PPAR and LXR-dependent pathways controlling lipid metabolism and development of atherosclerosis

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Abstract

The nuclear receptor superfamily is comprised of transcription factors that positively and negatively regulate gene expression in response to the binding of a diverse array of lipid-derived hormones and metabolites. Intense efforts are currently being directed at defining the biological roles and mechanisms of action Liver X receptors (LXRs) and Peroxisome Proliferator-Activated Receptors (PPARs). LXRs have been found to play essential roles in the regulation of whole body cholesterol absorption and excretion, in the efflux of cholesterol from peripheral cells, and in the biosynthesis and metabolism of very low density lipoproteins. PPARs have been found to regulate diverse aspects of lipid metabolism, including fatty acid oxidation, fat cell development, lipoprotein metabolism and glucose homeostasis. Intervention studies indicate that activation of PPAR\(\alpha\), PPAR\(\gamma\) and LXRs by specific synthetic ligands can inhibit the development of atherosclerosis in animal models. Here we review recent studies that provide new insights into the mechanisms by which these subclasses of nuclear receptors act to systemically influence lipid and glucose metabolism and regulate gene expression within the artery wall.

Introduction

On June 1, 1889, 72 year old Charles-Edouard Brown-Sequard reported to the Societe de Biologie of Paris that he had injected himself with aqueous extracts of guinea pig and dog testes and within a short period of time experienced a remarkable enhancement in physical strength, intellectual capacity, and sexual potency (1). Brown-Sequard’s self-experimentation was based on the then novel hypothesis that the testes were the source of a substance that was released into the circulation and exerted masculinizing effects on other tissues in the body. Subsequent investigation indicated that testicular extracts contain little or no active androgen and Brown-
Sequard’s ‘rejuvination’ is now considered to be a well-documented example of a placebo effect. Nevertheless, his concept of a chemical messenger was correct and greatly influenced studies of hormone-producing tissues and translational research. By 1891, Murray had successfully treated a hypothyroid patient with extracts made from the thyroid glands of sheep using Brown-Sequard’s methods, representing the first example of successful hormone replacement therapy (1). Thereafter, steady progress was made in the identification and characterization of the classical steroid and thyroid hormones, which were subsequently shown to have their activity by binding to and activating so called nuclear receptors that exerted their biological effects by regulating gene transcription. Nearly 100 years after Brown-Sequard’s experiments, cDNAs encoding the glucocorticoid receptor (GR), estrogen receptor (ER) and thyroid hormone (TR) receptors were cloned, leading to the recognition of a superfamily of nuclear receptors that responded not only to steroid hormones, but other classes of lipid-derived mediators that function to regulate development and homeostasis (2). Here, we review recent findings that provide new insights into roles of nuclear receptors in the control of lipid metabolism and atherosclerosis, focusing on liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs). While drugs that regulate LXRs and PPARs may not have the rejuvenating properties sought by Brown-Sequard, they do hold promise for new approaches for prevention of atherosclerosis, the leading cause of age-related morbidity and mortality in Western societies (3).

Pathogenesis of Atherosclerosis

Atherosclerosis has its origins in pathogenic interactions between circulating lipoproteins, hemodynamic factors, the arterial wall, and the immune system. The earliest visible lesion is the “fatty streak,” consisting predominately of monocyte-derived macrophages engorged with
lipoprotein-derived cholesterol (reviewed in (4)). The formation of fatty streaks is initiated by the adherence of circulating monocytes to activated endothelial cells at lesion-prone sites within large arteries (reviewed in (5)). Adherent monocytes subsequently migrate into the subendothelial space in response to locally produced chemo-attractant molecules, where they further differentiate into macrophages. This program of differentiation includes up-regulation of so-called “scavenger” receptors that normally function in the recognition and internalization of pathogens and apoptotic cells (6). However, scavenger receptors also recognize altered molecular patterns present on modified forms of LDL, such as oxidized (oxLDL), and mediate the massive accumulation of cholesterol characteristic of macrophage foam cells (7, 8). Macrophages in turn contribute to the formation of oxLDL, which can stimulate the expression of adhesion molecules on endothelial cells, such as VCAM-1 and promote secretion of chemotactic factors such as macrophage chemotactic protein 1 (MCP-1), leading to recruitment of additional circulating monocytes (7).

Fatty streak formation may occur transiently during fetal development, has been observed in children, and becomes common in adolescence and early adulthood in Westernized societies (9). Although not clinically significant in themselves, fatty streaks can evolve into more complex lesions. Lesion progression involves the influx of T-cells, which elaborate cytokines that influence the functional properties of nearby endothelial cells, macrophages and smooth muscle cells (10, 11). Smooth muscle cells migrate from the media into the intima, where they accumulate cholesterol and become smooth muscle cell-derived foam cells. The death of lipid-laden foam cells leads to the formation of a necrotic, cholesterol-rich core that becomes walled off by a fibrous cap of extracellular matrix proteins secreted by smooth muscle cells. The rupture of an advanced lesion can lead to thrombus formation that occludes the vessel lumen and
results in acute myocardial infarction. Several lines of evidence, including epidemiological studies in humans, strongly suggest that local inflammation contributes to the vulnerability of lesions to rupture (reviewed in (11)).

Risk factors such as hypercholesterolemia, smoking, genetic factors and male gender accelerate atherosclerosis (12). In addition, the growing incidence of obesity is driving an epidemic of a constellation of metabolic abnormalities, collectively referred to as the metabolic syndrome, that includes insulin resistance, hypertension, low HDL and hypertriglyceridemia. Each of these abnormalities also contributes to risk of atherosclerosis (13). Intriguingly, PPARs, LXR and other nuclear receptors not only influence lipid metabolism at a systemic level but also regulate lipid homeostasis and inflammation in macrophages, endothelial cells and smooth muscle cells within the artery wall. Drug therapy targeted at these receptors may therefore act at several levels to influence the development of atherosclerosis. Activation of PPARs and LXR by natural and synthetic ligands leads to multiple changes in gene expression, some of which are predicted to be atherogenic, and others anti-atherogenic. A major goal of investigation in the PPAR and LXR fields is to define the biological activities of each receptor subtype and the molecular mechanisms underlying their activities. Based on lessons from studies of steroid hormone receptors (14), this knowledge is likely to facilitate the development of selective PPAR and LXR modulators that exhibit novel and improved pharmaceutical profiles.

**PPARs and LXR – structure and function**

The PPAR and LXR subfamilies together account for five of the forty-eight nuclear receptors that have been identified in the human and mouse genomes. PPARs and LXR possess both the conserved DNA-binding and ligand binding domains that are characteristic of nuclear
receptors (Figure 1). The central DNA binding domain (DBD) consists of two zinc finger motifs that mediate sequence-specific recognition of hormone response elements in direct target genes. PPARs and LXRs bind to specific DNA response elements as heterodimers with retinoid X receptors (RXRs). The C-terminal ligand-binding domain (LBD) determines the specific ligand binding properties of each receptor and mediates ligand-regulated interactions with other proteins that act as effectors of transcriptional activation and/or repression. In contrast to steroid hormones that bind their respective receptors with high affinity (i.e., binding constants in the nanomolar range), natural ligands for PPARs and LXRs appear to consist of fatty acid and cholesterol metabolites, respectively, that bind with relatively low affinities (i.e., binding constants in the micromolar range) (15-17). These binding constants are in accordance with what are thought to be ambient concentrations of these metabolites within cells.

Ligand-induced allosteric changes in the LBD regulate interactions with co-activator and corepressor complexes that mediate the transcriptional activities of nuclear receptors (18, 19). PPARs and LXRs regulate gene expression through at least three distinct types of transcriptional activities (Figure 1). First, LXR/RXR and PPAR/RXR heterodimers can bind to specific response elements in target genes in the absence of ligands and actively repress transcription through interactions with corepressor complexes that contain the nuclear receptor co-repressors NCoR and/or SMRT (20-23). Second, PPARs and LXRs bind to hormone response elements in the presence of ligands and activate transcription (Figure 1). Transcriptional activation is linked to ligand-dependent allosteric changes in the LBD that lead to the recruitment of coactivator proteins (24). A large number of coactivator proteins have been identified that contribute to nuclear receptor function (18, 19). Many of these proteins are components of large multi-protein complexes with associated enzymatic activities, including
nucleosome remodeling activities, histone acetyltransferase activities, histone methyltransferase activities, and/or have the ability to recruit core transcription factors. The transition from active repression to ligand-dependent transcriptional activation requires both dissociation of copressors and recruitment of coactivators. Recent studies suggest that ligand-dependent corepressor-coactivator exchange requires ubiquitinylation machinery that targets the corepressor complex for proteosome-dependent destruction (25). Third, several members of the nuclear receptor family 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 1). This activity, referred to as transrepression, is thought to underlie anti-inflammatory actions of nuclear receptors such as the glucocorticoid receptor, PPARs and LXR (26-28). 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.

**Liver X Receptors**

The liver X receptor subfamily consists of two members, LXRα (NR1H3) and LXRβ (NR1H2). LXRα is expressed in a tissue-specific and autoregulated manner, while LXRβ is ubiquitously expressed (16, 29). LXR are regulated by oxysterols such as 24(s), 25-epoxycholesterol, 22(S)-hydroxycholesterol, and 24(S)-hydroxycholesterol that appear to be produced in proportion to cellular cholesterol content (17). Consistent with this, LXR have been documented to play important roles as effectors of feed forward mechanisms that protect cells from elevated cholesterol levels. A physiologic requirement for LXR in maintenance of cholesterol homeostasis is exemplified by the finding that LXRα-null mice fed a high cholesterol
diet develop massive hepatic accumulation of cholesterol, while wild type mice are highly resistant to cholesterol feeding (30). LXR$\text{S}$ positively regulate several hepatic and intestinal genes required for cholesterol excretion from the body, including Cyp7a, the rate limiting enzyme for bile acid biosynthesis (30), and ABC genes involved in cholesterol transport in liver and intestine (ABCG5, ABCG8) (31). In addition, LXR$\text{S}$ directly and indirectly regulate genes involved in fatty acid metabolism including sterol response element binding protein 1c (SREBP-1c), fatty acid synthase (FAS), stearoyl-coenzyme A desaturase (SCD), and acyl-coenzyme A carboxylase (ACC) (32), and regulate genes that control secretion and metabolism of triglyceride-rich lipoproteins, including lipoprotein lipase (LPL) (33), cholesterol ester transfer protein (CETP), phospholipid transfer protein (PLTP) (34) and the apolipoprotein E/C-I/C-IV/C-II gene cluster (35). The overall impact of administration of LXR agonists to mice is to stimulate fatty acid biosynthesis in liver and cause an increase in circulating triglyceride levels, at least in part as a consequence of inducing the expression and activity of SREBP-1c (32, 36).

LXR$\text{S}$ also appear to play important roles in regulation of cholesterol homeostasis in peripheral cells, including macrophages, by positively regulating the expression of ABCA1, ABCG1 and ABCG4 (Figure 2). ABCA1 mediates cholesterol efflux from cells to lipid-poor apolipoprotein AI (apoAI), representing a reverse cholesterol transport pathway that promotes the formation of high density lipoprotein (HDL) particles in peripheral tissues (37-39). Consistent with this, mutations in the ABCA1 gene are the molecular defect in patients with Tangier’s disease in 1998 (reviewed in (40)). Tangier’s disease patients have low HDL levels, accumulation of cholesterol esters in reticuloendothelial tissues such as tonsils and develop a peripheral neuropathy. Although phenotypes are not striking, overexpression of ABCA1 reduces
development of atherosclerosis in hypercholesterolemic mice, while loss of ABCA1 results in
more extensive atherosclerosis (41-43).

ABCA1 appears to be an important target of LXRs in macrophages (Figure 2). Macrophages express both LXR\(\alpha\) and \(\beta\) (29, 44) and treatment of RAW cells and murine peritoneal macrophages with oxysterols and synthetic LXR agonists was found to induce expression of ABCA1 (45-48). Recent studies suggest that ABCG1 and ABCG4 are also important targets of LXRs in regulation of cholesterol efflux in macrophages.

Anti-sense oligonucleotides directed against ABCG1 inhibited HDL\(_3\)-dependent efflux of cholesterol in human macrophages (49). Interestingly, expression of ABCG1 is increased in peripheral blood monocyte-derived macrophages isolated from Tangier’s patients, suggesting a compensatory role (50). More recently siRNA and overexpression studies suggest that ABCG1 and ABCG4 stimulate cholesterol efflux to HDL subclasses but not to lipid poor-apoA1 (51) (52). Thus, LXRs appear to regulate two independent cholesterol efflux pathways in macrophages (Figure 2). Finally LXR\(\alpha\) appears to induce synthesis of fatty acids that are preferential substrates of ACAT in cholesterol esterification reactions (32). In concert, LXRs function to reduce free cholesterol levels in macrophages and protect cells from its cytotoxic effects. These pathways may be of particular importance in macrophages because of the roles of the cells in phagocytosis of necrotic and apoptotic cells, which result in substantial cholesterol loads. The endogenous LXR ligands that are generated in macrophages in response to cholesterol loading remain to be clearly established. 27-hydroxycholesterol synthesized by Cyp27 may function as an endogenous ligand for LXR in macrophages mediating cholesterol efflux, although 27-hydroxy cholesterol is less active as an agonist of LXRs than synthetic and other naturally occurring LXR ligands (53).
Not surprisingly, LXRs have been shown to protect agonist development of atherosclerosis in mice. Treatment of hypercholesterolemic low density lipoprotein receptor-deficient (LDLR−/−) mice with a synthetic LXR agonist significantly reduced the development of atherosclerosis (54). Conversely, bone marrow transplantation experiments in hypercholesterolemic apolipoproteinE-deficient mice demonstrated that mice receiving LXR-null bone marrow progenitor cells developed more atherosclerosis than mice receiving wild type progenitor cells (55). These studies demonstrate clear anti-atherogenic roles of LXRs, raising the possibility that LXR agonists may be of therapeutic utility in humans if undesirable effects on circulating triglyceride levels can be reduced or eliminated through development of selective modulators (36).

In addition to regulating cholesterol homeostasis, recent studies suggest that LXRs may also antagonize inflammatory responses. Synthetic LXR agonists can inhibit induction of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), macrophage chemotactic protein-1 (MCP-1) and Gelatinase B by LPS or other proinflammatory cytokines (28, 56). These findings suggest previously unrecognized connections between cholesterol metabolism and inflammation.

**Peroxisome Proliferator-activated Receptors**

PPARα (NR1C1), PPARβ/δ (NR1C2, hereafter referred to as PPARδ), and PPARγ (NR1C3) comprise 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 (57, 58).
PPARα, the first PPAR to be identified, was named based on its ability to be activated by substances that drive peroxisome proliferation in rodents (59). The cloning of PPARα cDNAs also led to the recognition of PPARα as the molecular target of fibrates, such as gemfibrozil, that are used in the treatment of hypertriglyceridemia (60, 61). Many lines of evidence indicate that PPARα regulates lipid homeostasis in part by stimulating peroxisomal β-oxidation of fatty acids. In the liver, activation of PPARα leads to the upregulation of fatty acid transport protein and long-chain acyl-CoA synthetase genes (61, 62). By increasing β-oxidation, PPARα not only stimulates energy production but also shortens long chain fatty acids, thus preventing lipid accumulation and toxicity. Mitochondrial HMG-CoA synthase is also a target of PPARα and plays a role in the formation of ketone bodies (63). In addition to stimulating β-oxidation, activation of PPARα has been shown to increase apolipoprotein AI and AII and decrease apolipoprotein CIII, an inhibitor of lipoprotein lipase (LPL) (64, 65). These effects would tend to increase HDL levels and decrease triglyceride levels and are 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 of the expression of LXRα, which can then stimulate ABCA1 expression and promote efflux of cholesterol to apoAI (66). It has also been demonstrated that PPARα can inhibit esterification of free cholesterol by ACAT-1 and increase the efflux of free cholesterol by increasing the expression of scavenger receptor B-1 (SRB-1) (67, 68).

In addition to effects on cellular and circulating lipid levels, activation of PPARα has also been suggested to exert anti-diabetic effects by increasing insulin sensitivity. In PPARα-null mice, there is no gross alteration of insulin sensitivity (69). However, in Zucker obese fa/fa
rats and lipoatrophic mice (AZIP/F-1), activation of PPARα led to a significant improvement in insulin sensitivity (70, 71).

Studies performed in cultured cells suggest that PPARα regulates the expression of genes that control inflammatory responses in endothelial cells, smooth muscle cells and macrophages. PPARα has been shown to inhibit transcriptional responses to inflammatory stimuli by interfering with the activation of NF-kB, leading to the reduction of vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells (72). In vascular smooth muscle cells (VSMCs) PPARα agonists inhibited interleukin (IL-1)-induced production of IL-6 and and COX-2 expression (73). PPARα agonists also reduced the expression of tissue factor and matrix metalloproteinase in monocyte and macrophages, thereby decreasing thrombogenicity and plaque instability (74-76). On the other hand, some studies have suggested potential pro-atherogenic consequences of PPARα activation, such as the ability of PPARα agonists to stimulate the production of MCP-1 in endothelial cells (77), which would be expected to enhance recruitment of monocytes into lesions.

Identification of potential ligands for PPARs has primarily relied on screening candidate molecules. This approach has led to the identification of numerous natural compounds that can bind to PPARα and stimulate its transcriptional activities in cells, including polyunsaturated fatty acids such as linoleic acid, dodecاهexanoic acid (DHA) and eicosapentanoic acid (EPA) (15, 78). Because these substances bind to PPARα with relatively low affinity, it has been difficult to clearly establish their importance in vivo. An alternative strategy has been to evaluate enzymatic pathways that could potentially be involved in the local generation of ligands. Using this strategy, it was recently demonstrated that LPL releases fatty acids from triglyceride-rich lipoproteins such as VLDL and chylomicrons that activate PPARα and decrease VCAM-1
expression (79). Intriguingly, other lipases that were equally effective at generating free fatty acids were not effective at activating PPARα, suggesting selective utilization of fatty acids derived by LPL hydrolysis of triglyceride-rich lipoproteins.

In concert, the effects of PPARα agonists on lipid and carbohydrate metabolism would be expected to result in protection against development of atherosclerosis. Consistent with this, a clinical trial examining effects of the PPARα agonist gemfibrozil in men with a history of coronary heart disease and low HDL levels demonstrated a significant reduction in incidence of fatal and non-fatal myocardial infarction (80). These effects could only be partially explained by increased levels of HDL (81), and are consistent with actions in peripheral tissues, including macrophages. However, studies of the influence of PPARα on the development of atherosclerosis in animal models have yielded conflicting results. Surprisingly, PPARα−/−/apoE-deficient mice exhibited less atherosclerosis than control apoE-deficient mice, suggesting an atherogenic role of PPARα (82). PPARα−/− mice were found to be less insulin resistant and to have lower blood pressure compared to wild type controls (82), potentially at least partially explaining the unexpected outcome. In another study of apoE-deficient mice, treatment with ciprofibrate worsened diet-induced hyperlipidemia and increased atherosclerosis (83). In these studies, ciprofibrate treatment was associated with an increase in apo B48-containing lipoproteins, suggesting an effect of PPARα on editing of the apo B messenger RNA. Other studies have found that PPARα agonists are anti-atherogenic. In one study, fenofibrate had minimal anti-atherogenic effects in apoE-deficient mice (84, 85), but exerted a more pronounced effect in apoE-deficient mice carrying a fenofibrate-inducible human apoAI transgene.

Studies in our laboratories recently demonstrated that activation of PPARα by a highly specific and potent agonist (GW7647), inhibited atherosclerosis by nearly 50% throughout the
aortas of hyperlipidemic LDLR−/− mice (86). In these studies, activation of PPARα with GW7647 did not significantly alter diet-induced hyperlipidemia. However, improvement of insulin sensitivity was observed, which was associated with less weight gain in treated animals compared to control mice. 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 (85). In concert with its effects on the development of atherosclerosis, GW7647 inhibited the formation of macrophage foam cells in the peritoneal cavities of hypercholesterolemic mice. Through the use of macrophage transfer and bone marrow transfer experiments, this effect was shown to require macrophage expression of PPARα and LXRs (86).

Reduction of foam cell formation in peritoneal macrophages was independent of cholesterol esterification and cholesterol efflux through the ABCA1 pathway. Unexpectedly, when LXR-null bone marrow progenitor cells were transplanted into LDLR−/− mice or transferred into the peritoneal cavity, the majority of macrophage died when mice were treated with the PPARα agonist. The few cells that could be recovered were massively engorged with oil Red O-staining lipid. These studies suggested that PPARα negatively regulated macrophage foam cell formation through an LXR-dependent, ABCA1 independent pathway. It has been proposed that PPARα and PPARγ activation may promote macrophage apoptosis in a context-dependent manner (87). The sensitivity of LXR-deficient macrophages to apoptosis/necrosis following treatment with PPARα agonists raises the possibility that LXRs mediate a protective effect in wild-type macrophages.

Two significant differences may explain the apparent discrepancies observed regarding the influence of PPARα agonists on the development of atherosclerosis in mice. First, initial
studies were performed on a background of apoE deficiency, whereas our recent studies used LDLR-deficient mice. Apolipoprotein E is present in all lipoproteins except LDL and plays a role in the clearance of remnants and VLDL by the liver (reviewed in (88)). Apolipoprotein E is also a LXR target gene in macrophages and can potentially serve as an acceptor for cholesterol efflux. Expression of apoE may be required for inhibition of foam cell formation by PPARα agonists. A second significant difference concerns the specific PPARα agonists that were tested for effects on atherosclerosis. Fibrates such as fenofibrate and gemfibrozil are relatively low affinity ligands for PPARα, with binding constants in the micromolar range. When used in vivo, it may be difficult to achieve effective concentrations of these drugs in peripheral tissues in mouse models. In contrast, GW7647 binds to PPARα at low nanomolar concentrations and is likely to effectively activate PPARα throughout the body (reviewed in (89)). To distinguish between these two possibilities, it will be necessary to perform intervention studies of conventional fibrates in LDLR−/− mice and studies of high affinity PPARα agonists in apoE−/− mice. It is possible that bioavailable, 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 apoAI or HDL-dependent cholesterol efflux suggests that additional PPARα/LXR pathways controlling cholesterol homeostasis remain to be discovered.

**PPAR gamma**

PPARγ can be activated by a number of naturally occurring fatty acid metabolites, including oxidized linoleic acid (9- and 13- HODE) and 15 deoxy Δ2,14 prostaglandin J2 (15, 90, 91). Endogenous ligands remain poorly characterized however and there is considerable
evidence concerning the biological importance of agonist (15 deoxy Δ2,14 prostaglandin J2) having an important biological role (92). As exemplified by studies of LPL as a component of a pathway generating ligands for PPARα, it may be useful to identify enzymatic systems necessary for activation of endogenous PPARγ target genes. The 12/15 lipoxygenase, which can generate 13-HODE from linoleic acid, has been suggested to mediate formation of PPARγ ligands in specific contexts (93).

Two isoforms of PPARγ have been identified and are derived from the same gene by alternative promoter usage (94, 95). PPARγ2 is specifically expressed in adipose tissue and differs from PPARγ1 by the presence of 30 additional N-terminal amino acids that 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 (96-99).

PPARγ plays a critical role in glucose homeostasis, and is the molecular target of a class of insulin-sensitizing drugs referred to as thiazolidinediones (TZDs) (100). Consistent with this, a number of PPARγ polymorphisms are linked with features of the metabolic syndrome, including insulin resistance, hypertension and obesity and dominant negative mutations in PPARγ have been shown to cause severe insulin resistance (reviewed in (101)). Systemic deletion of the PPARγ gene results in embryonic lethality due to essential roles in adipose, kidney and placental development (97). 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 liver and skeletal muscle (102-105). The mechanisms by which PPARγ influences insulin action have been intensively studied and several potentially important targets
of regulation have been established. Activation of PPAR\(\gamma\) induces expression of the insulin-dependent glucose transporter GLUT4 (106), increases the release of free fatty acids from chylomicrons and VLDL (107), upregulates genes involved in intracellular fatty acid transport, synthesis and estrification (94, 108), and increases the expression of adiponectin (109, 110).

Several lines of evidence have also linked inflammatory mediators, such as TNF\(\alpha\), interleukin-6 and resistant to the insulin-resistant states associated with obesity and type 2 diabetes (reviewed in (111)). Activators of PPAR\(\gamma\) can inhibit a diverse array of inflammatory responses (reviewed in (112)) and block tumor necrosis factor \(\alpha\) (TNF\(\alpha\))-induced inhibition of insulin signaling (113), raising the possibility that some of the insulin-sensitizing actions of PPAR\(\gamma\) relate to negative regulation of inflammatory mediators. Consistent with these findings, thiazolidinediones reduce circulating levels of the inflammatory markers C-reactive protein and soluble gelatinase B in diabetic patients (114).

A role for PPAR\(\gamma\) in regulation of inflammation and immunity was initially suggested by the findings that it is expressed in macrophages and inhibits the expression of a number of pro-inflammatory genes, including TNF\(\alpha\), IL-1\(\beta\), iNOS and gelatinase B (27, 115). A large number of inflammatory responses have been shown to be subject to negative regulation by PPAR\(\gamma\) agonists (reviewed in (112, 116)). Initial studies of PPAR\(\gamma\)-deficient macrophages raised questions regarding whether PPAR\(\gamma\) agonists exerted effects on inflammatory response genes through PPAR\(\gamma\)-dependent or PPAR\(\gamma\)-independent mechanisms (117). Further investigation established that both types of mechanisms contribute to these actions. For example, the PPAR\(\gamma\) agonist 15-deoxy \(\Delta^{12,14}\) prostaglandin J\(_2\) was shown to inhibit NF-\(\kappa\)B signaling in a PPAR\(\gamma\)-independent manner by covalently modifying I\(\kappa\)B-kinase and NF-\(\kappa\)B subunits (118, 119). High concentrations of synthetic PPAR\(\gamma\) agonists can also exert anti-inflammatory effects by binding
to PPARδ (120). When used at receptor-specific concentrations, PPARγ ligands inhibited transcriptional responses of primary macrophages to bacterial lipopolysaccharide in a PPARγ-dependent manner (120).

Recent gene expression profiling studies suggest that anti-inflammatory actions of PPARγ in macrophages may be relevant to obesity-induced insulin resistance. Through a comparison of gene expression profiles of insulin-sensitive adipose tissue derived from lean animals and insulin-resistant adipose tissue derived from obese animals, the unexpected observation was made that macrophages accumulate in adipose tissue in the setting of obesity (121, 122). Adipose tissue macrophages were found to be a major source of inflammatory mediators that are linked to insulin resistance and are subject to counterregulation by PPARγ agonists.

In vitro studies of effects of PPARγ agonists on cholesterol homeostasis in macrophages suggested both atherogenic and antiatherogenic influences. PPARγ was found to stimulate transcription of the CD36 gene (90, 123), which is a macrophage scavenger receptor that contributes to macrophage foam cell formation and development of atherosclerosis in mice (124). In conjunction with the finding that PPARγ can be activated by 9- and 13-HODE present in oxLDL, a ‘PPARγ cycle’ was 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 (90, 123). This cycle would potentially promote foam cell formation and atherosclerosis. However, a recent finding suggests that PPARγ may actually decrease expression of CD36 protein in the context of the metabolic syndrome, and thereby reduce uptake of modified forms of LDL. Macrophages isolated from ob/ob mice exhibit impaired insulin signaling and increased expression of CD36. Treatment of peritoneal macrophages isolated from these mice with
rosiglitazone had no effect on CD36 mRNA levels but reduced protein expression at the cell surface (125). This would suggest a post-translational effect of rosiglitazone on CD36 via the insulin-signaling pathway. With respect to cholesterol efflux pathways, PPARα and PPARγ were shown to induce expression of LXRα and thereby stimulate ABCA1-dependent cholesterol efflux to apoAI (66, 126) (Figure 2), analogous to the PPARα/LXRα/ABCA1 pathway described above. The cytochrome p450 enzyme, Cyp27, which is the enzyme that catalyzes production of the weak LXR agonist 27OH-cholesterol, has recently been demonstrated to be activated by PPARγ (127). Cyp27 may thus function as an integrator of the PPAR/LXR cholesterol efflux pathway in macrophages by generating ligands that activate LXRs.

Large-scale clinical trials examining the effects of PPARγ agonists on cardiovascular endpoints have not been reported. Two small clinical studies found that treatment of diabetic patients with thiazolidinediones inhibited carotid intimal thickening (128, 129). Studies of effects of PPARγ agonists in hypercholesterolemic male mice have consistently demonstrated anti-atherogenic effects despite an increase expression of CD36 in atherosclerotic lesions (130-132). PPARγ agonists improved insulin sensitivity in these studies and inhibited the expression of inflammatory markers in the artery wall. Unexpectedly, in the one study that evaluated female mice, PPARγ agonists were not effective at inhibiting the development of atherosclerosis (130). These findings are consistent with the idea that PPARγ agonists can act to promote both atherogenic and antiatherogenic programs of gene expression with the net outcome being influenced by additional factors such as hormonal status.

Our laboratories recently demonstrated that PPARγ agonists inhibit the formation of macrophage foam cells within the peritoneal cavities of hypercholesterolemic LDLR−/− mice (86). In these studies, inhibition of foam cell formation by PPARγ occurred without changes in the expression
of LXRα or ABCA1. As LXRα and ABCA1 are already highly upregulated in this context, further activation of this pathway by PPARγ agonists may not be possible. In contrast to findings with PPARα agonists, however, was the observation that PPARγ agonists could significantly inhibit foam cell formation in macrophages lacking LXRα and LXRβ. Investigation of alternative pathways regulating cholesterol homeostasis revealed two additional PPARγ-sensitive targets. First, rosiglitazone inhibited cholesterol esterification in an LXR-independent manner. This effect was not due to down regulation of ACAT1 mRNA expression, and may result from indirect effects on transfer of cholesterol for esterification or post translational effects on ACAT1 activity. The effect of rosiglitazone on cholesterol esterification directly links PPARγ activity with inhibition of macrophage foam cell formation in vitro. Although studies of ACAT1-defeciency in the setting of atherosclerosis have been inconsistent (133, 134), there is also evidence that partial inhibition of ACAT1 activity may reduce lesion formation (135). In addition, these studies demonstrated that PPARγ agonists induce expression of ABCG1 in wild type and LXR-deficient macrophages and in the artery walls of hypercholesterolemic mice. It will be of interest to test the possibility that the induction of ABCG1 and inhibition of cholesterol esterification are mechanistically linked and represent components of a coordinated pathway for the regulation of macrophage cholesterol homeostasis. Together, these two PPARγ-sensitive targets provide a plausible mechanism for LXR-independent effects on macrophage foam cell formation (Figure 2).

The finding that PPARα and PPARγ agonists inhibit foam cell formation raises the possibility that combined therapy or use of PPARα/γ co-agonists could have additive or synergistic protective effects in the arterial wall in addition to beneficial effects on lipid and glucose metabolism. Recent animal studies using co-agonists demonstrated combined
improvements in insulin sensitivity, as well as fatty acid, glucose and lipoprotein metabolism (136, 137). The PPAR\(\alpha/\gamma\) co-agonist, GW2331 decreased atherosclerosis by 32% in apoE-deficient mice (84). Another PPAR\(\alpha/\gamma\) co-agonist resulted in a 2.5-fold reduction in lesion area in hypercholesterolemic apoE\(^-\) mice and was correlated with inhibition of interferon \(\gamma\) and beta 2 integrin CD11a gene expression (138).

**PPAR\(\delta\)**

PPAR\(\delta\) was the last of the PPARs to be identified (139). Gene deletion studies suggest important roles of PPAR\(\delta\) in skin biology, lipid metabolism, and energy homeostasis (140-143). Constitutive activation of PPAR\(\delta\) in adipose tissue led to an improvement of lipid profiles and a reduction of adiposity, whereas PPAR\(\delta\)-deficient mice exhibited a reduction in energy uncoupling and obesity (51, 140). The role of PPAR\(\delta\) in atherosclerosis is unclear at present. In vitro studies demonstrated that a PPAR\(\delta\)-specific agonist (GW501516) enhanced reverse cholesterol transport in a human macrophage cell line (THP-1), skin fibroblasts (1BR3N), and intestinal cells (FHS74). Administration of this compound to obese, insulin-resistant rhesus monkeys led to normalization of plasma HDL levels (141). On the other hand, a different PPAR\(\delta\) agonist promoted lipid accumulation in THP-1 cells and primary human macrophages (144). PPAR\(\delta\) was found to function as a lipid sensor of fatty acids derived from hydrolysis of VLDL-triglycerids, which in turn increased the expression of the adipophilin related protein (ADRP) (145). In addition to its role in lipid metabolism, PPAR\(\delta\) also has anti-inflammatory properties. Treatment of endothelial cells with a PPAR\(\delta\) agonist decreased VCAM-1 and MCP-1 expression (146) and a PPAR\(\delta\) agonist inhibited LPS-inducible genes such as COX-2 and iNOS in murine peritoneal macrophages (120). PPAR\(\delta\) has been suggested to act as molecular switch
between certain types of pro-inflammatory and anti-inflammatory contexts dependent on interactions with transcriptional repressors such as BCL-6 (147). In the absence of ligands, PPARδ binds to BCL-6, which is sequestered from target genes such as MCP-1, resulting in de-repression. In the presence of a PPARδ agonist, BCL-6 is released and can then repress MCP-1 expression.

Two studies have evaluated the consequences of PPARδ activation on development of atherosclerosis in mice. Transplantation of PPARδ-deficient bone marrow progenitor cells into hypercholesterolemic LDLR-deficient mice resulted in less atherosclerosis than in mice transplanted with wild type progenitor cells (147), suggesting that PPARδ is pro-atherogenic. This finding is consistent with the ability of the apo form of PPARδ to be anti-inflammatory, but implies that PPARδ is unliganded in lesion macrophages. Our laboratories investigated the influence of a potent and selective PPARδ agonist on development of atherosclerosis in hypercholesterolemic LDLR-deficient mice under conditions in which PPARα and PPARγ agonists were protective (86). Treatment with the PPARδ agonist did not alter the progression of atherosclerosis compared to untreated mice despite a decrease in inflammatory cytokines within the atherosclerotic lesions. These findings suggest that anti-inflammatory effects of PPARδ ligands are not sufficient to inhibit development of atherosclerosis in the setting of extreme hypercholesterolemia. Because of the marked anti-inflammatory effects of PPARδ agonists, additional studies in models of less extreme hypercholesterolemia would be of interest, however.

Conclusions

Rapid progress continues to be made with respect to defining the biological roles and mechanisms of action of PPARs and LXRs. The metabolic roles of PPARα and PPARγ are now
well established, and the ability to study consequences of loss of function of these receptors in
specific cell types in conjunction with large-scale gene expression analysis will allow the
development of an increasingly sophisticated understanding of how these receptors contribute to
metabolic control. These studies should be able to delineate the relative roles of transcriptional
activators of positively regulated genes and repression of inflammatory response genes in
mediating anti-diabetic actions. 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 support 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 PPARα and PPARγ
agonists and co-agonists in prevention of atherosclerosis will continue to be an important area for
clinical investigation. Similarly, LXRαs appear to hold significant promise as targets for new
classes of anti-atherogenic drugs. The major challenges here will be to identify LXR ligands that
have desirable effects on HDL metabolism and cholesterol efflux pathways, but do not cause
hypertriglyceridemia. The recent demonstration that LXRαs can exert potent inhibitory effects on
inflammatory responses also raises a number of interesting new directions connecting cholesterol
metabolism with immune function.
Bibliography


activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells, Circ Res. 87: 516-21.


Figure Legends

**Figure 1. Transcriptional activities of PPARs and LXRs.** PPARs and LXRs each possess the conserved DNA binding domain (black) and C-terminal ligand-binding domain (white) characteristic of nuclear hormone receptors. PPARs and LXRs bind to specific response elements in target genes as heterodimers with retinoid X receptors, which are also members of the nuclear receptor superfamily. Top: In the absence of ligands, PPAR/RXR and LXR/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). Middle: In the presence of ligands, PPAR/RXR and LXR/RXR heterodimers activate transcription through the recruitment of diverse coactivator complexes. These complexes contain enzymatic functions that include nucleosome remodeling activity, histone acetyltransferase and histone methyltransferase activities and directly or indirectly recruit core transcriptional machinery to the promoter. Bottom: PPARs and LXR agonists can inhibit the activities of other signal-dependent transcription factors, such as NF-κB and AP-1. This transrepression function contributes to anti-inflammatory actions of PPARs and LXRs.

**Figure 2. THE LXR subfamily of nuclear receptors.**
The figure illustrates major sites of expression in body, major biological functions, and naturally occurring ligands. LXRs are potential targets for new classes of anti-atherogenic drugs.

**Figure 3. The PPAR subfamily of nuclear receptors.**
The figure illustrates major sites of expression in the body, major biological roles, naturally occurring ligands, and classes of drugs that are in clinical use. PUFA; polyunsaturated fatty acids, 15dPGJ₂; 15-deoxy-Δ^{12,14} prostaglandin J₂, HODE: hydroxyoctadecadienoic acid, HETE; hydroxyeicosatetraenoic acid.

**Figure 4. Regulation of cholesterol efflux pathways in macrophages by PPARs and LXR s.**

LXR agonists stimulate expression of ABCA1, which facilitates efflux of cholesterol to lipid-poor apoAI, and ABCG1, which facilitates efflux of cholesterol to HDL. PPARα and PPARγ agonists can induce expression of LXRα, thereby stimulating cholesterol efflux to apoAI in an LXR-dependent manner. PPARγ ligands also directly activate ABCG1, enabling LXR-independent efflux to HDL. PPARα and PPARγ have also been shown to inhibit cholesterol esterification in cholesterol-loaded macrophages. This effect is not due to changes in ACAT expression and may result from altered cholesterol trafficking within the cell.
Active Repression

Ligand-dependent Transrepression

Ligand-dependent Transactivation

Li and Glass/Figure 1
The LXR subfamily of nuclear receptors

<table>
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<th>Tissue Expression</th>
<th>Liver</th>
<th>Macrophages</th>
<th>Broadly expressed</th>
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### Biological Functions
- Cholesterol absorption (intestine)
- Cholesterol excretion (liver)
- Cholesterol efflux (peripheral cells)
- VLDL synthesis (liver)
- Fatty acid biosynthesis (liver and peripheral cells)

### Ligands
- 24(S),25-epoxycholesterol
- 22(S)-hydroxycholesterol
- 24(S)-hydroxycholesterol

### Disease targets
- Atherosclerosis
## The PPAR subfamily of nuclear receptors

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<th>PPARγ</th>
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HDL-dependent cholesterol efflux

ABCG1

ABCA1

apoA-I-dependent cholesterol efflux

LXR α

PPAR γ

Free cholesterol efflux

Cholesterol ester

Li and Glass/Figure 4