatory properties of PPAR-γ are because of a generalized repression of NF-κB, CCAAT/enhancer binding protein, and activator protein 1-mediated gene transcription.103,104,105 Data regarding antiinflammatory effects of PPAR-γ in vivo are somewhat controversial. PPAR-γ ligands markedly reduced colonic inflammation in a mouse model of inflammatory bowel disease.106 In addition, in mice treated with the TZD rosiglitazone or GW7845, TNF-α and gelatinase B mRNA expression were significantly reduced in the aortic root.107 In contrast, PPAR-γ ligands did not suppress lipopolysaccharide-induced cytokine production in mice.107 Moreover, some antiinflammatory effects required high-ligand doses and did not appear mediated by receptor-dependent processes.108
In a variety of studies, PPAR-γ ligands have been shown to decrease atherosclerotic lesion formation in genetically prone mouse models. This effect occurs in insulin-sensitive or insulin-resistant models with or without diabetes. Ligands to the heterodimeric partners of PPAR-γ, RXR, also attenuate atherosclerosis. Intriguingly, less reduction in atherosclerosis in response to PPAR-γ ligand treatment was observed in female mice, indicating that additional factors, like hormonal status, may affect the outcome. PPAR-γ ligands also inhibited angiotensin II (Ang II)-accelerated atherosclerosis in LDLR−/− mice, whereas no effect on lipid profile, glucose, or blood pressure was observed. The attenuation of Ang II-accelerated atherosclerosis correlated with a downregulation of the proinflammatory transcription factor early growth response gene 1 and several of its target genes, indicating that inhibition of inflammation plays a crucial role for the antiatherosclerotic effect of PPAR-γ ligands. Ang II is known to be a major proatherogenic factor by inducing inflammation in the vessel wall and stimulating proliferation and migration of VSMCs and monocytes. Previous studies have shown that PPAR-γ ligands modulate Ang II signaling both at the receptor level and downstream of the Ang II type-1 receptor (AT1-R). PPAR-γ activators were found to downregulate AT1-R expression in VSMCs and block AT1-R-mediated extracellular signal regulated kinase 1/2 MAPK activation, which is crucial for VSMC proliferation and migration. However, a variety of in vitro studies regarding the effect of PPAR-γ agonists on cholesterol homeostasis in macrophages suggest both atherogenic and antiatherogenic influences. PPAR-γ has been shown to transcriptionally induce the expression of the macrophage scavenger receptor CD36, suggesting that PPAR-γ might promote foam cell formation and the development of atherosclerosis. However, Chowla et al demonstrated that, in addition to lipid uptake, PPAR-γ induces ATP binding cassette A-1 (ABCA1) expression and cholesterol efflux in macrophages through a transcriptional cascade mediated by liver X receptor α. Li et al demonstrated recently that PPAR-γ ligands inhibit the formation of macrophage foam cells in the peritoneal cavity of hypercholesterolemic LDLR−/− mice, possibly through transcriptional regulation of ABCG1, a transporter that mediates cholesterol efflux to HDL acceptors.

Large-scale clinical trials examining the effects of PPAR-γ agonists on cardiovascular end points are underway. In the meantime, small clinical studies in patients with type 2 diabetes demonstrated that troglitazone and pioglitazone have a potent inhibitory effect on the progression of common carotid arterial intima-media thickness. Recently, our group demonstrated that rosiglitazone treatment improved positron-emission tomography- assessed myocardial blood flow responses to the cold pressor test, which is largely endothelial dependent. In addition, various studies showed that treatment of patients with type 2 diabetes with TZDs reduced inflammatory surrogate parameters of atherosclerosis, such as C-reactive protein, TNF-α, serum amyloid A, and PAI-1 while increasing adiponectin. Although these effects were observed as early as 2 weeks after treatment, TZDs exhibit maximal glucose-lowering effects 8 to 12 weeks after the initiation of treatment. Satoh et al observed that pioglitazone treatment reduced C-reactive protein levels in both responders and nonresponders with respect to its antidiabetic effect. These findings suggest that the effect of TZDs on biomarkers of cardiovascular risk may be independent of their antidiabetic actions. Previous data indicate that throughout the spectrum of insulin resistance, from the metabolic syndrome to type 2 diabetes, PAI-1 levels are increased. Because PAI-1 promotes clot formation in plasma and various studies demonstrated an association between circulating PAI-1 levels and cardiovascular events, a TZD-mediated decrease in PAI-1 might play an important role in reducing the incidence of CAD and its complications in this population.

PPAR-α

PPAR-α, the first PPAR to be identified, is implicated in the regulation of lipid metabolism and glucose homeostasis by regulating the expression of proteins involved in the transport and β-oxidation of FFAs. PPAR-α is expressed predominately in liver and, to a lesser extent, in heart, skeletal muscle, and kidney, where it appears to play a crucial role in intracellular lipid metabolism. Fibrates like fenofibrate or bezafibrate are weak activators of PPAR-α and are widely used to treat hypertriglyceridemia in patients. Fibrates lower circulating triglyceride levels by increasing the activity of the enzyme lipoprotein lipase (LPL), which is the key enzyme in the hydrolysis of triglycerides. PPAR-α agonists directly enhance LPL activity by increasing gene transcription and indirectly by decreasing apolipoprotein (apo) C-III, an inhibitor of LPL activity. PPAR-α agonists also upregulate apo A-1 and A-II synthesis, major apos of the HDL fraction, in the liver, thus contributing to an increase in serum HDL levels. In addition, LDL particle size was significantly increased with fenofibrate therapy. However, a common observation in fibrate-treated patients is the considerable variation in induced lipid changes, indicating that polymorphisms in the PPAR-α gene may contribute to the different response to fibrate treatment. Numerous studies have investigated the antiinflammatory properties of PPAR-α. By decreasing the expression of several cytokines and proteins involved in monocyte activation, VSMC proliferation, and inflammation, PPAR-α agonists might, consequently, inhibit atherosclerosis. Although data in mouse models are controversial, trial in humans demonstrated a significant reduction in the progression of coronary atherosclerosis by treatment with fibrates. In addition, Kobayashi et al have shown that fibrates also improve glucose tolerance in type 2 diabetic patients, although this activity may not be attributable to enhanced fatty acid oxidation related to PPAR-α activation, because some of these compounds also have modest PPAR-γ activity.

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In the liver, activation of PPAR-α induces the expression of the fatty acid transport protein and fatty acid translocase, proteins that facilitate the transport of FFAs across the cell membrane. PPAR-α activation also directly increases the transcription of enzymes of the peroxisomal β-oxidation
pathway, such as long-chain acyl-CoA synthetase or acyl-CoA oxidase, the rate-limiting enzyme in the peroxisomal β-oxidation pathway. Furthermore, carnitine palmitoyl transferase I, which catalyzes the rate-limiting step in the translocation of activated fatty acids across the mitochondrial membranes, is upregulated by PPAR-α. A functional PPRE has been identified in the promoter region of this gene. Other PPAR-α responsive genes in the β-oxidation pathway, like various acyl-CoA dehydrogenases and hydroxymethylglutaryl-CoA synthase, have been identified. Functional PPREs have also been identified in the promoter of the CYP4A6 gene, which encodes the cytochrome P450 fatty acid ω-hydroxylase. Moreover, PPAR-α reduces de novo fatty acid synthesis by blocking enzymes like acetyl-CoA carboxylase and fatty acid synthase. These observations are supported by a study by Lee et al., demonstrating that PPAR-α null mice did not display increased transcription of fatty acid metabolizing enzymes in response to treatment with PPAR-α agonists.

Obesity is known to be associated with increased plasma concentrations of FFAs. Whereas acute elevation of FFAs moderately stimulates insulin release, chronic exposure to FFAs impairs insulin secretion. In addition to FFA-induced β-cell dysfunction, excess FFAs have been shown to induce β-cell apoptosis. Through stimulation of β-oxidation, PPAR-α agonists might decrease tissue lipid content, thus preventing lipid accumulation and toxicity. However, overexpression of PPAR-α in the heart is cardiotoxic. The cardiac-specific PPAR-α transgenic mouse could not increase glucose metabolism normally in response to stress. The continued FFA β-oxidation resulted in cardiac hypertrophy and heart failure. The use of PPAR-α null mice has additionally contributed to defining the role of PPAR-α. Although the phenotype of PPAR-α null mice on a regular diet was not different from wild-type mice, starvation induced major changes. In fasted conditions, the liver and heart of PPAR-α null mice were steatotic, and the mice displayed severe hypoglycemia, hypothermia, hypoketonemia, and elevated plasma FFAs levels, indicating a dramatic inhibition of fatty acid uptake and oxidation. These results demonstrate a critical role for PPAR-α in the transcriptional response to fasting. Studies over the past few years in different mouse models have shown that PPAR-α agonists markedly reduce plasma triglyceride levels, prevent high-fat diet-induced increase of body weight, and improve hepatic and muscle steatosis, consequently improving insulin sensitivity. However, in contrast to these results indicating a beneficial effect of PPAR-α activation on insulin sensitivity, PPAR-α null mice have also been shown to be protected from high-fat diet-induced insulin resistance.

In summary, PPAR-α functions as a fatty acid sensor and important regulator of fatty acid metabolism and energy homeostasis. However, although fibrates, like fenofibrate or bezafibrate, are widely used to treat hypertriglyceridemia in patients, their effects on insulin sensitivity in humans have not been extensively investigated.

**Inflammation and Atherosclerosis**

Earlier studies suggested that PPAR-α is involved in inflammation, because leukotriene B4, a potent chemotactic agent, was shown to be an activating ligand for PPAR-α. Mice lacking PPAR-α display a prolonged response to inflammatory stimuli, indicating that PPAR-α has antiinflammatory effects. Like PPAR-γ, PPAR-α is expressed in VSMCs, endothelial cells, monocytes/macrophages, and T lymphocytes. In VSMCs, PPAR-α ligands inhibit IL-1-induced IL-6 and prostaglandin production and cyclooxygenase-2 expression. In endothelial cells, PPAR-α agonists reduce cytokine-induced expression of vascular cell adhesion molecule 1 (VCAM-1), thus decreasing the adhesion of monocyte-like cells to endothelial cells. In contrast, some studies also suggest that PPAR-α activation may be potentially proinflammatory and proatherogenic by stimulating the production of MCP-1 in endothelial cells.

PPAR-α activators may also be involved in the regression of fatty streaks by regulating genes implicated in cholesterol efflux. Activation of PPAR-α results in the induction of both the HDL receptor CLA-1/SR-BI and ABCA1, a transporter that is involved in cholesterol efflux from macrophages. PPAR-α also decreases the ratio of intracellular cholesteryl ester to free cholesterol by reducing the activity of the Acyl-CoA:cholesterol acyltransferase-1, resulting in an enhanced availability of free cholesterol for efflux and subsequent reverse transport. Despite the antiinflammatory properties of PPAR-α ligands and their effect on lipid metabolism and reverse cholesterol transport, surprisingly, atherosclerotic lesion areas at the aorta were less in PPAR-α-null mice on an apoE−/− background compared with their wild-type littermates. Accordingly, atherosclerotic lesions in cibrofibrate-treated apoE-deficient mice were considerably advanced compared with untreated animals, whereas plasma cholesterol levels were also increased. Other studies, however, found that PPAR-α agonists are antiatherogenic. Treatment of apoE-deficient mice with the PPAR-α agonist fenofibrate reduced the atherosclerotic lesion surface area in the descending aorta, whereas the PPAR-α agonist GW7647 reduced atherosclerosis in LDLR−/− mice throughout the aorta. Recently, Wu et al. reported accelerated atherosclerosis in apoE−/−;db/db double knockout mice compared with their apoE−/− littermates, which was reduced by fenofibrate treatment. Although the data on the effects of PPAR-α on atherosclerosis development in rodents are controversial, fibrates have been shown in a variety of clinical trials to reduce the progression of atherosclerosis both in nondiabetic and type 2 diabetic patients and reduce the risk of coronary events.

In angiographic end point trials like the Diabetes Atherosclerosis Intervention study or the Bezafibrate Coronary Atherosclerosis Intervention trial, fibrates have been shown to result in a significant reduction in lesion development and lumen narrowing in coronary arteries. Clinical end-point trials, like the Helsinki Heart Study or the Veterans Affairs HDL Intervention Trial, demonstrated that fibrates significantly reduce the incidence of cardiovascular disease and coronary events among patients with a history of CAD and low HDL serum levels. Whether the results of human studies are attributable to an increase in HDL and altered lipid metabolism or because of the antiinflammatory
effects of PPAR-α activation or both remain to be determined.

**PPAR-δ**

PPAR-δ, the third isoform of the PPAR nuclear receptor family, shows a widespread tissue distribution. PPAR-δ is implicated in fatty acid-controlled adipogenesis, skin biology, lipid metabolism, and energy homeostasis. Treatment with a synthetic PPAR-δ agonist has been shown to improve the lipid profile in mice and monkeys and to reverse diet-induced obesity and insulin resistance in mice. Other than its profound role in fat homeostasis, increasing evidence suggest a role for PPAR-δ in inflammation. Treatment with a PPAR-δ agonist reduced VCAM-1 and monocyte chemoattractant protein 1 (MCP-1) expression in endothelial cells and inhibited inflammatory gene expression in peritoneal macrophages. However, despite its antiinflammatory effects, the role of PPAR-δ in atherosclerosis is still unclear. The findings from cell culture and animal models have translated into clinical trials to assess the effect of PPAR-δ on obesity and hyperlipidemic patients. Thus, PPAR-δ agonists might be important candidates for the treatment of obesity, insulin resistance, and dyslipidemia.

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Treatment of obese rhesus monkeys with the specific PPAR-δ agonist GW501516 decreased fasting insulin and serum LDL while increasing serum HDL levels. Other than the beneficial effect on HDL concentration, PPAR-δ compounds also favor the production of large HDL particles in primates. In humans, small HDL particles are associated with CAD progression, whereas large HDL particles are thought to be protective.

Administration of PPAR-δ agonists to mice fed a high-fat diet ameliorated diet-induced obesity and insulin resistance, probably through enhanced FFA oxidation and reduction in lipid content in skeletal muscle. Transgenic mice, which selectively express a constitutive active form of PPAR-δ in adipose tissue, display a lean phenotype and are protected from high-fat diet-induced and genetically predisposed obesity. The activation of PPAR-δ in adipose tissue specifically induces the expression of genes required for FFA oxidation and energy expenditure. In parallel, PPAR-δ deficient mice challenged with a high-fat diet show reduced energy uncoupling and are prone to dramatic weight gain.

In summary, these data identify PPAR-δ as a key metabolic regulator of fat burning and activator of thermogenesis, suggesting PPAR-δ agonists as strong candidates for the treatment of obesity and diabetes.

**Inflammation and Atherosclerosis**

Although results from both PPAR-δ-overexpressing macrophages and PPAR-δ−/− macrophages suggest that PPAR-δ is proinflammatory, treatment with a synthetic PPAR-δ ligand decreased the expression of inflammatory molecules like MCP-1 and IL-1β. In addition, PPAR-δ ligands also inhibited cytokine-induced MCP-1 and VCAM-1 expression in endothelial cells. Lee et al proposed that PPAR-δ regulates an inflammatory switch by binding or releasing transcriptional repressors. In the absence of ligand, PPAR-δ sequesters a transcriptional repressor of the inflammatory response, leading to inflammation. In the presence of ligand, PPAR-δ releases the repressor, which is then free to exert its antiinflammatory effects. Up to now, 3 studies investigated the effect of PPAR-δ activation on the development of atherosclerosis in mice. Lee et al demonstrated that PPAR-δ−/− bone marrow-transplanted mice revealed less atherosclerosis than wild-type C57 bone marrow-transplanted animals, suggesting a proatherogenic effect of PPAR-δ. Accordingly, in male hypercholesterolemic LDLR−/− mice under conditions in which PPAR-α and PPAR-γ ligands reduced lesion development, PPAR-δ agonists failed to inhibit lesion formation. However, PPAR-δ ligands inhibited inflammatory gene expression in atherosclerotic lesions like TNF-α, MCP-1, or ICAM-1, although it did not result in an overall antiatherogenic effect. These results indicate that PPAR-δ agonists might not be sufficient to inhibit the development of atherosclerosis in the setting of extreme hypercholesterolemia. Recently, Graham et al demonstrated a potent antiatherogenic effect of PPAR-δ ligands in LDLR−/− mice, with a reduction in the lesion area up to 50%.

The PPAR-δ compound GW501516 has been shown to increase expression of ABCA1 and induce apo A1-specific cholesterol efflux from different cell types. In contrast, Vosper et al found that another PPAR-δ compound promotes lipid accumulation in macrophages by increasing the expression of genes involved in lipid uptake and storage, such as the class A and B scavengers receptors (SR-A and CD36) and adipophilin. However, the expression levels of SR-A and CD36 have been shown to be similar in wild-type and PPAR-δ−/− macrophages. Expression and activation of the ABCA1 transporter by liver X receptor ligands was also not changed in PPAR-δ−/− macrophages compared with wild-type cells, suggesting that PPAR-δ has no effect on cholesterol homeostasis in the macrophage. In concert, PPAR-δ agonist treatment also did not inhibit the formation of macrophage foam cells in the peritoneal cavity. In conclusion, the role of PPAR-δ agonists in atherosclerosis and their potential therapeutic value in treatment and prevention of CAD is still unclear. Additional studies are necessary to determine the role of PPAR-δ in modulating development, stability, or regression of atherosclerotic lesions.

**Conclusions**

CAD is the leading cause of death in Americans, accounting for ~500,000 deaths every year. Obesity and diabetes mellitus, significant risk factors for the development of CAD, are becoming a global epidemic, which is related to environmental, behavioral, and genetic factors. Although changes in lifestyle are effective in preventing both diabetes and obesity in high-risk adults with impaired glucose tolerance, achieving modifications in lifestyle have proven to be difficult. Current recommendations suggest that in addition to nonpharmacological methods, drug therapy should be considered for patients with a BMI ≥30 kg/m² or a BMI of 27 to 30 kg/m² with ≥1 obesity-related disorders. Currently available antiobesity medications either decrease food intake or reduce intestinal fat absorption. However,
short-term clinical trials evaluating antidiabetes medications demonstrated only modest weight loss compared with placebo, and there are no long-term clinical trials to examine mortality and cardiovascular morbidity. Thus, more effective and better-tolerated drugs are urgently needed to control obesity and the metabolic syndrome. The adipose tissue plays a crucial role in the regulation of food intake, because it secretes a number of endocrine and paracrine mediators, including leptin, adiponectin, resistin, and TNF-α, which have been shown to influence appetite. Understanding the complex signaling system that underlies appetite control will likely offer new approaches for treatment strategies. The ability of PPAR-δ agonists to induce adaptive thermogenesis and protect against both diet-induced and genetically predisposed obesity in animal models suggest that PPAR-δ might be an exiting new target in the treatment of obesity. Of particular interest are the dual PPARs, a single ligand activating both γ and α and the panPPARs, activating α, γ, and δ.

All 3 of the PPAR isotypes attenuate inflammatory responses, which is important, because inflammation is intimately connected to appetite, insulin resistance, obesity, and atherosclerosis. These anti-inflammatory actions result in improvement of atherosclerosis in some animal models, although the effect on atherosclerosis is also related to the PPAR ability to regulate foam cell formation. Future studies are required to elucidate these interactions and the role of PPAR ligands as potential candidates for treatment of obesity, type 2 diabetes, and atherosclerosis. The enthusiasm for the use of PPARs is dampened by their potential oncologic effects. Whereas long-term administration of fibrates in humans revealed no peroxisome proliferation or any other morphological changes in the liver, fibrates have been shown to cause cancer in rodents. However, epidemiological studies did not reveal a statistically significant increase in cancer up to 8 years after initiation of therapy. Treatment with PPAR-γ agonists increased the frequency and size of colon tumors in mice but caused a significant reduction in the growth of human cancer cell lines. Whereas the synthetic PPAR-δ agonist GW501516 increased the number and size of intestinal polyps in a cancer-prone mouse model, suggesting that PPAR-δ might be a transducer in colorectal carcinogenesis, PPAR-δ has been shown to be dispensable for colon polyp formation. However, extrapolation of the evidence of carcinogenesis from rodents to humans is an uncertain process, and additional studies are necessary. In the meantime, ligands that activate and modify PPARs are being actively pursued for clinical development.

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