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PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS: How Their Effects on Macrophages Can Lead to the Development of a New Drug Therapy Against Atherosclerosis

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■ **Abstract** Peroxisome proliferator-activated receptors (PPARs) alpha (α), beta/delta (β/δ), and gamma (γ) are members of the nuclear receptor superfamily, which also includes the estrogen, androgen, and glucocorticoid receptors. Recent evidence suggests that PPARs regulate genes involved in lipid metabolism, glucose homeostasis, and inflammation in various tissues; however, the mechanisms involved are not completely understood. Anti-diabetic drugs, called glitazones, can selectively activate PPARγ, and hypolipidemic drugs, called fibrates, can weakly activate PPARα. Both classes of drugs can decrease insulin resistance and dyslipidemias, which also makes them attractive for treating the metabolic syndrome. The metabolic syndrome exhibits a constellation of risk factors for atherosclerosis that include obesity, insulin resistance, dyslipidemias, and hypertension. Interestingly, all three PPARs are present in macrophages and can therefore have a profound effect on several disease processes, including atherosclerosis. Macrophages are key players in atherosclerotic lesion development. Currently, the first line of defense in reducing the risk of atherosclerosis is aimed at lowering low-density lipoproteins (LDL) and raising high-density lipoproteins (HDL), but a large percentage of patients on statins still succumb to coronary artery disease. However, with the development of drugs selectively activating PPARs, a new arsenal of drugs specifically targeting to the macrophage/foam cell may potentially have a profound impact on how we treat cardiovascular disease.

INTRODUCTION

Atherosclerosis is a complex chronic inflammatory process responsible for most clinical manifestations of coronary artery disease (CAD) and ischemic strokes, and one of the leading causes of morbidity and mortality in Western societies (1). The earliest atherosclerotic lesion is the "fatty streak," rich in monocyte-derived macrophages engorged with lipids (2). Fatty streak formation may begin during

fetal development and is prevalent in childhood (3, 4). The intima, the innermost layer of the artery adjacent to the lumen, consists of a monolayer of endothelial cells and an internal elastic membrane (Figure 1*a*). In humans and primates, the intima also contains proteoglycan-rich connective tissue, in particular near branch sites. Lipid accumulation is generally absent from the normal intima, as are macrophages, except for occasional patrolling monocytes (Figure 1*b*). Underneath the intima, a thick arterial media, consisting mainly of smooth muscle cells interwoven with lamellae of elastin and collagen, conveys mechanical stability to the arterial wall (Figure 1*a*).

The accumulation of lipids in the arterial intima is dependent on the interaction of low-density lipoproteins (LDL) with proteoglycans within the extracellular matrix (5). Indeed, LDL receptor-deficient mice expressing proteoglycan-defective-binding LDL develop less atherosclerosis (4). This accumulation of lipids may then undergo modification and begin to recruit monocytes into the vessel wall (Figures 1*c* and *d*). Once LDL begins to accumulate and be oxidized in the artery wall, circulating monocytes begin to roll on, and adhere to, endothelial cells expressing adhesion molecules (4). Penetration of monocytes through the endothelium is promoted by chemokines, such as monocyte chemoattractant protein 1 (MCP-1) (Figure 1*e*). The chemokine C-C receptor (CCR2) is present in monocytes and has previously been shown to play an important role in atherosclerosis by binding to MCP-1 (6). Once monocytes have taken residence in extracellular matrix, they undergo phenotypic transformation into macrophages, internalize large amounts of cholesterol via a family of scavenger receptors, and become foam cells (Figure 1*c*,*d*). If atherogenic conditions persist, fatty streaks can progress to advanced stages of atherosclerotic lesions susceptible to plaque rupture and thrombosis.

Current guidelines by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recommend aggressive intervention in people whose LDL cholesterol is greater than 200 mg/dl or who have a family history of early cardiovascular disease (7). Prevailing current medical therapy is aimed at lowering LDL and raising high-density lipoproteins (HDL) with medications such as bile acid sequestrants, niacin, fibrates, and statins. For the past 20 years, statins have been the mainstay for the treatment of hypercholesterolemia, mainly owing to their relatively low incidence of side effects. The statins inhibit HMG-CoA reductase, the rate-limiting enzyme involved in the cholesterol biosynthetic pathway. Brown & Goldstein's (8) seminal work demonstrated that by reducing the cell's own ability to synthesize cholesterol, LDL receptors are upregulated, particularly in hepatocytes, and remove the circulating proatherogenic LDL particles more rapidly. Several large clinical trials have established that statins markedly reduce the clinical manifestations of atherosclerosis (9), and that other lipid-lowering drugs also do so, albeit less effectively. However, despite a significant decrease in the risk of CAD overall, some 60% of the patients on statins still develop coronary events. This may reflect significant lesions already present before cholesterol-lowering therapy and the partial ineffectiveness of the statins to regress or stabilize

the plaque, or to reduce plaque rupture or its thrombotic consequences. In a study that recruited 321 men who were less than 60 years of age and developed premature CAD, approximately 48% of the patients had cholesterol levels less than 200 mg/dl, suggesting that factors other than elevated cholesterol levels, such as low HDL and lipoprotein (a) [Lp(a)], contribute to CAD (10). In recent years, there has been a significant push toward the development of a new class of drugs targeted at other pathways involved in atherogenesis that can be used in combination with statins. For instance, the drug ezetimibe selectively inhibits intestinal uptake and absorption of dietary and biliary cholesterol in small intestinal enterocytes (11). The drug inhibited cholesterol absorption >90% and reduced atherosclerosis 97% in apolipoprotein E (apoE)-deficient mice fed different cholesterol-rich diets (12). Coadministration of a statin and ezetimibe synergistically decreased LDL and raised HDL levels, compared to single-drug treatment (13). Another potential drug that is undergoing clinical trials is torcetrapib. This drug can increase HDL levels by inhibiting the cholesteryl transfer protein (CETP), which is present in humans but not in mice. CETP is a plasma glycoprotein that facilitates transfer of cholesteryl esters from HDL cholesterol to apolipoprotein B–containing lipoproteins, such as very low-density lipoprotein (VLDL) and LDL. By inhibiting this process, cholesterol from peripheral tissues can be returned to the liver and secreted in the bile. A single-blinded, placebo-controlled study was performed using torcetrapib in patients with low HDL levels (14). In these subjects, torcetrapib increased HDL levels and decreased LDL levels when administered as a monotherapy or in combination with a statin.

Although the strategy of reducing plasma cholesterol levels and cholesterol absorption is quite effective in reducing the risk of CAD in a majority of patients, developing drugs specifically targeting cells that are involved in the development of atherosclerosis is an attractive idea. The development of the atherosclerotic lesion is a complex process involving interaction between endothelial cells, smooth muscle cells, lymphocytes, and macrophages (15).

Drug therapy can be aimed at several sites: at the vessel wall, by decreasing inflammation and the adherence of monocytes, by increasing vasodilatation, or by removing cholesterol from atherosclerotic lesions; at the liver, by regulating apolipoproteins and lipoprotein synthesis or excreting cholesterol through bile; or at the small intestines, decreasing dietary cholesterol absorption. Additionally, reducing other risk factors, such as the metabolic syndrome, diabetes, and hypertension, can also reduce the development of atherosclerotic lesions.

CONTRIBUTION OF THE METABOLIC SYNDROME TO ATHEROGENESIS

In addition to hypercholesterolemia, other risk factors, such as dyslipidemias, diabetes, hypertension, smoking, and male gender, can also accelerate atherosclerosis.

Developing drugs that improve insulin sensitivity, reduce hypertension, and correct the dyslipidemias may therefore also have an impact on atherosclerosis. In recent years, there has been great concern about the growing incidence of obesity and the metabolic syndrome, which has become a major health problem in the United States, especially in children. Obesity, insulin resistance, hypertension, low HDL, hypertriglyceridemia, and hypercoagulability define the metabolic syndrome. It is estimated that more than 30% of adults in the United States are obese and are therefore at a higher risk of developing noninsulin-dependent diabetes mellitus (NIDDM) (16). The pathogenic mechanisms through which insulin resistance (IR) and diabetes may promote atherosclerosis include impairment of nitric oxide–mediated vasodilatation, increased expression of the plasminogen activator inhibitor-1 (PAI-1), and increased smooth muscle cell proliferation (by affecting the phosphatidylinositol 3 kinase pathway). In addition to endothelial dysfunction, atherogenesis, and thrombosis, IR may cause increased production or decreased uptake of free fatty acids (FFAs) by adipocytes (17). Increased plasma FFAs lead to increased hepatic uptake, which in turn increases hepatic production of triglyceride-rich lipoproteins, such as VLDL, resulting in dyslipidemia. The increase in VLDL is often associated with smaller HDL particles that are ineffective in removing cholesteryl esters from peripheral tissues, including the arterial wall, and with smaller, denser LDL particles that are more susceptible to oxidation. Both are considered atherogenic factors. However, new evidence suggests that obesity may be exacerbated by inflammation within adipose tissues causing a dysregulation in the insulin-signaling pathway, and that macrophages contribute to the obesity and the metabolic imbalance, leading to insulin resistance in adipocytes. Xu et al. and Weisberg et al. (18, 19) demonstrated that macrophages infiltrate adipose tissue in mice and are an important source of inflammation in this tissue. By separating adipocytes from their stromal supportive connective tissues, Xu et al. determined that the majority of inflammatory genes being expressed were localized in the stromal-vascular fraction. Histological analysis revealed that the small-nucleated cells present in this fraction were macrophages and that the relative number of macrophages increased as the animals gained more weight and adipose tissue. Weisberg et al. performed bone marrow reconstitution experiments, using two antigenetically distinct forms of the CD45 protein, a leukocyte-specific antigen. They transplanted $CD45.1^+$ cells into $CD45.2^+$ mice. After placing these animals on a high-fat diet, they used a macrophage-specific antibody (F4/80) and determined that 85% of the macrophages isolated from adipose tissue were $CD45.1^+$. It is therefore possible that macrophages play an analogous role in the development of obesity and insulin resistance in adipose tissue as in the arterial wall, in particular with regard to the recruitment of additional monocyte/macrophages. Therefore, the macrophage would be an attractive target for drug therapy aimed at both atherosclerosis as well as the metabolic syndrome.

THE MACROPHAGE IS A KEY PLAYER IN ATHEROGENESIS AND A POTENTIAL TARGET FOR DRUG THERAPY

Macrophages play several important beneficial roles in the immune system; e.g., they present antigens to immunocompetent cells and act as powerful effector cells in cell-mediated immune response. However, they also play a pathogenic role in several chronic inflammatory conditions, including atherosclerosis (20). In response to the expression of adhesion molecules on endothelial cells, such as vascular cell adhesion molecule-1 (VCAM-1); intercellular adhesion molecule 1 (ICAM-1); P and E selectins; and the presence in the subendothelial space of cytokines, such as tumor necrosis factor alpha (TNFα) and MCP-1, circulating monocytes enter the arterial wall (Figure 1*e*). There, they differentiate into macrophages and take up cholesterol. Scavenger receptors, such as scavenger receptor A (SRA), class B, type 1 (SR-BI), and CD36, are responsible for the massive uptake of lipoprotein cholesterol and induce foam cell formation (21) (Figure 1*f*). This is in part due to the fact that unlike LDL receptors, scavenger receptors are not regulated in response to high levels of intracellular cholesterol. The major ligand for scavenger receptors, such as CD36, appears to be oxidized LDL (oxLDL), which is present in atherosclerotic lesions (22). Of particular importance for early lesion formation, macrophages are a major source of reactive oxygen species (ROS) and can also oxidize LDL through 12/15 lipoxygenase and/or inducible nitric oxide synthase (iNOS) (23, 24). Therefore LDL oxidation represents a critical event in the development of the fatty streak lesion. Macrophages also promote the growth of atherosclerotic lesions by interacting with and profoundly influencing other intimal cells, such as smooth muscle cells (SMC) and T-lymphocytes, through expression of an array of cytokines and other factors. As macrophages become engorged with lipids, a program of cell death or apoptosis may occur (25, 26). This may have a beneficial effect in early lesions, preventing the extracellular accumulation of cellular components and oxidized lipids. However, in advance atheromas, apoptosis may contribute to weakening of the fibrous cap. Even in the most advanced plaques there are metabolically active macrophages, predominately at the shoulders of the lesion. It is mainly at the shoulders of the lesion that plaque ruptures occur (2/3 shoulder, 1/3 cap), as shown by Davies (27). Macrophages can produce reactive forms of oxygen (e.g., O_2^-), proteases, and metalloproteinases (MMPs), such as gelatinase B. These enzymes can remodel the extracellular matrix, which can rupture the plaque and release thrombogenic material from the necrotic lipid core into the lumen of the artery and cause occlusion, ultimately leading to myocardial infarction or stroke.

The rapid uptake of the modified lipoproteins leading to foam cell formation is the hallmark of atherosclerosis. The cholesterol liberated from the modified lipoproteins by lysosomal hydrolase needs to be reesterified into cholesteryl esters for intracellular storage, otherwise the free cholesterol will be toxic to the cell.

There is a dynamic balance between the amount of free cholesterol and cholesteryl esters within the cell, which is regulated by two enzymes located in the endoplasmic reticulum: Acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) and neutral cholesterol ester hydrolases (nCEH). An example of an nCEH is hormone sensitive lipase (HSL). Removal of cholesterol from macrophages can theoretically lead to the regression of atherosclerosis or stabilization of the plaque. For instance, by inhibiting cholesterol esterification, free cholesterol can exit from foam cells, return to the liver by HDL, and be secreted in the bile. Studies in primary human monocyte-derived macrophages demonstrated that an ACAT1-selective inhibitor limited the uptake of modified LDL and enhanced cholesterol efflux (28). Another ACAT inhibitor (F-1394) decreased atherosclerosis in apoE-deficient mice (29). However, transfer of ACAT1$^{-/-}$ bone marrow progenitor cells into apoE and LDL receptor-deficient mice promoted atherosclerosis, compared to ACAT1$^{+/+}$ wild-type controls (30, 31). This would suggest that partial inhibition of ACAT1 might be effective, but complete deletion of the gene may affect downstream genes that may completely dysregulate cholesterol trafficking and efflux. In a follow-up paper, Dove et al. (32) determined that cholesterol efflux was reduced in ACAT1-deficient macrophages despite an upregulation of ABCA1 gene expression. On the other hand, manipulating ACAT2 expression, which is primarily found in the liver and small intestine, may prove to be beneficial. ACAT2$^{-/-}$ mice backcrossed to apoE-deficient mice had fewer lesions, compared with the wild-type controls (33). Further analysis revealed that these mice expressed higher levels of lecithin/cholesterol acyltransferase (LCAT) activity, which increased HDL cholesteryl esters and lowered total cholesterol levels owing to the decrease in cholesterol intestinal absorption. A recent study showed that avasimibe enhances the lipid lowering effect of the statin atorvastatin in homozygous familial hypercholesterolemia patients (34). Alternatively, other investigators are examining ways of increasing the free cholesterol content by increasing nCEH activity. However, animal studies have also demonstrated paradoxical results. Escary et al. (35) initially demonstrated that overexpression of HSL in a murine macrophage cell line (RAW 264.7) increased cholesterol ester hydrolysis by two- to threefold. However, atherosclerosis in transgenic female C57BL6 mice overexpressing HSL increased 2.5 times in size, compared to the control wild-type littermates (36). They concluded that the increase in free cholesterol resulted in cell death or was rapidly reesterified by ACAT1. When only a HSL transgenic mouse was crossed with a transgenic mouse overexpressing human apolipoprotein A-I, a component of HDL, that a reduction in atherosclerosis occurred, indicating a concomitant increase in cholesterol acceptors is also required (37).

Enhancing cholesterol efflux from foam cells and its return to the liver would be a major potential target for drug therapy. Several pathways have been proposed that are commonly referred to as reverse cholesterol transport (RCT). In 1999, several laboratories identified the molecular defect in Tangier's patients, *ATP binding cassette transporter A1* (*ABCA1*) gene (38–41). Patients with Tangier's disease

present with low HDL levels and sterol accumulation in tissue macrophages and are theoretically at a higher risk of developing cardiovascular disease. The apoAI-specific active transport of cholesterol assisted by ABCA1 plays an important role in RCT. Overexpression of the human *ABCA1* gene in apoE-deficient mice resulted in a reduction in total cholesterol levels and atherosclerosis (42, 43). However, knocking out the gene has led to some curious results. Bone marrow transplantation of ABCA1$^{-/-}$ progenitor cells into apoE- and LDL receptor–deficient mice resulted in an increase in atherosclerosis without any changes in total cholesterol levels (44, 45). Furthermore, complete absence of ABCA1 in either animal model resulted in no difference in atherosclerosis, compared to wild-type controls, despite a significant reduction in total cholesterol levels (45). This would suggest that complete absence of ABCA1 in animals has a profound effect on lipoprotein homeostasis without increasing atherosclerosis, whereas selective disruption of ABCA1 in macrophages results in an increased lesion size independent of total cholesterol levels.

Other possible RCT pathways include the hydroxylation of cholesterol by the cytochrome p450 enzyme, sterol 27-hydroxylase (Cyp27), into 27-hydroxycholesterol. This oxysterol can serve two functions. First, 27-hydroxycholesterol can integrate itself into the plasma membrane where it can be transferred to pre-HDL particles and returned back to the liver for bile acid secretion (46). More recently, it has been demonstrated that 27-hydroxycholesterol is a weak agonist for the nuclear receptor, liver X receptor (LXR) (47). When activated, LXR increases ABCA1 expression (48–50). Treatment of apoE- and LDL receptor–deficient mice with a synthetic LXR agonist decreases atherosclerosis by more than 50% through the upregulation of ABCA1 (51). Unfortunately, a major side effect of LXR agonists is the increase of VLDL and severe lipogenesis observed in rodents through the activation of the SREBP pathway, which may be a contraindication in patients with the metabolic syndrome (52, 53). Finally, SR-BI may also play a role in cholesterol efflux in macrophages and can be a target for drug therapy. SR-BI is predominately expressed in hepatocytes as well as steroidogenic tissues, and it mediates the selective uptake of cholesteryl esters into HDL (54). Overexpression of SR-BI in hepatocytes led to the reduction of cholesterol levels, enhanced cholesterol secretion into the bile, and reduced atherosclerosis in mice (55, 56). Deleting the gene caused abnormally large HDL particles, impaired cholesterol secretion into the bile, and increased atherosclerosis in mice (57–59). SR-BI is also present in macrophages. Cell culture studies demonstrated that SR-BI enhanced cholesterol efflux through a concentration gradient (60, 61). Zhang et al. (62) demonstrated that SR-BI knockout bone marrow progenitor cells, when transplanted into apoE-deficient mice, produced more lesions compared with animals that received wild-type macrophages.

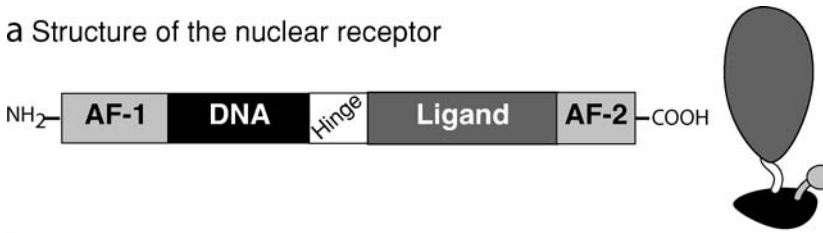
In summary, the macrophage/foam cell is clearly a potential target for therapeutic intervention. Identifying the genes that play a role in cholesterol homeostasis and understanding the regulation of these genes that can ultimately lead to the

development of drug therapies has been a major thrust in the field of atherosclerosis. One area of interest is nuclear receptors, and in recent years there has been a flourish of publications on nuclear receptors and lipid metabolism. Not only do nuclear receptors respond to steroid hormones that function to regulate development and homeostasis but they can also respond to other classes of lipid-derived mediators that regulate lipid and glucose homeostasis (63). Lipoproteins can provide a source of lipid-derived mediators that can activate these receptors and therefore play a role in atherogenesis. Here, we review recent findings that provide new insights into the role of nuclear receptors, particularly focusing on the PPARs and their effects on lipid metabolism and inflammation within macrophages, and their overall effect on atherosclerosis.

NUCLEAR RECEPTORS

Forty-eight nuclear receptors in the human and mouse genomes have been identified thus far (64). They are divided into three categories based on the identification of known ligands, their target genes, and their physiological function: the endocrine receptors, the adopted orphan receptors, and the orphan receptors. All members of this superfamily of nuclear receptors have similar characteristics (Figure 2*a*). In general, nuclear receptors possess a ligand-independent transcriptional activation function domain (AF-1) located in the N terminus, a core DNA-binding domain consisting of two highly conserved zinc-finger motifs that bind the receptor to specific DNA sequences, a hinge region that allows flexibility of the nuclear receptors to dimerize to other nuclear receptors and binding to DNA, a large region containing the ligand-binding domain (LBD) and a second dimerization interface, and finally a second ligand dependent activation function domain (AF-2) at the carboxy terminus.

The first group of nuclear receptors is considered the classic group of "endocrine receptors," which includes the estrogen (ER), progesterone (PR), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. These receptors play an important role in the development of sexual maturity and reproduction, carbohydrate metabolism, immunity, and water and electrolyte balance. Ligands activating these receptors are known as the steroid hormones that are regulated through the hypothalamic-pituitary axis. The endocrine receptors bind to their respective ligands with high affinity in the nanomolar range. Furthermore, the endocrine receptors bind to DNA as "homodimers." This is in contrast to the members of the "adopted orphan nuclear receptors." These include LXR, PPAR, retinoid X receptor (RXR), farnesyl X receptor (FXR), xenobiotics receptor/pregnane X receptor (SXR/PXR), constitutive androstane receptor (CAR), and most recently the "deorphanized" retinoic acid receptor-related orphan receptor (ROR). Instead of binding to DNA as a homodimer, the adopted orphan nuclear receptors bind to DNA as "heterodimers" with the RXR as an obligate partner. The endogenous ligands



b Subgroups of PPAR

	PPARα	PPARγ	PPARδ
Tissue Expression	Liver Heart Kidney Adrenal	Adipose tissue Spleen Adrenal Colon	Many tissues
Cell-specific Expression	Endothelial cells Macrophages Smooth muscle cells	Macrophages T cells Smooth muscle cells	Many cell types
Biological Functions	Triglyceride-rich lipoprotein synthesis and metabolism β-oxidation Anti-inflammation	Fat cell development Glucose homeostasis Anti-inflammation	Energy utilization Lipid metabolism Anti-inflammation
Endogenous Ligands	PUFAs 8(S)-HETE	PUFAs 15d-PGJ2 13-HETE 9-HODE	PUFAs Carboprostacyclin
Disease targets	Hypertriglyceridemia	Type 2 diabetes	Metabolic syndrome?
Drugs	Fibrates	Glitazones	

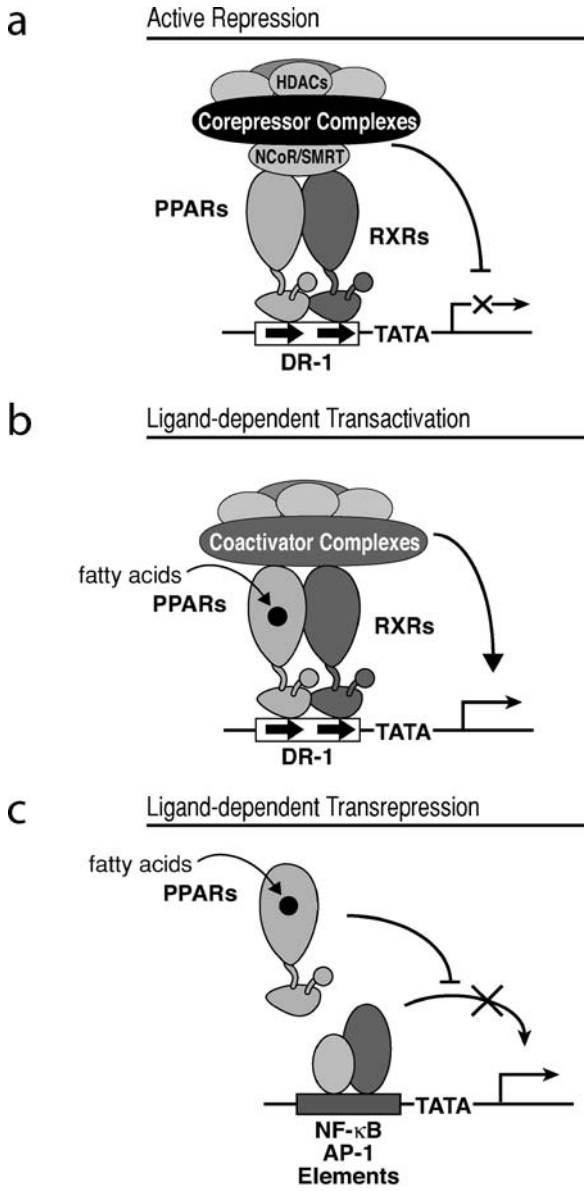
Figure 2 Peroxisome proliferator-activated receptors (PPARs). (*a*) Generalized structure of a nuclear receptor. Each nuclear receptor possesses a conserved DNA binding domain and C-terminal ligand binding domain characteristic of each nuclear receptor. (*b*) The PPAR subfamily of nuclear receptors. Major sites of expression in the body, major biological functions, and naturally occurring ligands and classes of drugs that are in clinical use are listed. Polyunsaturated fatty acids: PUFAs.

consist of low-affinity (micromolar range) dietary lipids and their metabolites, and it appears that the role of the adopted orphan nuclear receptors is to regulate lipid and glucose homeostasis in the liver, adipocytes, skeletal muscle, and in macrophages (Figure 2*b*).

The regulation of gene expression by nuclear receptors is a complex process. The nuclear receptor is activated by its ligand, but in addition to directly binding to the nuclear receptor, the ligand also induces allosteric changes within the LBD that regulate interactions with coactivator and corepressor complexes, which can further mediate the transcriptional activities of the nuclear receptors (65, 66). The PPARs regulate gene expression through at least three distinct types of transcriptional activities (Figure 3). First, PPAR/RXR heterodimers bind to a specific response element (AGGTCA-N-AGGTCA) identified as a direct repeat 1 (DR-1) within the promoter/enhancer sequence of target genes. In the absence of ligands, this heterodimer actively represses transcription through interactions with corepressor complexes that contain the nuclear receptor corepressors NCoR and/or SMRT (Figure 3*a*) (67–70). Second, in the presence of ligands, PPARs bind to the DR-1 element and activate transcription of the target gene (Figure 3*b*). Transcriptional activation is linked to the recruitment of coactivator proteins as well as the dissociation of corepressors (71). Recent studies suggest that ligand-dependent corepressor-coactivator exchange requires ubiquitinylation machinery that targets the corepressor complex for proteosome-dependent destruction (72). A large number of coactivator proteins have been identified that contribute to nuclear receptor function (65, 66). Many of these proteins are components of large multiprotein complexes with associated enzymatic activities, including nucleosome remodeling activities, histone acetyltransferase activities, histone methyltransferase activities, and/or these proteins have the ability to recruit core transcription factors. Third, the PPARs have the ability to negatively regulate gene expression in a ligand-dependent manner by antagonizing the activities of other classes of signal-dependent transcription factors, such as NF-κB and AP-1 (Figure 3*c*). This

Figure 3 Regulation of gene expression by PPARs. PPARs as heterodimers with RXRs bind to a specific DNA binding site, known as DR-1 element, in target genes. (*a*) In the absence of ligands, PPAR/RXR heterodimers can bind to target genes and actively repress transcription through the recruitment of corepressor complexes that contain NcoR, SMRT, and histone deacetylases (HDACs). (*b*) In the presence of ligands, PPAR/RXR heterodimers activate transcription through the recruitment of a coactivator complex, which contains enzymatic functions, which include nucleosome remodeling activity, histone acetylase transferase, and histone methyltransferase activities, and directly or indirectly recruit core transcriptional machinery to the promoter. (*c*) PPARs can inhibit the activities of other signal-dependent transcription factors, such as NF-kB and AP-1. This transrepression function contributes to the anti-inflammatory actions of PPARs. Figure 3 is reprinted from Li & Glass (204) with permission from The American Society for Biochemistry and Molecular Biology.

activity, referred to as transrepression, is thought to underlie antiinflammatory actions of nuclear receptors, such as the glucocorticoid receptor, PPARs, and LXRs (73–75). The molecular mechanisms responsible for transrepression remain less well understood than mechanisms responsible for transcriptional activation, but do not appear to involve sequence-specific binding to DNA.



PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

PPARα (NR1C1), PPARβ/δ (NR1C2, hereafter referred to as PPARδ), and PPARγ (NR1C3) compose the PPAR subfamily of nuclear receptors. Although there is overlap in natural ligands that are capable of activating the three PPARs, each receptor subtype exhibits distinct patterns of expression and overlapping but distinct biological activities (76, 77) (Figure 4).

PPARα

PPARα, the first PPAR to be identified, was named based on its ability to be activated by substances that drive peroxisome proliferation in rodents (78). Endogenous ligands for PPARα include polyunsaturated fatty acids, such as linoleic acid, dodecahexanoic acid (DHA), and eicosapentanoic acid (EPA). Many lines of

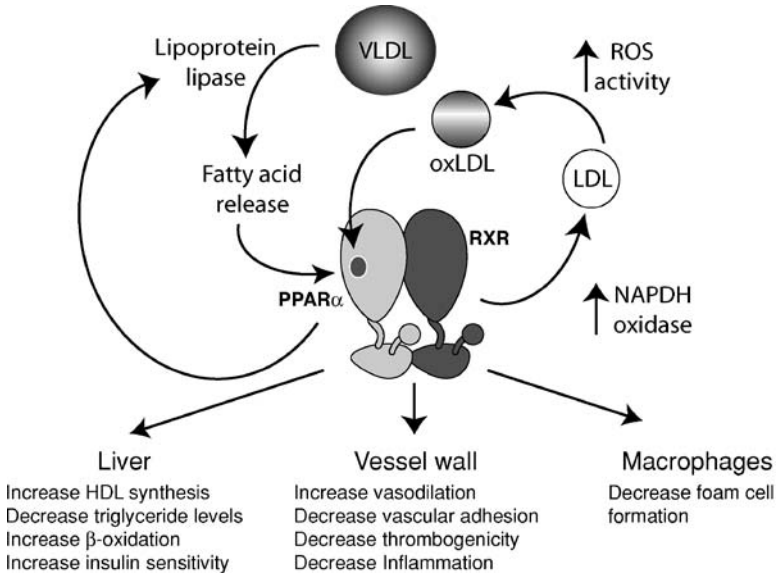


Figure 4 Modulation of lipid metabolism and inflammatory genes by PPARα. Synthetic ligands, such as fibrates, can activate PPARα. As for endogenous ligands, it is hypothesized that fatty acids released from triglyceride-rich lipoproteins, such as VLDL, can activate PPARα, which would in turn increase LPL expression/activity and continue releasing more ligands from triglyceride-rich lipoproteins. Additionally, PPARα can also generate its own ligand by increasing NADPH oxidase expression and LDL oxidation. When activated, PPARα can exert it effects on different tissues affecting insulin sensitivity, lipoprotein synthesis and metabolism, β-oxidation, vasodilation, inflammation, and cholesterol efflux.

evidence indicate that PPARα regulates lipid homeostasis, in part by stimulating peroxisomal fatty acid β oxidation. In the liver, activation by PPARα leads to the upregulation of fatty acid transport proteins and long-chain acyl-CoA synthase (79, 80). By increasing β oxidation, PPARα not only provides energy for cells but also shortens the long-chain fatty acids, thus preventing lipid accumulation and toxicity in the cells. Mitochondrial HMG-CoA synthase is also a target of PPARα; it plays a role in the formation of ketone bodies and provides energy in extrahepatic tissues (81). It also has been proposed that PPARα possesses antidiabetic properties, such as increasing insulin sensitivity. In Zucker obese fa/fa rats and lipoatrophic mice (AZIP/F-1), activation of PPARα led to a significant improvement in insulin sensitivity (82, 83). Yet, in PPARα null mice, there is no gross alteration of insulin sensitivity (84). With respect to atherosclerosis, improvement of insulin sensitivity counterbalanced endothelial dysfunction, e.g., by increasing nitric oxide synthesis and endothelin-1 expression (85, 86).

PPARα is also the molecular target of fibrates that are used in the treatment of hypertriglyceridemia (87). They include gemfibrozil, fenofibrate, cofibrate, bezafibrate, and ciprofibrate. Additionally, derivatives from fatty acids can also activate PPARα. Ziouzenkova et al. (88) demonstrated that LPL provides fatty acid metabolites from triglyceride-rich lipoproteins, such as VLDL and chylomicrons, which can activate PPARα. At the same time, PPARα, as well as PPARγ, can increase LPL gene expression/activity and therefore can provide more ligands for PPAR activation by a positive feedback mechanism (89, 90). Activation of PPARα has been shown to increase apolipoprotein AI and AII and decrease apolipoprotein CIII, an inhibitor of LPL (91–93). This would in turn increase HDL and decrease triglyceride levels and is thought to contribute to beneficial effects of fibrates on lipoprotein levels in hypertriglyceridemic individuals. PPARα agonists have also been shown to regulate cholesterol homeostasis in cultured macrophages. Activation of PPARα can lead to induction and the expression of LXRα, which can then stimulate ABCA1 expression and promote efflux of cholesterol to apo-AI in human primary monocyte-derived macrophages (94). It has also been demonstrated that PPARα can inhibit esterification of free cholesterol by ACAT1 and increase the efflux of free cholesterol by increasing the expression of SR-BI (94–96).

Studies in vitro suggest that PPARα also regulates the expression of genes that control inflammatory responses in endothelial cells, smooth muscle cells and macrophages (Figure 4). PPARα has been shown to inhibit transcriptional responses to inflammatory stimuli by interfering with the activation of NFκB, leading to the reduction of VCAM-1 in endothelial cells (88, 97). In vascular smooth muscle cells (VSMCs), PPARα agonists inhibited interleukin (IL)-1-induced IL-6 expression and cyclooxygenase-2 (COX-2) and prostaglandin production (98). In monocytes and macrophages, PPARα agonists reduced the expression of tissue factor and matrix metalloproteinase, thereby decreasing thrombogenicity and plaque instability (99, 100). All of the above effects should reduce atherogenesis and plaque rupture. On the other hand, other investigators reported potential proatherogenic effects, such as the ability of PPARα agonists to stimulate the

production of MCP-1 in endothelial cells (101), which would be expected to enhance recruitment of monocytes into lesions. These discrepancies may be due to PPARα's ability to regulate genes differently, based on coactivators and corepressors present or absent in macrophages versus endothelial cells. It may also be due to the type of ligands being generated in vivo. In agreement with Ziouzenkova et al. (88), Teissier et al. demonstrated that lipoproteins provide ligands that activate PPARα and that PPARα, but not γ synthetic agonists, induced ROS activity by increasing NADPH oxidase expression (102). They also showed that induction of NADPH oxidase in both human and murine primary and macrophage cell lines increased LDL oxidation, which can further promote atherogenesis. At the same time, oxLDL activated PPARα, which in turn inhibited LPS-induced iNOS expression. The generation of ligands for PPAR activation from oxLDL is quite intriguing because oxLDL is generally considered to be proatherogenic. It is possible that PPAR activation is a protective response that limits the pathogenic effects of oxLDL, but that this becomes ineffective with progressive atherogenesis, possibly owing to the downregulation of the receptor itself. To reactivate PPARα, a more potent PPARα ligand, such as a synthetic ligand, may be required. Further investigation is needed to determine if these possibilities are true.

In concert, the effects of PPARα agonists on lipid and carbohydrate metabolism would be expected to result in protection against atherosclerosis. Epidemiological studies have demonstrated that PPARα agonists reduce the progression of atherosclerosis (103, 104) and decrease the incidence of coronary heart disease (105). Furthermore, treatment of nondiabetic and type 2 diabetic patients with fibrates resulted in an improvement in insulin sensitivity as well as vascular function (106–108). However, studies of PPARα in animal models of atherosclerosis have yielded conflicting information on the mechanisms responsible for the inhibition of atherosclerosis observed in clinical trials or predicted on the basis of in vitro studies. Genes that play a role in HDL synthesis, such as Apo-AI and AII, are responsive to PPARα agonists in humans but not in mice (109). Tordjman et al. (110) made the surprising observation that PPAR$\alpha-$null/apoE-deficient mice have fewer atherosclerotic lesions, a decrease in insulin resistance, and a decrease in blood pressure compared with wild-type controls. Fu et al. (111) found that treatment of apoE-deficient mice with a PPARα agonist, ciprofibrate, worsened hyperlipidemia and increased atherosclerosis. In another study, Claudel et al. (112) found no effect on atherosclerosis in apoE-deficient mice treated with fenofibrate.

On the other hand, other laboratories demonstrated that PPARα and its agonists are antiatherogenic. Duez et al. (113) found that fenofibrate exerted minimal antiatherogenic effects in apoE-deficient mice, but a more pronounced effect in apoE-deficient mice carrying a fenofibrate-inducible human apo-AI transgene. We demonstrated that activation of the murine PPARα by a highly specific and potent agonist (GW7647) inhibited atherosclerosis in hyperlipidemic LDL receptor-deficient mice by almost 50%, as determined by cross-sectional analysis through the aortic origin and by en face analysis of the entire aorta (114). We also observed

a decreased expression of inflammatory genes in atherosclerotic lesions by real-time PCR. With respect to lipid metabolism, PPARα did not significantly alter the diet-induced hyperlipidemia, but we did observe an improvement of insulin sensitivity, which may be associated with less weight gain compared to control mice. This effect was not due to a decrease in food intake in the drug treated group. Although treatment of mice with this PPARα agonist induced expression of LXRα in the arterial walls of hypercholesterolemic mice exhibiting extensive lesion formation, expression of ABCA1 did not change, consistent with previous studies using fenofibrate (113, 114). In concert with its effects on the development on atherosclerosis, GW7647 inhibited the formation of macrophage foam cells in the peritoneal cavities of hypercholesterolemic mice. Through the use of macrophage and bone marrow transfer experiments, this effect was shown to require macrophage expression of PPARα and LXR (114). Reduction of foam cell formation in peritoneal macrophages was independent of cholesterol esterification and cholesterol efflux via the ABCA1 pathway. Unexpectedly, when LXR-null bone marrow progenitor cells were transplanted into LDL receptor-deficient mice or transferred into their peritoneal cavity, the majority of donor macrophages died when recipient mice were treated with the PPARα agonist. The few cells that could be recovered were massively engorged with oil red O-staining lipid, suggesting that PPARα negatively regulated macrophage foam cell formation through an LXR-dependent, ABCA1-independent pathway. PPARα and PPARγ activation may promote macrophage apoptosis in a context-dependent manner (115). The decreased number of LXR-deficient macrophages recovered following treatment with the LXR-specific agonist therefore also raises the possibility that LXRs mediate an antiapoptotic effect in macrophages.

The discrepancies observed between the in vivo atherosclerosis models might be due to the animal models used, apoE-deficient versus the LDL receptor-deficient mice. ApoE is present in all lipoproteins except LDL (116). It plays a role in the clearance of remnants and VLDL by the liver. ApoE, which is a target gene of LXR, is also synthesized in macrophages (117, 118). It can potentially serve as an acceptor for cholesterol efflux. Therefore expression of apoE may be required for inhibition of foam cell formation by the PPARα agonists. Another possibility is the type of agonist used and its potency and bioavailability. The fibrates activate PPARα at concentrations in the high micromolar range in vitro and 200–1200 mg/day in the clinical settings, whereas PPARα-selective agonists do so in the low nanomolar range (119). The fibrates used in the reported studies are considered to be very weak activators of PPARα. To determine if fibrates have a similar effect observed in the apoE-deficient mice, LDL receptor-deficient mice will have to be treated with fibrates. It is also possible that the bioavailability of high affinity agonists of PPARα will have an expanded profile of pharmacological activities, compared to fibrates currently in clinical use. The ability of GW7647 to inhibit foam cell formation without affecting cholesterol esterification or apo-AI or HDL-dependent cholesterol efflux suggests that additional PPARα/LXR pathways controlling cholesterol homeostasis remain to be discovered.

As for side effects, fibrates are well tolerated in patients, but can cause gastrointestinal discomfort. In rodents, fibrates induce proliferation of peroxisomes in the liver, which can lead to hepatomegaly and hepatocarcinogenesis with chronic exposure (120), but no such cases were reported in humans. However, fibrates are contraindicated in patients with renal insufficiency, gallstones, abnormal liver function tests, and pregnancy. Fibrates are catabolized primarily in the kidneys. There are a few case reports in which fibrates caused muscle toxicity (myopathy) and inflammation (myositis), which can lead to muscle tissue breakdown (rhabdomyosis) causing muscle weakness and acute kidney failure. This is especially seen with statin/fibrate combination therapy (121). Prueksaritanont et al. (122) demonstrated that fibrates inhibit glucuronidation in dogs, a pathway that is necessary for statin clearance. Clinical studies assessing the safety of the more potent PPARα-selective agonists are currently under way.

PPARγ

PPARγ can be activated by a number of naturally occurring fatty acid metabolites, including 9- and 13-hydroxyoctadecadenoic acids (9- and 13-HODE) and 15 deoxy $\Delta^{2,14}$ prostaglandin J_2 (15dPGJ2) (123–125). Endogenous ligands remain poorly characterized, however, and considerable negative evidence concerning the biological importance of 15dPGJ2 has been reported recently (126). The 12/15 lipoxygenase, which can generate 13-HODE from linoleic acid, has been suggested to mediate formation of PPARγ ligands in specific contexts (127). Synthetic ligands of PPARγ include a new class of insulin-sensitizing drugs, referred to as thiazolidinediones (TZDs) or glitazones, which activate PPARγ and play a critical role in glucose homeostasis (128). Two glitazones are currently available in the United States, rosiglitazone and pioglitazone.

Two isoforms of PPARγ have been identified that are derived from the same gene by alternative promoter usage and differential mRNA splicing (129, 130). PPARγ2 is specifically expressed in adipose tissue and differs from PPARγ1 by the presence of 30 additional N-terminal amino acids at the N-terminal region, which confer a tissue-specific transactivation function. PPARγ1 is the predominant, if not exclusive, PPARγ isoform in all other tissues, including skeletal muscle and liver. PPARγ promotes adipocyte differentiation in vitro and has been shown to be essential for the development of adipose tissue in vivo (131–134).

The presence of PPARγ in adipocytes, skeletal muscle, and liver would suggest crosstalk among these tissues involving lipid metabolism and glucose homeostasis, thereby increasing the overall response to insulin. For instance, several PPARγ polymorphisms in the population can be correlated with the metabolic syndrome (135). Systemic deletion of the PPARγ gene results in embryonic lethality owing to essential roles in adipose, kidney, and placental development (132). The analysis of mice with deletions of PPARγ in specific tissues indicates major roles in controlling insulin resistance in adipose tissue, with contributions also observed in the liver and skeletal muscle (136–139). The mechanisms by which PPARγ influences

insulin action have been intensively studied, and several potentially important targets of regulation have been established. Under noninsulin resistance conditions, insulin reduces hepatic glucose output while at the same time it promotes the uptake of circulating glucose into skeletal muscle and adipocytes. GLUT4, an insulin-dependent glucose transporter, may play a central role in glucose disposal in the peripheral tissues (140). Insulin binding to its tyrosine kinase receptor leads to a cascade of intracellular phosphorylation, including phosphatidylinositol-3-OH kinase [(PI(3)K]) and movement of GLUT4 from the cytoplasm to the cell membrane. Disposal of glucose in the skeletal muscle and adipocytes is also dependent on a number of adipocyte derived factors or adipokines. Insulin sensitivity may be due in part to the balance between impaired insulin effects due to an increase of TNFα and free fatty acids versus insulin sensitizing effects and an increase in adiponectin levels. Activation of PPARγ can improve insulin sensitivity through several pathways: activation of GLUT4 (141); induction of fatty acid release from chylomicrons and VLDL and its uptake through the fatty acid transporter CD36; upregulation of genes involved in intracellular fatty acid transport proteins, fatty acid synthesis, and esterification, such as aP2, acyl-Co-A synthase, and PEPCK (142, 143); inhibition of TNFα (144) and leptin expression (145); and induction of adiponectin expression (146). With respect to adiponectin and atherosclerosis, Ouchi et al. (147) first demonstrated that this adipocyte-derived plasma protein reduced SRA expression in macrophages without altering CD36 expression. Introduction of recombinant adenovirus overexpressing human adiponectin in apoE-deficient mice resulted in a 30% reduction of atherosclerosis after 14 days (148). Furthermore, mice overexpressing adiponectin in an apoE-deficient background had a 50% reduction of atherosclerosis after 12 weeks owing to the decrease of SRA and TNFα expression in atherosclerotic lesions (149). In summary, the effects of the PPARγ agonists on atherosclerosis are both indirect, improving insulin sensitivity, and direct, affecting gene expression in the vessel wall.

In addition to being present in skeletal muscle, PPARγ1 is also upregulated during monocyte-macrophage differentiation and is found in atherosclerotic lesions (74, 150). The role of PPARγ in regulation of inflammation and immunity was initially suggested by the finding that it is expressed in macrophages and inhibits the expression of a number of proinflammatory genes, including TNFα, IL-1β, iNOS, and gelatinase B (74, 151). These findings suggest that PPARγ functions as a negative regulator of macrophage activation and that synthetic PPARγ ligands might exert antiinflammatory and antiatherogenic effects. Stauss et al. and Rossi et al. (152, 153) demonstrated that 15dPGJ2 covalently modified components of the inflammatory pathway, such as IκB-kinase and NFκB subunits.

PPARγ is also expressed in SMCs, endothelial cells, and T lymphocytes. Marx et al. (154) demonstrated that endothelial cells isolated from human saphenous veins responded to PPARγ, but not α agonists, and decreased interferon gamma-induced expression of IP-10, Mig, and I-Tac, thought to play a role in activated T cell recruitment. PPARγ activation has also been shown to inhibit vascular SMC proliferation by arresting the cell cycle through the induction of cyclin-dependent

kinase inhibitor p27 and retinoblastoma phosphorylation (97, 155, 156). There is evidence to suggest that PPARγ can also regulate the renin-angiotensin pathway and vascular remodeling. Sugawara et al. (157) determined that PPARγ agonists inhibited the expression of the angiotensin II, type I receptor (AT1R) in rat vascular SMCs through the inhibition of Sp1 binding to the AT1R's promoter. In transgenic mice overexpressing human renin and angiotensinogen, rosiglitazone decreased systolic and mean blood pressure, but it had no effect in normotensive control mice (158).

PPARγ agonists also regulate gene expression in macrophages. In addition to inflammatory genes, PPARγ agonists regulate both pro- and antiatherogenic genes involved in cholesterol homeostasis (Figure 5). PPARγ was found to stimulate transcription of the *CD36* gene (123, 159). CD36 is a macrophage scavenger receptor that contributes to foam cell formation and development of atherosclerosis in mice (160). In conjunction with the finding that PPARγ can be activated by 9- and 13-HODE present in oxLDL, a PPARγ cycle has been proposed in which oxLDL lipids would induce the activity of PPARγ, leading to increased expression of CD36, which in turn would increase the uptake of oxLDL. This cycle would thus promote foam cell formation and atherosclerosis (159). However, new evidence indicates that activation of PPARγ by TZDs may actually decrease the uptake of modified lipoproteins. Liang et al. (161) recently proposed that the metabolic syndrome increases CD36 protein expression. Using macrophages isolated from *ob/ob* mice, they determined that higher levels of CD36 expression disrupted the insulin-signaling pathway. Furthermore, they observed a higher level of CD36 expression in insulin receptor knockout mice. Ex vivo experiments using peritoneal macrophages isolated from *ob/ob* and *ob/ob*/LDLR$^{-/-}$ mice treated with rosiglitazone indicated a decrease in CD36 protein expression, but not RNA message. This would suggest a posttranslational effect of rosiglitazone on CD36 through the insulin-signaling pathway. They therefore concluded that the antidiabetic effect of the PPARγ agonist is targeted to the reduction of CD36 expression, leading to the decrease rather than an increase uptake of modified lipoproteins and inhibition of atherosclerosis.

Although early clinical studies reported that patients treated with troglitazone demonstrated a decrease in intimal thickening in their carotids (167), it wasn't until animal studies were performed in both the LDL receptor and apoE-deficient mice that it became clear that PPARγ is antiatherogenic, even though it increases expression of CD36 in atherosclerotic lesions (163–165). Interestingly, in our study (163) only male mice treated with the PPARγ agonists demonstrated a reduction in atherosclerosis, which we initially attributed to a decreased expression of TNFα and gelatinase B. We also observed an improvement of insulin sensitivity, which was not dependent on weight reduction, as seen with the PPARα agonist. In conjunction with our initial study, Han et al. (166) demonstrated that rosiglitazone decreased the oxLDL-induced expression of the MCP-1 receptor CCR2 in human and murine monocytes. Curiously, lesion size in female mice was unaffected. However, lipid levels, particularly VLDL, worsened in the drug-treated animals,

and control females did not develop hyperinsulinemia, in contrast to the male control group. This suggests that estrogens may counteract the antiatherogenic effect of the glitazone, but in ovariectomized females, rosiglitazone still had no effect, even though lipid levels no longer worsened and the animals became more insulin sensitive (A.C. Li, unpublished results). Determining whether this observation is species specific (mice versus humans) or inherent to the particular animal model (LDL receptor–deficient mice versus apoE-deficient mice) requires more study.

With respect to cholesterol efflux pathways, PPARγ was shown to induce expression of LXRα and thereby to stimulate ABCA1-dependent cholesterol efflux to apo-AI in vitro (94, 167) (Figure 5), analogous to the PPARα/LXRα/ABCA1 pathway described above. PPARγ can also induce Cyp27 expression, which can lead to the generation of 27-hydroxycholesterol and activate the LXR/ABCA1 pathway (168). Cyp27 may thus function as an integrator of the PPAR/LXR cholesterol efflux pathway in macrophages by generating ligands that activate LXRs. Although we were able to induce cholesterol efflux by ABCA1 in cholesterol-loaded murine peritoneal macrophages by the PPARγ agonist in vitro, we were unable to detect induction of ABCA1 in atherosclerotic lesions and peritoneal macrophage/foam cells isolated from LDL receptor–deficient mice treated with either the PPARα, γ, or δ agonists (114). Furthermore, unlike LXR agonists, PPARs were unable to induce ABCA1 expression in peritoneal macrophages that were not loaded with cholesterol, suggesting that induction of ABCA1 is a two-step process. First, PPARγ agonists increase the expression of LXRs, but activation of LXRs and induction of ABCA1 require oxysterols. When we compared macrophages from normo- and hyperlipdemic mice, we found message and protein expression of ABCA1 highly induced in untreated hyperlipidemic mice. However, in peritoneal macrophage/foam cells isolated from hyperlipidemic mice treated with rosiglitazone, ABCA1 expression did not further increase. We therefore postulated that under conditions of extreme hyperlipidemia, ABCA1 is already maximally upregulated and that further induction of LXRα expression would be ineffective. Another possibility for the ineffectiveness of the PPARγ agonist to increase ABCA1 expression in vivo is the presence of unsaturated fatty acids in macrophage/foam cells. Wang et al. (169) demonstrated that polyunsaturated fatty acids, such as linoleic acid, increase proteolytic degradation of ABCA1 protein levels but not mRNA levels. They also demonstrated that stearyl-CoA desaturase (SCD) can desaturate saturated fatty acids, such as palmitate and stearate, into monounsaturated fatty acids, which can also degrade ABCA1 (170). Interestingly, endogenous LXR and RXR ligands induced the expression of SCD. They hypothesized that under these conditions, a vicious cycle is created whereby ABCA1 is generated and degraded at the same time. Wang et al. further demonstrated that the addition of troglitazone with palmitate or stearate prevented the degradation of ABCA1. On the other hand, when troglitazone was added to cultured murine J774 macrophages in the presence of linoleic acid, ABCA1 degradation still occurred. They concluded that PPARγ-selective agonists prevent ABCA1 destabilization through the inhibition of the SCD pathway. Contrary to these results, Joseph et al. did see a decrease

in atherosclerosis associated with an increase of ABCA1 expression in lesions in LDL receptor– and apoE-deficient mice, using a synthetic LXR agonist. Yet, these animals did not show any evidence of sustained levels of hyperinsulinemia, hypertriglyceridemia, or elevated levels of free fatty acids that can potentially destabilize ABCA1 (51). In contrast, LDL receptor-deficient mice in our study developed hyperinsulinemia and hypertriglyceridemia associated with a significant increase in weight gain when fed a high-cholesterol/fat diet (163). However, despite the PPARγ agonists' ability to improve insulin levels in these mice, VLDL and free fatty acid levels did not decrease, which may explain why we did not see an increase in ABCA1 expression in both atherosclerotic lesions and peritoneal macrophage/foam cells in response to rosiglitazone (114).

It is also plausible that the increase in fatty acid accumulation in the macrophage/foam cell can also increase the rate of cholesterol esterification, which then reduces cholesterol efflux. We demonstrated that esterification was inhibited only by the PPARγ agonist to an extent comparable to that achieved by an ACAT1 inhibitor (114). This result was seen in LXR-wild-type and LXR-null peritoneal macrophages. Although we were unable to detect changes in ACAT1 gene expression, it does not rule out the possibility that PPARγ may play a role in post-translational modification or by exerting its effect on genes involved in cholesterol trafficking or increasing nCEH expression/activity. Most importantly, however, we identified the existence of an alternate LXR/ABCA1 independent pathway. Given that free cholesterol accumulated in the macrophages and ABCA1 expression did not change, another mechanism for cholesterol efflux must account for the observed decrease in foam cell formation. Using real-time PCR, we measured ABCG1 gene expression and found it increased in peritoneal macrophage/foam cells and atherosclerotic lesions isolated from drug-treated animals. Recent results suggest that ABCG1 may also play a role in cholesterol efflux from macrophages. By using antisense oligonucleotides against ABCG1, Klucken et al. (171) showed that HDL_3-dependent efflux of cholesterol decreased. Interestingly, Lorkowski et al. (172) found that despite a significant decrease in ABCA1 expression in Tangier's patients, ABCG1 expression increased, perhaps as a compensatory response to the defective *ABCA1* gene. Venkateswaran et al. (173) demonstrated that the murine homolog of ABCG1, ABC8, is present in macrophages and responds to LXR ligands. More recently, Wang and Nakamura (174, 175) both demonstrated, with the use of siRNAs and overexpression, that ABCG1 and ABCG4 are involved in cholesterol efflux to HDL subclasses but not to lipid-poor apo-A1. Welch et al. (176) demonstrated in PPARγ-null macrophages that ABCG1 is also a direct target gene of PPARγ. When we treated LXR-null peritoneal macrophages with a PPARγ agonist, ABCG1 but not ABCA1 expression increased (114). The increase in ABCG1 may be responsible for the enhanced HDL-specific cholesterol efflux that we observed. Kennedy et al. (177) developed mice that were either ABCG1-deficient or overexpressed human ABCG1. When they fed their animal models with a high-cholesterol/fat diet, they found a massive increase in lipid accumulation in hepatocytes and macrophages isolated from ABCG1-deficient mice

but not in tissues isolated from mice overexpressing ABCG1. Clearly, ABCA1 and ABCG1 have a profound impact on cholesterol efflux in macrophage/foam cells. However, to conclusively determine if PPARγ agonists inhibit atherosclerosis through the upregulation of ABCG1 rather than ABCA1, one would need to perform bone marrow transplants of ABCA1 and ABCG1-null cells into LDL receptor–deficient mice.

Recent clinical studies also support that PPARγ agonists are beneficial. Treatment of diabetic patients with glitazones reduced serum levels of C-reactive protein, gelatinase B/MMP-9, and soluble CD40 ligand (sCD40L) (178–180). In a Japanese study, non-diabetic patients treated for six months with troglitazone after stent placement had a significantly reduced narrowing of the coronary lumen, compared with placebo treated patients (181). Additionally, with both PPARα and γ agonists possessing antiatherogenic as well as antidiabetic properties, investigators are currently looking into the development of coagonists to further enhance the antiatherogenic and antidiabetic properties. Recent animal studies using coagonists demonstrate an improvement in insulin sensitivity as well as fatty acid, glucose, and lipoprotein metabolism (182, 183). Claudel et al. (112) demonstrated that a PPARα/γ coagonist, GW2331, decreased atherosclerosis by 32% in apoE-deficient mice. Zuckerman et al. (184) demonstrated a 2.5-fold reduction of atherosclerosis by en face analysis using another PPARα/γ coagonist, and attributed this to the inhibition of the interferon gamma induction of nitric oxide synthesis and beta 2 integrin CD11a expression. Finally, another PPARα/γ coagonist, tesaglitazar, is currently undergoing phase III clinical trials (185). These studies in animal models and humans are intriguing, but larger clinical trials are needed to determine if glitazones have an impact on CAD and reduce cardiovascular morbidity and mortality.

Although glitazones may seem the panacea for the treatment of diabetes, atherosclerosis, and other chronic inflammatory processes, there are side effects that may limit their use. Troglitazone, the first TZD to enter the U.S. market in 1997, was removed by the FDA in 2000 owing to severe liver toxicity leading to liver failure and death. At that time, there were more than 130 reported cases worldwide of severe hepatic failure leading to liver transplantation and six deaths attributed to the drug (186). In controlled trials, it was observed that other glitazones, such as rosiglitazone and pioglitazone, increased liver enzyme levels, particularly alanine aminotransferase (ALT), to the upper limit of normal, whereas troglitazone increased ALT levels threefold above the upper limits of normal. It is thought that the α-tocopherol moiety and drug interactions with the cytochrome P450 isoform 3A4, which is partly responsible for its TZD elimination, may be playing a role in its hepatotoxicity.

Another side effect of glitazones is fatty infiltration in the bone marrow. Bone marrow–derived stromal cells lead to decreased bone volume and increased fat content volume in mice (187). This was attributed to the changes in the expression of genes in osteoblasts, such as reduction of Runx2/Cbfa1, Dlx5, and α1(I) collagen, and an induction of aP2 expression in adipocytes. We also observed this effect

in our initial intervention study, and this was associated with an increase in spleen size and disruption of white and red pulps' architecture without fatty infiltration (163).

Finally, major concerns of using glitazones are increasing visceral adiposity, fluid retention, and hemodilution, which can lead to congestive heart failure (188). It was originally hypothesized that glitazones increase plasma vascular endothelial growth factor (VEGF) levels, which in turn increases vascular permeability. More recently, it was determined in normotensive rats that rosiglitazone activated genes involved in the sodium and water reabsorption in the kidneys in response to a drop in mean arterial blood pressure (MAP) (189). Song et al. found that high doses of rosiglitazone decreased urine volume by 22%, sodium excretion by 44%, creatinine clearance by 35%, and kidney weight by 9% without a decreasing water intake. The authors attributed the decrease in the glomerular filtration rate to increased protein expression of α-1 subunit of Na-K-ATPase, bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2), sodium hydrogen exchanger (NHE3), aquaporins 2 and 3, and endothelial nitric oxide synthase.

PPARδ

PPARδ was the last of the PPARs to be identified (190) and has been shown to play a role in epidermal maturation (191, 192). PPARδ also appears to play important roles in lipid and energy homeostasis. Targeted activation of PPARδ in adipose tissue led to an improvement of lipid profiles and a reduction of adiposity, whereas PPARδ-null mice showed a reduction in energy uncoupling and obesity (193, 194). However, its role in atherosclerosis is unclear at present. In vitro studies demonstrated that a PPARδ-specific agonist, GW501516, enhanced reverse cholesterol transport in a human macrophage cell line (THP-1), skin fibroblasts (1BR3N), and intestinal cells (FHS74), and administration of this compound to obese, insulin-resistant rhesus monkeys led to normalization of plasma HDL levels (195). On the other hand, a different PPARδ agonist promoted lipid accumulation in THP-1 cells and primary human macrophages (196). Chawla et al. hypothesized that PPARδ acts as a lipid sensor, where fatty acids derived from VLDL can activate PPARδ, which in turn can increase the expression of the adipophilin related protein (ADRP) and store triglycerides in macrophages and possibly promote atherosclerosis (197). In addition to its role in lipid metabolism, it appears that PPARδ also has antiinflammatory properties. Rival et al. (198) determined that treatment of endothelial cells with a PPARδ agonist decreases VCAM-1 and MCP-1 expression. In addition, Welch et al. (176) demonstrated that a PPARδ agonist inhibited LPS-inducible genes, such as COX-2 and iNOS, in murine peritoneal macrophages.

Three studies have evaluated the consequences of PPARδ activation on development of atherosclerosis in mice. Transplantation of PPARδ-null bone marrow progenitor cells into LDL receptor–deficient mice resulted in less atherosclerosis than in LDL receptor–deficient mice transplanted with wild-type progenitor cells,

suggesting that PPARδ is proatherogenic (199). Lee and colleagues further postulated that PPARδ acts as a molecular switch between proinflammatory and antiinflammatory activities and is dependent on the presence of the agonist and binding of transcriptional repressors such as BCL-6 (Figure 6). In the absence of its agonist, PPARδ binds to BCL-6, which in turn is unable to inhibit MCP-1 expression, but in the presence of the agonist, BCL-6 is released and blocks MCP-1 expression. Therefore, in the absence of PPARδ, BCL-6 would be free

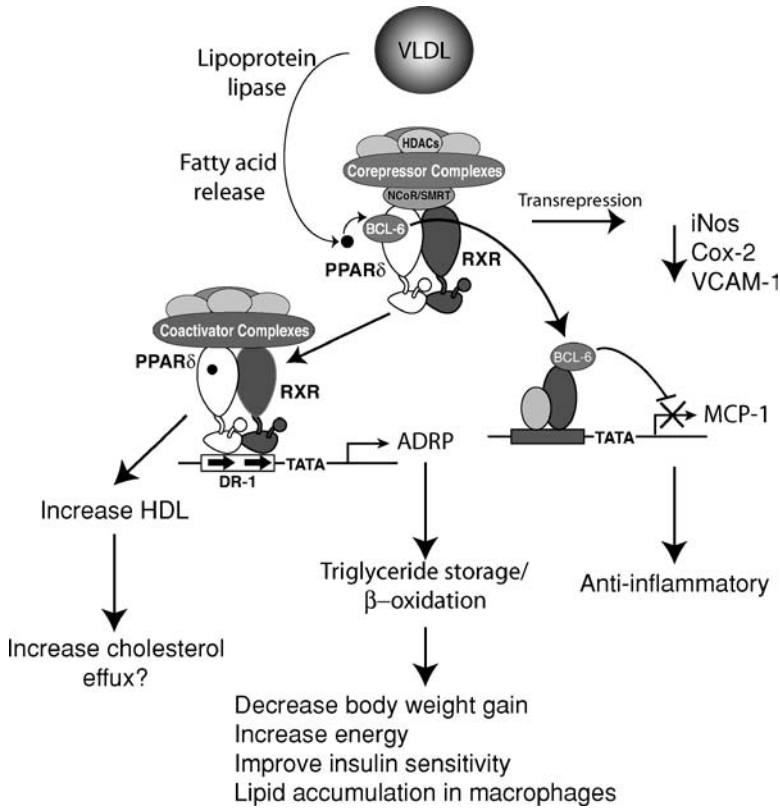


Figure 6 Modulation of lipid metabolism and inflammatory genes by PPARδ. Like PPARα, fatty acids from VLDL can also activate PPARδ. When activated, PPARδ has been demonstrated to increase HDL and cholesterol efflux and to activate genes involved in triglyceride storage and β-oxidation. This can lead to an increase in energy, improvement in insulin sensitivity, and lipid accumulation in macrophages. PPARδ can also inhibit genes involved in the inflammatory process, either recruiting corepressors or causing the displacement of BCL-6 from the activated PPARδ/RXR complex, which would in turn bind to an activator complex on the MCP-1 promoter and prevent its activation, and thus possibly inhibit atherosclerosis.

to inhibit MCP-1 expression. Knocking out the *MCP-1* gene has been shown to inhibit atherosclerosis in hypercholesterolemic mice overexpressing human apolipoprotein B (200). Interestingly, overexpression of BCL-6 did not inhibit the regulation of ADRP by PPARδ. In our experiments, we found that in hyperlipidemic LDL receptor–deficient mice, the PPARδ agonist GW7842 did not alter the progression of atherosclerosis, compared with untreated mice, despite a decrease in inflammatory cytokines within atherosclerotic lesions (114). Furthermore, the agonist did not have an effect on insulin sensitivity or on cholesterol efflux, but may increase modified LDL uptake and esterification. Although activation of PPARδ proved to be ineffective under high levels of hyperlipidemia, we hypothesized that under moderate levels of hypercholesterolemia, the PPARδ agonist might inhibit atherosclerosis solely by its antiinflammatory properties. In a recent paper by Graham et al. (201), they performed a study using a diet that induced cholesterol levels to only 25 mmol/l (970 mg/dl) in LDLR$^{-/-}$ female mice. They measured the extent of atherosclerosis at two different time points and treated the animals at two different drug concentrations. The serum drug level was 0.805–1.25 μM for the low dose (6 mg/kg/day); however, at the higher dose (60 mg/kg/day), the drug level was high enough (13.99–21.15 μM) to activate both murine PPARα and γ. At 10 weeks of diet and drug therapy, only the high dose significantly decreased atherosclerosis by 55%; however, at 16 weeks the reduction of atherosclerosis was less pronounced, 28%, whereas the low dose significantly reduced atherosclerosis by 33%. Furthermore, at 6 mg/kg/day, total cholesterol and VLDL levels decreased significantly, whereas the higher dosage had no effect. The authors also examined gene expression and found a decrease in MCP-1 and ICAM-1 expression in atherosclerotic lesions and a decrease of TNFα expression in macrophages. In summary, these results further support our hypothesis that although PPARδ agonists may be effective in patients who have mild to moderate levels of hypercholesterolemia, it may not be as effective as the PPARα- and γ-selective agonists. This agonist could be used in conjunction with statins. However, it may be contraindicated because the agonist that is metabolized in the liver increased liver weight by 20% at 6 mg/kg/day and nearly twofold at 60 mg/kg/day.

CONCLUSIONS

PPARs may influence atherogenesis in many complex ways because they can activate genes that are both pro- and antiatherogenic. Table 1 summarizes the effects of the PPAR-selective agonists on two atherosclerosis models, the apoE- and LDL receptor–deficient mice (114). Interestingly, not all three PPAR-selective agonists had the same effect on atherosclerosis, and even the PPARα-selective agonist gave conflicting results in the two models. One can conclude that even though the PPARs have the same potential to bind to the same DR-1 element on a specific gene, not all three PPARs can regulate the same gene. This may suggest the presence of PPAR-specific coactivators, which adds an additional step

TABLE 1 Summary of the effects of the PPAR-selective agonists on murine models of atherosclerosis

PPAR	Agonist	Animal model	Lesion size	Reference
Alpha	None	PPAR$\alpha^{-/-}$ × apoE$^{-/-}$ male and female mice, western-style diet	Decreased	(110)
	Fenofibrate	ApoE$^{-/-}$ female mice, normal chow	No change	(112)
	Fenofibrate	ApoE$^{-/-}$ female mice, western-style diet	Slightly decreased	(113)
		ApoE$^{-/-}$ × apoA1 transgenic female mice, western-style diet	Decreased	(113)
	Ciprofibrate	ApoE$^{-/-}$ male and female mice, normal chow	Increased	(111)
	GW7647	LDLR$^{-/-}$ male mice, western-style diet	Decreased	(114)
Gamma	Rosiglitazone and GW7845	LDLR$^{-/-}$ male mice, western-style diet	Decreased	(163)
		LDLR$^{-/-}$ female mice, western-style diet	No change	
	Troglitazone	LDLR$^{-/-}$ male mice, western-style and high-fructose diets	Decreased	(164)
	Troglitazone	ApoE$^{-/-}$ male mice, western-style diet	Decreased	(165)
	None	Bone marrow transplant: PPAR$\gamma^{-/-}$ cells → LDLR$^{-/-}$ male mice, western-style diet	Increased	(166)
	Rosiglitazone	ApoE$^{-/-}$ female mice, normal chow	Decreased	(112)
Delta	None	Bone marrow transplant: PPAR$\delta^{-/-}$ cells → LDLR$^{-/-}$ male mice, western-style diet	Decreased	(199)
	GW0742	LDLR$^{-/-}$ male mice, western-style diet	No change	(114)
	GW0742	LDLR$^{-/-}$ female mice, low cholesterol diet	Decreased	(201)
Alpha/ gamma	GW2331	ApoE$^{-/-}$ female mice, normal chow	Decreased	(112)
	LY465608	ApoE$^{-/-}$ male mice, normal chow	Decreased	(184)

in gene regulation. On the other hand, transrepression of certain genes involved in inflammation is similar for all three PPAR-selective agonists, suggesting similar pathways or recruitment of corepressors.

Other differences between PPARs are seen in specific pathways involved in atherogenesis (Table 2). We have identified an LXR/ABCA1-independent pathway

TABLE 2 Summary of the effects of the PPAR-selective agonists on atherogenesis in LDL receptor-deficient male mice*

Effect on	PPARα	PPARγ	PPARδ
Atherosclerosis	↓	↓	—
Body weight	↓	—	—
Insulin levels	↓	↓	—
Cholesterol levels	—	—	—
CD36 expression	[a]—	[a]↑↑	[a]↑
Inflammation	[b]↓	[b]↓	[b]↓
Foam cell formation	[a]↓	[a]↓	[a]—
ABCA1 expression	[a]—	[a]—	[a]—
ABCG1 expression	[a]—	[a]↑	[a]—
Cholesterol esterification	—	↓	—
LXR required	Yes	No	Unknown

[a]In macrophage foam cells derived from hypercholesterolemic mice.

[b]In atherosclerotic lesions.

*Table 2 is reprinted from Li et al. (114) with permission from The American Society for Clinical Investigation.

by which PPARγ may regulate foam cell formation and also found that PPARγ regulates cholesterol esterification. It is unclear whether ACAT1 is a direct target gene, or whether PPARγ regulates genes involved in cholesterol trafficking within the cell that are either upstream or downstream of ACAT1. PPAR-selective agonists may also regulate different genes in humans and mice, e.g., apoA-I and ABCA1. Although we were unable to detect upregulation of ABCA1 by the PPAR-agonists in vivo, it is possible that in earlier lesions and under milder levels of hypercholesterolemia PPARs may influence ABCA1 gene expression in mice. Another potential difference concerns the expression of the PPARs in macrophages, especially PPARα. In human primary monocyte-derived macrophages from hyperglycemic patients, the expression of PPARα and δ was higher, whereas PPARγ was downregulated (89). In murine peritoneal macrophage/foam cells, we found PPARδ was higher, followed by γ, and α was barely detectable (114). Although studies were done in vitro in primary human and murine macrophages, further evidence to document that PPARα and gamma-selective agonists exert their antiatherogenic effects through ABCA1 is needed, such as measuring the expression of ABCA1 in atherectomy specimens isolated from patients treated with PPARγ or α agonists.

Rapid progress continues to be made with respect to defining the biological roles and mechanisms of action of PPARs. Surprises continue to emerge, such as the identification of new pathways for control of cholesterol homeostasis in macrophage foam cells. Emerging evidence from animal models supports the concept that PPARα and PPARγ not only act to control lipid and glucose at a systemic

level but also have important actions within cells that determine the development and clinical course of atherosclerosis. The potential use of PPARs agonists (used singly or as coagonists) alone or in combination with statins in the prevention of atherosclerosis will continue to be an important area for clinical investigation, and clinical studies are currently under way to determine their potential in reducing the risk of CAD. Potential areas for drug development include identifying PPAR-specific coactivators and the development of selective PPAR modulators (SPPARMs), analogous to the concept and development of selective estrogen receptor modulators (SERMs). The SPPARMs should only activate antiatherogenic and antidiabetic pathways and not pathways that increase CD36 expression, visceral obesity, fluid retention, and fatty infiltration in the bone marrow. For instance, Pascual et al. (202) recently demonstrated that transrepression of inflammatory genes by PPARγ is dependent on sumolyation of a specific lysine residue within the PPARγ ligand-binding domain, which prevents the recruitment of the ubiquitylation/19S proteosome machinery and removal of the corepressor complex from an inflammatory target gene. They found that the PPARγ antagonist, GW0072, continued to exert its transrepressive effect on inflammatory genes without activating PPARγ target genes, such as CD36. This is in agreement with other laboratories in which this compound still demonstrated its antidiabetic effect but prevented adipocyte differentiation (203). These results clearly have important therapeutic implications and open up an exciting new area in PPAR research.

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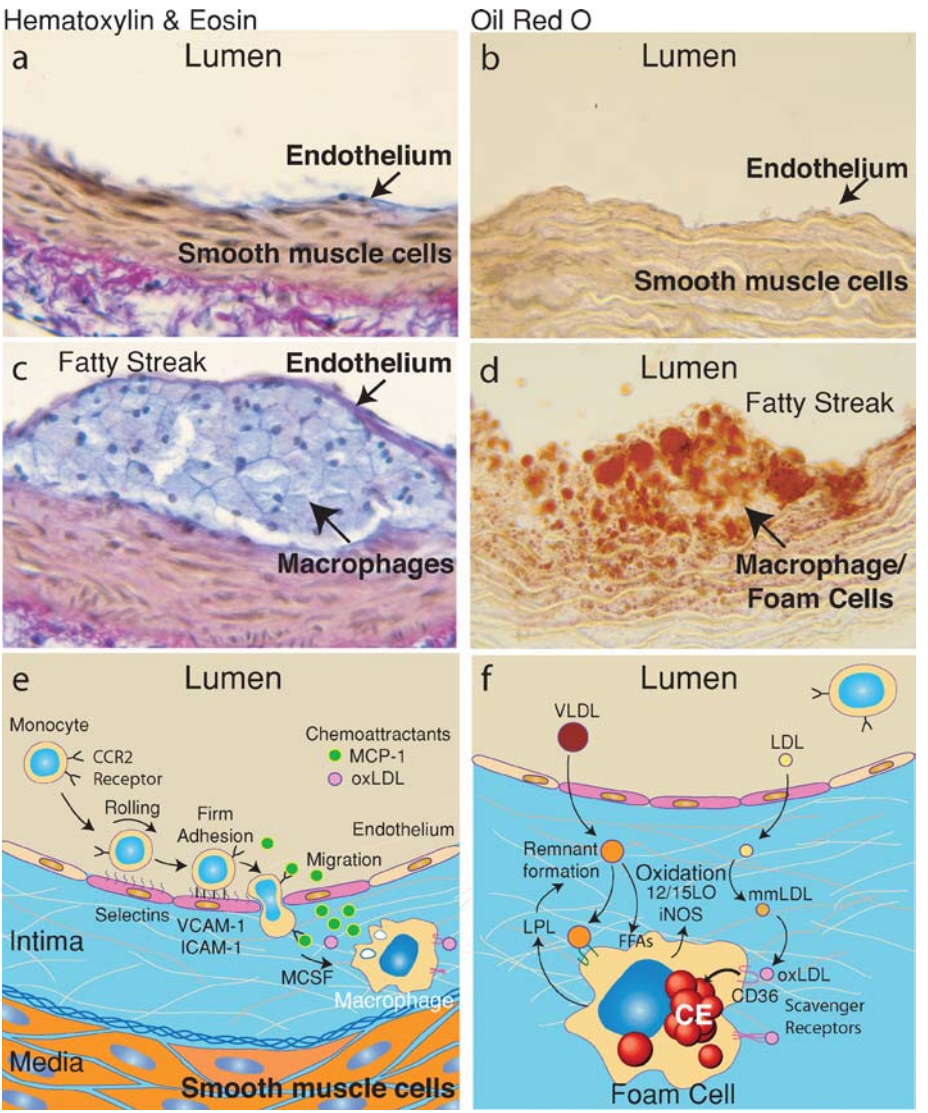

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Figure 1 Development of the atherosclerotic lesion. Atherogenesis is a chronic inflammatory process. (*a*) The normal arterial wall, in this example the aorta of LDL receptor-deficient mice, consists of the intima (a single layer of endothelial cells attached to an internal elastic lamina), followed by the media (layers of smooth muscle cells) and adventitia. (*b*) Very few macrophages and oil-red O-positive lipid infiltrations are found in the normal intima. (*c*) The earliest stage of atherosclerotic lesion, the fatty streak, is dominated by subendothelial macrophage/foam cell accumulation. (*d*) These macrophages contain massive amounts of lipids. (*e*) Under conditions of hypercholesterolemia, LDL begins to accumulate in the intima and is progressively oxidized by endothelial and other arterial cells. Endothelial cells also become activated and increase the expression of selectins and adhesion molecules, such as VCAM-1 and ICAM-1, on their surfaces. Chemoattractants in the subendothelial space, such as oxLDL and MCP-1, attract circulating monocytes to the endothelial surface, where they become attached via selectins and adhesion molecules. CCR2, the receptor for MCP-1, is upregulated in circulating monocytes and further increases their rate of recruitment. Once attached to the endothelial surface, monocytes transmigrate between intact endothelial cells and take up residence in the extracellular space where they are phenotypically transformed into macrophages and begin producing enzymes that oxidatively modify LDL, such as 12/15 LO and iNOS. (*f*) Oxidized LDL is rapidly taken up by scavenger receptors, such as CD36. The rapid accumulation of cholesteryl esters (CE) results in foam cell formation. Endothelial cells as well as foam cells can secrete LPL, which can increase the uptake of FFAs. The uptake of FFAs and oxLDL in macrophage/foam cells provide a source of lipids that can activate the PPARs. Remnants from triglyceride-rich lipoproteins (except chylomicrons) can also accumulate in the vessel. Original magnification of microphotographs is 40x. Figures 1*b* and *d* are reprinted from Li & Glass (20) with permission from Nature Publishing group. Figure 1*e* is reprinted from Glass & Witztum (15) with permission from Elsevier.

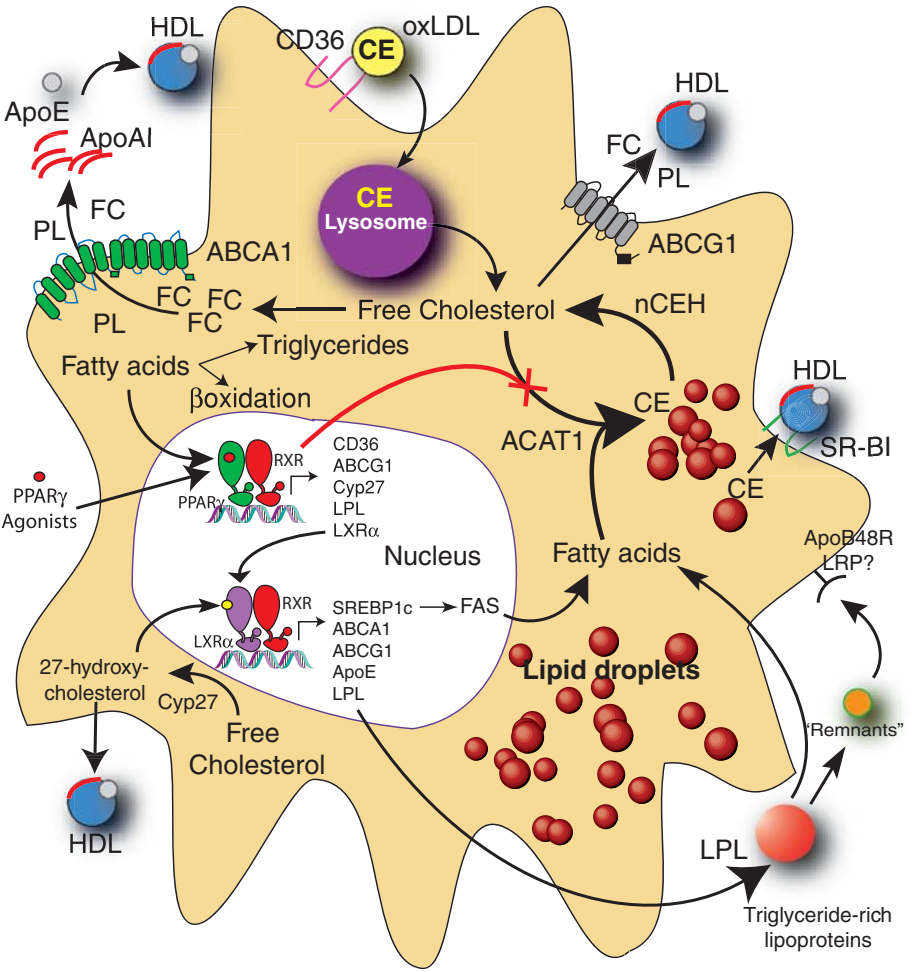

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Figure 5 The coordinated regulation of lipid metabolism in macrophages by PPARγ and LXRα. Cholesterol is taken up through scavenger receptors, such as CD36, that recognize oxLDL. Remnants of triglyceride-rich lipoproteins can also be taken up by macrophages through either the apolipoprotein B48 receptor or the LDL receptor-related protein (LRP) and contribute to foam cell formation. The components of the oxLDL are degraded within lysosomes and the cholesterol is released. The free cholesterol (FC) is reesterified and stored as cholesteryl esters (CE). The esterification is done by ACAT1 located in the endoplasmic reticulum. It is possible that cholesteryl esters can leave the foam cell through SR-BI and bind to HDL. HDL may deliver cholesterol to the liver where it is converted and secreted into bile. Alternatively, nCEH can release the cholesterol from the ester form. The FC can leave the macrophage/foam cell through several reverse cholesterol transport pathways: In the presence of a cholesterol acceptor, such as apo-A1 or HDL, the free cholesterols along with phospholipids (PL) can leave through ATP binding cassette transporters, such as ABCA1 and ABCG1. Cyp27 can also hydroxylate FC into oxysterols. The oxysterols either diffuse across the cell membrane or activate LXRs, which may in turn increase the expression of ABCs and apoE. PPARγ can influence different pathways involved in promoting and inhibiting foam cell formation. First, in the presence of endogenous ligands or glitazones, PPARγ can upregulate the uptake of oxLDL by increasing CD36 expression. Additionally, components of oxLDL, such as 9- and 13-HODE, can further activate PPARγ, thus generating the PPARγ cycle, which can further increase oxLDL uptake and accelerate foam cell formation. Second, PPARγ can increase the expression of LXRs. When activated by oxysterols, the LXRs increase the following: cholesterol efflux through ABCA1, ABCG1, and apoE synthesis; fatty acid synthesis through upregulation of SREBP1c and fatty acid synthase (FAS); and fatty acid uptake through the upregulation of LPL expression/activity, which can then metabolize triglyceride-rich lipoproteins, such as VLDL and chylomicrons. The free fatty acids released can be used for β-oxidation in the mitochondria, stored as triglycerides, or used for cholesterol esterification. PPARγ can also regulate the expression of Cyp27. Finally, PPARγ can decrease cholesterol esterification and induce HDL-specific cholesterol efflux by increasing the expression of ABCG1, both in an LXR-independent manner.

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