thematic review

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

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Abstract The nuclear receptor superfamily is composed 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 of 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 PPARα, PPARγ, 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.—Li, A. C., and C. K. Glass. **PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. *J. Lipid Res.* 2004.** 45: **2161–2173.**

Supplementary key words peroxisome proliferator-activated receptors • liver X receptors • foam cell formation

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, estrogen receptor, and thyroid hormone receptors were cloned, leading to the recognition of a superfamily of nuclear receptors that responded not only to steroid hormones but to other classes of lipid-derived mediators that function to regulate development and homeostasis (2). Here, we review recent findings that provide new insights into the roles of nuclear receptors in the control of lipid metabolism and atherosclerosis, focusing on liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs). Although drugs that regulate LXRs and PPARs may not have the rejuvenating properties sought by Brown-Sequard, they do hold promise for new approaches for the 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 predominantly of monocyte-derived macrophages engorged with lipoprotein-derived cholesterol (reviewed in 4). The formation of

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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 chemoattractant molecules, where they further differentiate into macrophages. This program of differentiation includes the upregulation 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 LDL (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 vascular cell adhesion molecule-1 (VCAM-1), and promote the secretion of chemotactic factors such as macrophage chemotactic protein-1 (MCP-1), leading to the 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 the risk of atherosclerosis (13). Intriguingly, PPARs, LXRs, and other nuclear receptors not only influence lipid metabolism at the 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 LXRs by natural and synthetic ligands leads to multiple changes in gene expression, some of which are predicted to be atherogenic and others antiatherogenic. 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 LXRS: STRUCTURE AND FUNCTION

The PPAR and LXR subfamilies together account for 5 of the 48 nuclear receptors that have been identified in the human and mouse genomes. PPARs and LXRs possess both the conserved DNA binding and ligand binding domains that are characteristic of nuclear receptors (**Fig. 1**). The central DNA binding domain 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 coactivator 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 (Fig. 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 corepressors NCoR and/or SMRT (20–23). Second, PPARs and LXRs bind to hormone response elements in the presence of ligands and activate transcription (Fig. 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 multiple-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 corepressors and recruitment

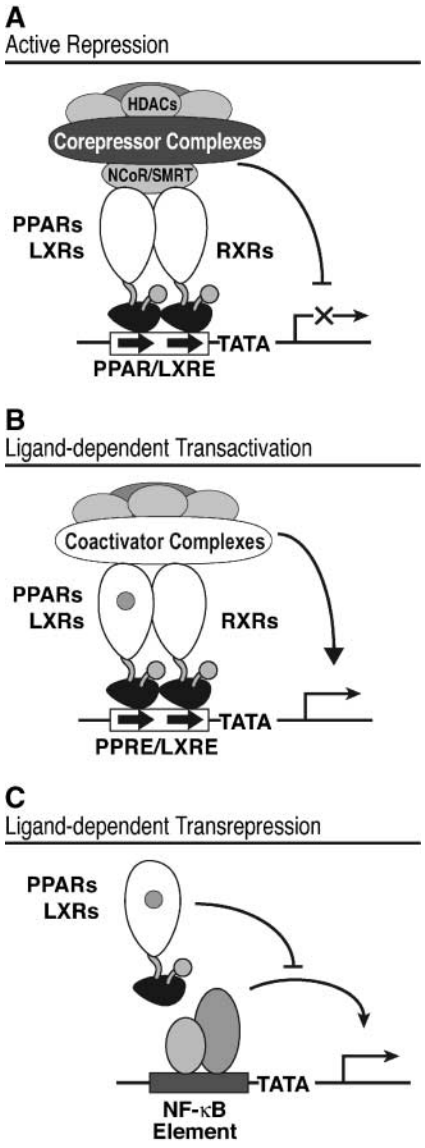

Fig. 1. Transcriptional activities of peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs). PPARs and LXRs each possess the conserved DNA binding domain (black) and C-terminal ligand binding domain (white) characteristic of nuclear hormone receptors. PPARs and LXRs bind to specific response elements in target genes as heterodimers with retinoid X receptors (RXRs), which are also members of the nuclear receptor superfamily. A: 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). B: 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. C: PPARs and LXR agonists can inhibit the activities of other signal-dependent transcription factors, such as nuclear factor κB (NF-κB) and activator protein-1 (AP-1). This transrepression function contributes to the anti-inflammatory actions of PPARs and LXRs.

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 nuclear factor κB (NF-κB) and activator protein-1 (AP-1) (Fig. 1). This activity, referred to as transrepression, is thought to underlie anti-inflammatory actions of nuclear receptors such as the glucocorticoid receptor, PPARs, and LXRs (26–28). The molecular mechanisms responsible for transrepression are less well understood than the mechanisms responsible for transcriptional activation, but they do not appear to involve sequence-specific binding to DNA.

LXRS

The LXR subfamily consists of two members, LXRα (NR1H3) and LXRβ (NR1H2) (**Fig. 2**). LXRα is expressed in a tissue-specific and autoregulated manner, whereas LXRβ is ubiquitously expressed (16, 29). LXRs 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, LXRs have been documented to play important roles as effectors of feed-forward mechanisms that protect cells from increased cholesterol levels. A physiological requirement for LXRs in the maintenance of cholesterol homeostasis is exemplified by the finding that LXRα-null mice fed a high-cholesterol diet develop massive hepatic accumulation of cholesterol, whereas wild-type mice are highly resistant to cholesterol feeding (30). LXRs 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 ATP binding cassette (ABC) genes involved in cholesterol transport in liver and intestine (ABCG5, ABCG8) (31). In addition, LXRs directly and indirectly regulate genes involved in fatty acid metabolism, including sterol response element binding protein-1c (SREBP-1c), fatty acid synthase, stearoyl-CoA desaturase, and acyl-CoA carboxylase (32), and regulate genes that control the secretion and metabolism of triglyceride-rich lipoproteins, including LPL (33), cholesteryl ester transfer protein, phospholipid transfer protein (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).

LXRs also appear to play important roles in the regulation of cholesterol homeostasis in peripheral cells, including macrophages, by positively regulating the expression of ABCA1, ABCG1, and ABCG4. ABCA1 mediates cholesterol efflux from cells to lipid-poor apolipoprotein A-I

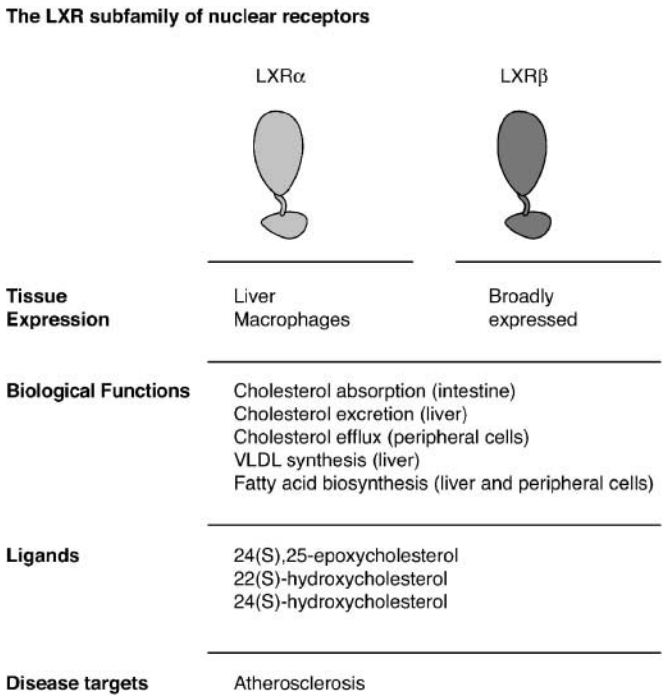

Fig. 2. The LXR subfamily of nuclear receptors. Major sites of expression in the body, major biological functions, and naturally occurring ligands are listed. LXRs are potential targets for new classes of antiatherogenic drugs.

(apoA-I), representing a reverse cholesterol transport pathway that promotes the formation of HDL particles in peripheral tissues (37–39). Consistent with this, mutations in the ABCA1 gene are the molecular defect in patients with Tangier disease (reviewed in 40). Tangier disease patients have low HDL levels, accumulation of cholesteryl esters in reticuloendothelial tissues such as tonsils, and develop a peripheral neuropathy. Although phenotypes are not striking, overexpression of ABCA1 reduces the development of atherosclerosis in hypercholesterolemic mice, whereas loss of ABCA1 results in more extensive atherosclerosis (41–43).

ABCA1 appears to be an important target of LXRs in macrophages. Macrophages express both LXRα and LXRβ (29, 44), and treatment of RAW cells and murine peritoneal macrophages with oxysterols and synthetic LXR agonists was found to induce the expression of ABCA1 (45–48). Recent studies suggest that ABCG1 and ABCG4 are also important targets of LXRs in the regulation of cholesterol efflux in macrophages. Antisense 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 disease patients, suggesting a compensatory role (50). More recently, small interfering RNA (siRNA) and overexpression studies have suggested that ABCG1 and ABCG4 stimulate cholesterol efflux to HDL subclasses but not to lipid-poor apoA-I (51, 52). Thus, LXRs appear to regulate two independent cholesterol efflux pathways in macrophages. Finally, LXRα appears to induce the 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-hydroxycholesterol is less active as an agonist of LXRs than are synthetic and other naturally occurring LXR ligands (53).

Not surprisingly, LXRs have been shown to protect against the 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 apoE-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 antiatherogenic roles of LXRs, increasing 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 the 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 the induction of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), MCP-1, and gelatinase B by LPS or other proinflammatory cytokines (28, 56). These findings suggest previously unrecognized connections between cholesterol metabolism and inflammation.

PPARS

PPARα (NR1C1), PPARβ/δ (NR1C2; hereafter referred to as PPARδ), and PPARγ (NR1C3) constitute the PPAR subfamily of nuclear receptors (**Fig. 3**). Although there is overlap in the 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-

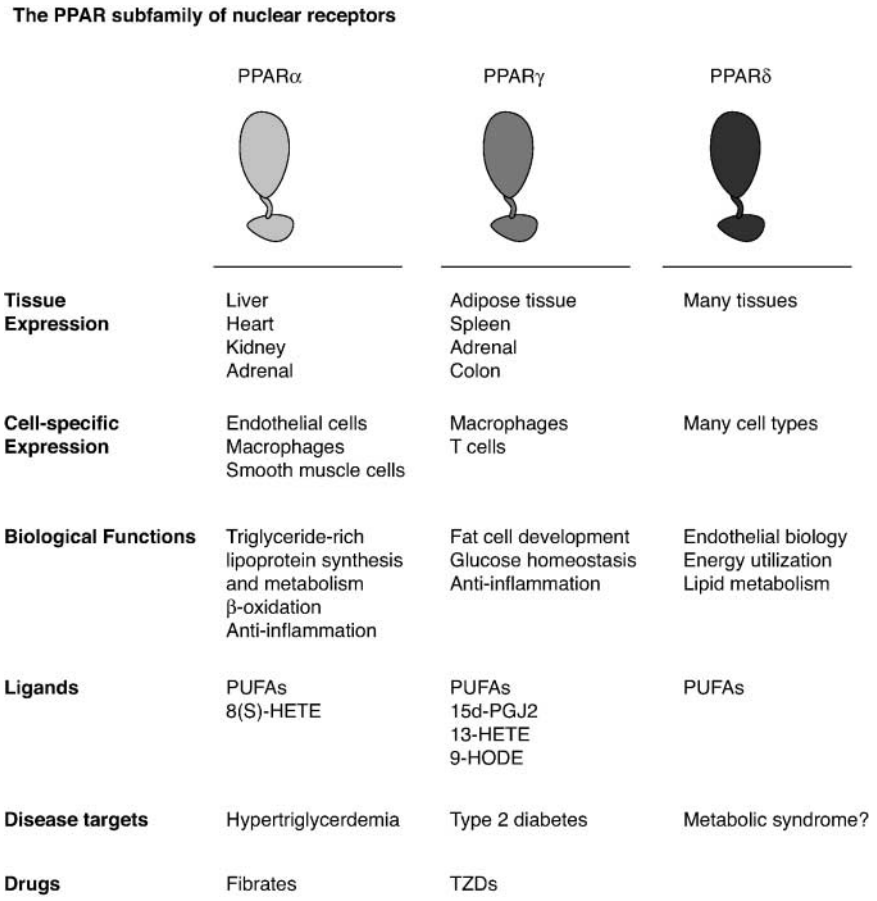

Fig. 3. The PPAR subfamily of nuclear receptors. Major sites of expression in the body, major biological roles, naturally occurring ligands, and classes of drugs that are in clinical use are listed. 15dPGJ$_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid.

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 apoA-I and apoA-II and decrease apoC-III, an inhibitor of LPL (64, 65). These effects would tend to increase HDL levels and decrease triglyceride levels and are thought to contribute to the 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 the induction of expression of LXRα, which can then stimulate ABCA1 expression and promote efflux of cholesterol to apoA-I (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 class B type 1 (67, 68).

In addition to effects on cellular and circulating lipid levels, activation of PPARα has also been suggested to exert antidiabetic 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-κB, leading to the reduction of VCAM-1 in endothelial cells (72). In vascular smooth muscle cells, PPARα agonists inhibited interleukin (IL-1)-induced production of IL-6 and COX-2 expression (73). PPARα agonists also reduced the expression of tissue factor and matrix metalloproteinase in monocytes and macrophages, thereby decreasing thrombogenicity and plaque instability (74–76). On the other hand, some studies have suggested potential proatherogenic 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 the 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 tran-

scriptional activities in cells, including polyunsaturated fatty acids such as linoleic acid, dodecahexanoic acid, and eicosapentanoic acid (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 the 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 the incidence of fatal and nonfatal 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$^{-/-}$ mice exhibited less atherosclerosis than control apoE$^{-/-}$ mice, suggesting an atherogenic role of PPARα (82). PPARα$^{-/-}$ mice were found to be less insulin resistant and to have lower blood pressure compared with wild-type controls (82), potentially at least partially explaining the unexpected outcome. In another study of apoE$^{-/-}$ mice, treatment with ciprofibrate worsened diet-induced hyperlipidemia and increased atherosclerosis (83). In these studies, ciprofibrate treatment was associated with an increase in apoB-48-containing lipoproteins, suggesting an effect of PPARα on editing of the apoB mRNA. Other studies have found that PPARα agonists are antiatherogenic. In one study, fenofibrate had minimal antiatherogenic effects in apoE$^{-/-}$ mice (84, 85) but exerted a more pronounced effect in apoE$^{-/-}$ mice carrying a fenofibrate-inducible human apoA-I 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 with control mice. Although treatment of mice with this PPARα agonist induced the 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 macrophages 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 after 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$^{-/-}$ mice. ApoE is present in all lipoproteins except LDL and plays a role in the clearance of remnants and VLDL by the liver (reviewed in 88). ApoE 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 the 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 with fibrates currently in clinical use. The ability of GW7647 to inhibit foam cell formation without affecting cholesterol esterification or apoA-I- or HDL-dependent cholesterol efflux suggests that additional PPARα/LXR pathways controlling cholesterol homeostasis remain to be discovered.

PPARγ

PPARγ can be activated by a number of naturally occurring fatty acid metabolites, including oxidized linoleic acid (9- and 13-HODE) and 15-deoxy-$\Delta^{12,14}$-prostaglandin

J_2 (15, 90, 91). Endogenous ligands remain poorly characterized, however, and there is considerable evidence concerning the biological importance of agonist (15-deoxy-$\Delta^{2,14}$-prostaglandin J_2) (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 the activation of endogenous PPARγ target genes. The 12/15-lipoxygenase, which can generate 13-HODE from linoleic acid, has been suggested to mediate the 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 (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 attributable 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 PPARγ induces the 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 esterification (94, 108), and increases the expression of adiponectin (109, 110). Several lines of evidence have also linked inflammatory mediators, such as tumor necrosis factor-α (TNFα), interleukin-6, and resistan, to the insulin-resistant states associated with obesity and type 2 diabetes (reviewed in 111). Activators of PPARγ can inhibit a diverse array of inflammatory responses (reviewed in 112) and block TNFα-induced inhibition of insulin signaling (113), raising the possibility that some of the insulin-sensitizing actions of PPARγ relate to the 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 PPARγ in the 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 proinflammatory genes, including TNFα, IL-1β, iNOS, and gelatinase B (27, 115). A large number of inflammatory responses have been shown to be subject to negative regulation by PPARγ agonists (reviewed in 112, 116). Initial studies of PPARγ-deficient macrophages raised questions regarding whether PPARγ agonists exerted effects on inflammatory response genes through PPARγ-dependent or PPARγ-independent mechanisms (117). Further investigation established that both types of mechanisms contribute to these actions. For example, the PPARγ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 was shown to inhibit NF-κB signaling in a PPARγ-independent manner by covalently modifying IκB-kinase and NF-κB subunits (118, 119). High concentrations of synthetic PPARγ 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 the 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 the 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 the 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 the expression of CD36 protein in the context of the metabolic syndrome and thereby reduce the 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 suggests a posttranslational effect of rosiglitazone on CD36 via the insulin-signaling pathway. With respect to cholesterol efflux pathways, PPARα and PPARγ were shown to induce the expression of LXRα and thereby stimulate ABCA1-dependent cholesterol efflux to apoA-I (66, 126) (**Fig. 4**), analogous to the PPARα/LXRα/ABCA1 pathway

described above. The cytochrome P450 enzyme Cyp27, which catalyzes production of the weak LXR agonist 27-hydroxycholesterol, 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 (Fig. 4).

Large-scale clinical trials examining the effects of PPARγ agonists on cardiovascular end points have not been reported. Two small clinical studies found that treatment of diabetic patients with thiazolidinediones inhibited carotid intimal thickening (128, 129). Studies of the effects of PPARγ agonists in hypercholesterolemic male mice have consistently demonstrated anti-atherogenic effects despite an increased 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 attributable to downregulation of ACAT1 mRNA expression and may result from indirect effects on the transfer of cholesterol for esterification or posttranslational effects on ACAT1 activity. The effect of rosiglitazone on cholesterol esterification directly links PPARγ activity with the inhibition of macrophage foam cell formation in vitro. Although studies of ACAT1 deficiency 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 the 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 the 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 (Fig. 4).

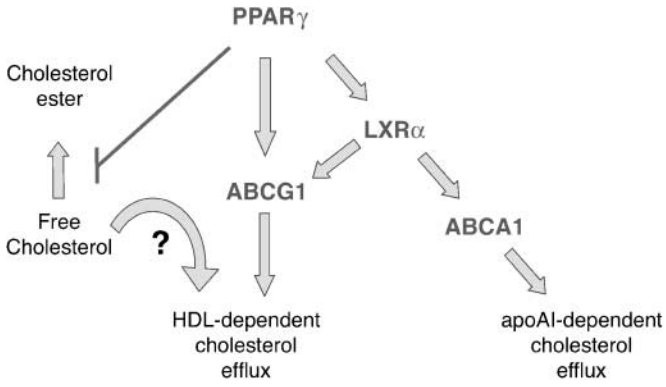

Fig. 4. Regulation of cholesterol efflux pathways in macrophages by PPARs and LXRs. LXR agonists stimulate the expression of ABCA1, which facilitates the efflux of cholesterol to lipid-poor apolipoprotein A-I (apoA-I), and ABCG1, which facilitates the efflux of cholesterol to HDL. PPARα and PPARγ agonists can induce the expression of LXRα, thereby stimulating cholesterol efflux to apoA-I 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 attributable to changes in ACAT expression and may result from altered cholesterol trafficking within the cell.

The finding that PPARα and PPARγ agonists inhibit foam cell formation raises the possibility that combined therapy or use of PPARα/γ coagonists 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 coagonists demonstrated combined improvements in insulin sensitivity as well as fatty acid, glucose, and lipoprotein metabolism (136, 137). The PPARα/γ coagonist GW2331 decreased atherosclerosis by 32% in apoE-deficient mice (84). Another PPARα/γ coagonist resulted in a 2.5-fold reduction in lesion area in hypercholesterolemic apoE$^{-/-}$ mice and was correlated with the inhibition of interferon-γ and β2 integrin CD11a gene expression (138).

PPARδ

PPARδ was the last of the PPARs to be identified (139). Gene deletion studies suggest important roles of PPARδ in skin biology, lipid metabolism, and energy homeostasis (140–143). Constitutive activation of PPARδ in adipose tissue led to an improvement of lipid profiles and a reduction of adiposity, whereas PPARδ-deficient mice exhibited a reduction in energy uncoupling and obesity (51, 140). The role of PPARδ 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), in skin fibroblasts (1BR3N), and in 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 PPARδ agonist promoted lipid accumulation in THP-1 cells and primary human macrophages (144). PPARδ was found to function as a lipid sensor of fatty acids derived from hydrolysis of VLDL-triglycerides, which in turn increased the expression of the adipophilin-related protein (145). In addition to its role in lipid metabolism, PPARδ also has anti-inflammatory properties. Treatment of endothelial cells with a PPARδ agonist decreased VCAM-1 and MCP-1 expression (146), and a PPARδ agonist inhibited LPS-inducible genes such as COX-2 and iNOS in murine peritoneal macrophages (120). PPARδ has been suggested to act as molecular switch between certain types of proinflammatory 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 derepression. 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 the development of atherosclerosis in mice. Transplantation of PPARδ$^{-/-}$ bone marrow progenitor cells into hypercholesterolemic LDLR$^{-/-}$ 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 apolipoprotein form of PPARδ to be anti-inflammatory, but it implies that PPARδ is unliganded in lesion macrophages. Our laboratories investigated the influence of a potent and selective PPARδ agonist on the development of atherosclerosis in hypercholesterolemic LDLR$^{-/-}$ 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 with 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 the 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.

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 the 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 antidiabetic actions. Surprises continue to emerge, such as the identification of new pathways for the 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 the 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 coagonists in the prevention of atherosclerosis will continue to be an important area for clinical investigation. Similarly, LXRs appear to hold significant promise as targets for new classes of antiatherogenic 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 LXRs can exert potent inhibitory effects on inflammatory responses also raises a number of interesting new directions connecting cholesterol metabolism with immune function.

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REFERENCES

1. Wilson, J. D. 1990. Charles-Edouard Brown-Sequard and the centennial of endocrinology. *J. Clin. Endocrinol. Metab.* **71:** 1403–1409.
2. Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science.* **240:** 889–895.
3. Hennekens, C. H. 1998. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. *Circulation.* **97:** 1095–1102.
4. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
5. Glass, C., and J. Witztum. 2001. Atherosclerosis: the road ahead. *Cell.* **104:** 503–516.
6. Gough, P. J., and S. Gordon. 2000. The role of scavenger receptors in the innate immune system. *Microbes Infect.* **2:** 305–311.
7. Boullier, A., D. A. Bird, M. K. Chang, E. A. Dennis, P. Friedman, K. Gillotre-Taylor, S. Horkko, W. Palinski, O. Quehenberger, P. Shaw, D. Steinberg, V. Terpstra, and J. L. Witztum. 2001. Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann. N.Y. Acad. Sci.* **947:** 214–223.
8. Linton, M. F., and S. Fazio. 2001. Class A scavenger receptors, macrophages, and atherosclerosis. *Curr. Opin. Lipidol.* **12:** 489–495.
9. Napoli, C., C. K. Glass, J. L. Witztum, R. Deutsch, F. P. D'Armiento, and W. Palinski. 1999. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood. Fate of Early Lesions in Children (FELIC) Study. *Lancet.* **354:** 1234–1241.
10. Hansson, G. K. 2001. Immune mechanisms in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1876–1890.
11. Libby, P. 2002. Inflammation in atherosclerosis. *Nature.* **420:** 868–874.
12. Kannel, W. B., and M. Larson. 1993. Long-term epidemiologic prediction of coronary disease. The Framingham experience. *Cardiology.* **82:** 137–152.
13. Grundy, S. M. 2002. Obesity, metabolic syndrome, and coronary atherosclerosis. *Circulation.* **105:** 2696–2698.
14. McDonnell, D. P. 1999. The molecular pharmacology of SERMs. *Trends Endocrinol. Metab.* **10:** 301–311.

15. Kliewer, S. A., S. S. Sundseth, S. A. Jones, P. J. Brown, G. B. Wisely, C. S. Koble, P. Devchand, W. Wahli, T. M. Willson, J. M. Lenhard, and J. M. Lehman. 1997. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. USA.* **94:** 4318–4323.
16. Peet, D. J., B. A. Janowski, and D. J. Mangelsdorf. 1998. The LXRs: a new class of oxysterol receptors. *Curr. Opin. Genet. Dev.* **8:** 571–575.
17. Janowski, B. A., M. J. Grogan, S. A. Jones, G. B. Wisely, S. A. Kliewer, E. J. Corey, and D. J. Mangelsdorf. 1999. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc. Natl. Acad. Sci. USA.* **96:** 266–271.
18. Rosenfeld, M. G., and C. K. Glass. 2001. Coregulator codes of transcriptional regulation by nuclear receptors. *J. Biol. Chem.* **276:** 36865–36868.
19. McKenna, N. J., and B. W. O'Malley. 2002. Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell.* **108:** 465–474.
20. Krogsdam, A. M., C. A. Nielsen, S. Neve, D. Holst, T. Helledie, B. Thomsen, C. Bendixen, S. Mandrup, and K. Kristiansen. 2002. Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor delta-mediated transactivation. *Biochem. J.* **363:** 157–165.
21. Shi, Y., M. Hon, and R. M. Evans. 2002. The peroxisome proliferator-activated receptor delta, an integrator of transcriptional repression and nuclear receptor signaling. *Proc. Natl. Acad. Sci. USA.* **99:** 2613–2618.
22. Wagner, B. L., A. F. Valledor, G. Shao, C. L. Daige, E. D. Bischoff, M. Petrowski, K. Jepsen, S. H. Baek, R. A. Heyman, M. G. Rosenfeld, I. G. Schulman, and C. K. Glass. 2003. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol. Cell. Biol.* **23:** 5780–5789.
23. Hu, X., S. Li, J. Wu, C. Xia, and D. S. Lala. 2003. Liver X receptors interact with corepressors to regulate gene expression. *Mol. Endocrinol.* **17:** 1019–1026.
24. Glass, C. K., and M. G. Rosenfeld. 2000. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev.* **14:** 121–141.
25. Perissi, V., A. Aggarwal, C. K. Glass, D. W. Rose, and M. G. Rosenfeld. 2004. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell.* **116:** 511–526.
26. De Bosscher, K., W. Vanden Berghe, and G. Haegeman. 2003. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr. Rev.* **24:** 488–522.
27. Ricote, M., A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass. 1998. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature.* **391:** 79–82.
28. Joseph, S. B., A. Castrillo, B. A. Laffitte, D. J. Mangelsdorf, and P. Tontonoz. 2003. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat. Med.* **9:** 213–219.
29. Laffitte, B. A., S. B. Joseph, R. Walczak, L. Pei, D. C. Wilpitz, J. L. Collins, and P. Tontonoz. 2001. Autoregulation of the human liver X receptor alpha promoter. *Mol. Cell. Biol.* **21:** 7558–7568.
30. Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J-M. A. Lobaccaro, R. E. Hammer, and D. J. Mangelsdorf. 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα. *Cell.* **93:** 693–704.
31. Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Richardson, H. Hobbs, and D. J. Mangelsdorf. 2002. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. *J. Biol. Chem.* **277:** 18793–18800.
32. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J-M. A. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c (SREBP-1c) by oxysterol receptors LXRα and LXRβ. *Genes Dev.* **14:** 2819–2830.
33. Zhang, Y., J. J. Repa, K. Gauthier, and D. J. Mangelsdorf. 2001. Regulation of lipoprotein lipase by the oxysterol receptors, LXR-alpha and LXRbeta. *J. Biol. Chem.* **276:** 43018–43024.
34. Mak, P. A., H. R. Kast-Woelbern, A. M. Anisfeld, and P. A. Edwards. 2002. Identification of PLTP as an LXR target gene and apoE as an FXR target gene reveals overlapping targets for the two nuclear receptors. *J. Lipid Res.* **43:** 2037–2041.
35. Mak, P. A., B. A. Laffitte, C. Desrumaux, S. B. Joseph, L. K. Curtiss, D. J. Mangelsdorf, P. Tontonoz, and P. A. Edwards. 2002. Regulated expression of the apolipoprotein E/C-I/C-IV/C-II gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors alpha and beta. *J. Biol. Chem.* **277:** 31900–31908.
36. Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, and B. Shan. 2000. Role of LXRs in control of lipogenesis. *Genes Dev.* **14:** 2831–2838.
37. Oram, J. F., and A. M. Vaughan. 2000. ABCA1-mediated transport of cellular cholesterol and phospholipids to HDL apolipoproteins. *Curr. Opin. Lipidol.* **11:** 253–260.
38. Schmitz, G., W. E. Kaminski, and E. Orso. 2000. ABC transporters in cellular lipid trafficking. *Curr. Opin. Lipidol.* **11:** 493–501.
39. Attie, A. D., J. P. Kastelein, and M. R. Hayden. 2001. Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis. *J. Lipid Res.* **42:** 1717–1726.
40. Young, S. G., and C. J. Fielding. 1999. The ABCs of cholesterol efflux. *Nat. Genet.* **22:** 316–318.
41. McNeish, J., R. J. Aiello, D. Guyot, T. Turi, C. Gabel, C. Aldinger, K. L. Hoppe, M. L. Roach, L. J. Royer, J. de Wet, C. Broccardo, G. Chimini, and O. L. Francone. 2000. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci. USA.* **97:** 4245–4250.
42. Singaraja, R. R., C. Fievet, G. Castro, E. R. James, N. Hennuyer, S. M. Clee, N. Bissada, J. C. Choy, J. C. Fruchart, B. M. McManus, B. Staels, and M. R. Hayden. 2002. Increased ABCA1 activity protects against atherosclerosis. *J. Clin. Invest.* **110:** 35–42.
43. Joyce, C. W., M. J. Amar, G. Lambert, B. L. Vaisman, B. Paigen, J. Najib-Fruchart, R. F. Hoyt, Jr., E. D. Neufeld, A. T. Remaley, D. S. Fredrickson, H. B. Brewer, Jr., and S. Santamarina-Fojo. 2002. The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. *Proc. Natl. Acad. Sci. USA.* **99:** 407–412.
44. Whitney, K. D., M. A. Watson, B. Goodwin, C. M. Galardi, J. M. Maglich, J. G. Wilson, T. M. Willson, J. L. Collins, and S. A. Kliewer. 2001. Liver X receptor (LXR) regulation of the LXRalpha gene in human macrophages. *J. Biol. Chem.* **276:** 43509–43515.
45. Schwartz, K., R. M. Lawn, and D. P. Wade. 2000. ABC1 gene expression and apoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem. Biophys. Res. Commun.* **274:** 794–802.
46. Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275:** 28240–28245.
47. Venkateswaran, A., J. J. Repa, J. M. Lobaccaro, A. Bronson, D. J. Mangelsdorf, and P. A. Edwards. 2000. Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. *J. Biol. Chem.* **275:** 14700–14707.
48. Laffitte, B. A., J. J. Repa, S. B. Joseph, D. C. Wilpitz, H. R. Kast, D. J. Mangelsdorf, and P. Tontonoz. 2001. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc. Natl. Acad. Sci. USA.* **98:** 507–512.
49. Klucken, J., C. Buchler, E. Orso, W. E. Kaminski, M. Porsch-Ozcurumez, G. Liebisch, M. Kapinsky, W. Diederich, W. Drobnik, M. Dean, R. Allikmets, and G. Schmitz. 2000. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. *Proc. Natl. Acad. Sci. USA.* **97:** 817–822.
50. Lorkowski, S., M. Kratz, C. Wenner, R. Schmidt, B. Weitkamp, M. Fobker, J. Reinhardt, J. Rauterberg, E. A. Galinski, and P. Cullen. 2001. Expression of the ATP-binding cassette transporter gene ABCG1 (ABC8) in Tangier disease. *Biochem. Biophys. Res. Commun.* **283:** 821–830.
51. Wang, N., D. Lan, W. Chen, F. Matsuura, and A. R. Tall. 2004. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **101:** 9774–9779.
52. Nakamura, K., M. A. Kennedy, A. Baldan, D. D. Bojanic, K. Lyons, and P. A. Edwards. 2004. Expression and regulation of multiple murine ATP-binding cassette transporter G1 mRNAs/isoforms that stimulate cellular cholesterol efflux to high density lipoprotein. *J. Biol. Chem.* In press.
53. Fu, X., J. G. Menke, Y. Chen, G. Zhou, K. L. MacNaul, S. D. Wright, C. P. Sparrow, and E. G. Lund. 2001. 27-Hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. *J. Biol. Chem.* **276:** 38378–38387.

54. Joseph, S. B., E. McKilligin, L. Pei, M. A. Watson, A. R. Collins, B. A. Laffitte, M. Chen, G. Noh, J. Goodman, G. N. Hagger, J. Tran, T. K. Tippin, X. Wang, A. J. Lusis, W. A. Hsueh, R. E. Law, J. L. Collins, T. M. Willson, and P. Tontonoz. 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. USA.* **99:** 7604–7609.
55. Tangirala, R. K., E. D. Bischoff, S. B. Joseph, B. L. Wagner, R. Walczak, B. A. Laffitte, C. L. Daige, D. Thomas, R. A. Heyman, D. J. Mangelsdorf, X. Wang, A. J. Lusis, P. Tontonoz, and I. G. Schulman. 2002. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **99:** 11896–11901.
56. Castrillo, A., S. B. Joseph, C. Marathe, D. J. Mangelsdorf, and P. Tontonoz. 2003. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.* **278:** 10443–10449.
57. Desvergne, B., and W. Wahli. 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20:** 649–688.
58. Willson, T., P. Brown, D. Sternbach, and B. Henke. 2000. The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.* **43:** 527–550.
59. Issemann, I., and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* **347:** 645–650.
60. Issemann, I., R. A. Prince, J. D. Tugwood, and S. Green. 1993. The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J. Mol. Endocrinol.* **11:** 37–47.
61. Schoonjans, K., M. Watanabe, H. Suzuki, A. Mahfoudi, G. Krey, W. Wahli, P. Grimaldi, B. Staels, T. Yamamoto, and J. Auwerx. 1995. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J. Biol. Chem.* **270:** 19269–19276.
62. Motojima, K., P. Passilly, J. M. Peters, F. J. Gonzalez, and N. Latruffe. 1998. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J. Biol. Chem.* **273:** 16710–16714.
63. Rodriguez, J. C., G. Gil-Gomez, F. G. Hegardt, and D. Haro. 1994. Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J. Biol. Chem.* **269:** 18767–18772.
64. Berthou, L., N. Duverger, F. Emmanuel, S. Langouet, J. Auwerx, A. Guillouzo, J. C. Fruchart, E. Rubin, P. Denefle, B. Staels, and D. Branellec. 1996. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J. Clin. Invest.* **97:** 2408–2416.
65. Vu-Dac, N., S. Chopin-Delannoy, P. Gervois, E. Bonnelye, G. Martin, J. C. Fruchart, V. Laudet, and B. Staels. 1998. The nuclear receptors peroxisome proliferator-activated receptor alpha and Rev-erb-alpha mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J. Biol. Chem.* **273:** 25713–25720.
66. Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H. B. Brewer, J. C. Fruchart, V. Clavey, and B. Staels. 2001. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7:** 53–58.
67. Chinetti, G., F. G. Gbaguidi, S. Griglio, Z. Mallat, M. Antonucci, P. Poulain, J. Chapman, J. C. Fruchart, A. Tedgui, J. Najib-Fruchart, and B. Staels. 2000. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation.* **101:** 2411–2417.
68. Chinetti, G., S. Lestavel, J. C. Fruchart, V. Clavey, and B. Staels. 2003. Peroxisome proliferator-activated receptor alpha reduces cholesterol esterification in macrophages. *Circ. Res.* **92:** 212–217.
69. Guerre-Millo, M., P. Gervois, E. Raspe, L. Madsen, P. Poulain, B. Derudas, J. M. Herbert, D. A. Winegar, T. M. Willson, J. C. Fruchart, R. K. Berge, and B. Staels. 2000. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J. Biol. Chem.* **275:** 16638–16642.
70. Ye, J. M., P. J. Doyle, M. A. Iglesias, D. G. Watson, G. J. Cooney, and E. W. Kraegen. 2001. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes.* **50:** 411–417.
71. Chou, C. J., M. Haluzik, C. Gregory, K. R. Dietz, C. Vinson, O. Gavrilova, and M. L. Reitman. 2002. WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *J. Biol. Chem.* **277:** 24484–24489.
72. Marx, N., G. K. Sukhova, T. Collins, P. Libby, and J. Plutzky. 1999. PPARalpha activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. *Circulation.* **99:** 3125–3131.
73. Staels, B., W. Koenig, A. Habib, R. Merval, M. Lebret, I. P. Torra, P. Delerive, A. Fadel, G. Chinetti, J. C. Fruchart, J. Najib, J. Maclouf, and A. Tedgui. 1998. Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. *Nature.* **393:** 790–793.
74. Marx, N., N. Mackman, U. Schonbeck, N. Yilmaz, V. Hombach, P. Libby, and J. Plutzky. 2001. PPARalpha activators inhibit tissue factor expression and activity in human monocytes. *Circulation.* **103:** 213–219.
75. Neve, B. P., D. Corseaux, G. Chinetti, C. Zawadzki, J. C. Fruchart, P. Duriez, B. Staels, and B. Jude. 2001. PPARalpha agonists inhibit tissue factor expression in human monocytes and macrophages. *Circulation.* **103:** 207–212.
76. Shu, H., B. Wong, G. Zhou, Y. Li, J. Berger, J. W. Woods, S. D. Wright, and T. Q. Cai. 2000. Activation of PPARalpha or gamma reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem. Biophys. Res. Commun.* **267:** 345–349.
77. Lee, H., W. Shi, P. Tontonoz, S. Wang, G. Subbanagounder, C. C. Hedrick, S. Hama, C. Borromeo, R. M. Evans, J. A. Berliner, and L. Nagy. 2000. Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. *Circ. Res.* **87:** 516–521.
78. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA.* **94:** 4312–4317.
79. Ziouzenkova, O., S. Perrey, L. Asatryan, J. Hwang, K. L. MacNaul, D. E. Moller, D. J. Rader, A. Sevanian, R. Zechner, G. Hoefler, and J. Plutzky. 2003. Lipolysis of triglyceride-rich lipoproteins generates PPAR ligands: evidence for an antiinflammatory role for lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* **100:** 2730–2735.
80. Robins, S. J., D. Collins, J. T. Wittes, V. Papademetriou, P. C. Deedwania, E. J. Schaefer, J. R. McNamara, M. L. Kashyap, J. M. Hershman, L. F. Wexler, and H. B. Rubins. 2001. Relation of gemfibrozil treatment and lipid levels with major coronary events. VA-HIT: a randomized controlled trial. *J. Am. Med. Assoc.* **285:** 1585–1591.
81. Robins, S. J., H. B. Rubins, F. H. Faas, E. J. Schaefer, M. B. Elam, J. W. Anderson, and D. Collins. 2003. Insulin resistance and cardiovascular events with low HDL cholesterol. The Veterans Affairs HDL Intervention Trial (VA-HIT). *Diabetes Care.* **26:** 1513–1517.
82. Tordjman, K., C. Bernal-Mizrachi, L. Zemany, S. Weng, C. Feng, F. Zhang, T. C. Leone, T. Coleman, D. P. Kelly, and C. F. Semenkovich. 2001. PPARalpha deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. *J. Clin. Invest.* **107:** 1025–1034.
83. Fu, T., P. Kashireddy, and J. Borensztajn. 2003. The peroxisome-proliferator-activated receptor alpha agonist ciprofibrate severely aggravates hypercholesterolaemia and accelerates the development of atherosclerosis in mice lacking apolipoprotein E. *Biochem. J.* **373:** 941–947.
84. Claudel, T., M. D. Leibowitz, C. Fievet, A. Tailleux, B. Wagner, J. J. Repa, G. Torpier, J. M. Lobaccaro, J. R. Paterniti, D. J. Mangelsdorf, R. A. Heyman, and J. Auwerx. 2001. Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor. *Proc. Natl. Acad. Sci. USA.* **98:** 2610–2615.
85. Duez, H., Y. S. Chao, M. Hernandez, G. Torpier, P. Poulain, S. Mundt, Z. Mallat, E. Teissier, C. A. Burton, A. Tedgui, J. C. Fruchart, C. Fievet, S. D. Wright, and B. Staels. 2002. Reduction of atherosclerosis by the peroxisome proliferator-activated receptor alpha agonist fenofibrate in mice. *J. Biol. Chem.* **277:** 48051–48057.
86. Li, A. C., C. J. Binder, A. Gutierrez, K. K. Brown, C. R. Plotkin, J. W. Pattison, A. F. Valledor, R. A. Davis, T. M. Willson, J. L. Witztum, W. Palinski, and C. K. Glass. 2004. Differential inhibition of macrophage foam cell formation and atherosclerosis in mice by PPAR a, b/d, and g. *J. Clin. Invest.* In press.
87. Chinetti, G., S. Griglio, M. Antonucci, I. P. Torra, P. Delerive, Z.

Majd, J-C. Fruchart, J. Chapman, J. Najib, and B. Staels. 1998. Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. *J. Biol. Chem.* **273:** 25573–25580.
88. Reardon, C. A., and G. S. Getz. 2001. Mouse models of atherosclerosis. *Curr. Opin. Lipidol.* **12:** 167–173.
89. Kliewer, S. A., J. M., Lehmann, M. V. Milburn, and T. M. Willson. 1999. The PPARs and PXRs: nuclear xenobiotic receptors that define novel hormone signaling pathways. *Recent Prog. Horm. Res.* **54:** 345–368.
90. Nagy, L., P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans. 1998. Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR-gamma. *Cell.* **93:** 229–240.
91. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. *Cell.* **83:** 803–812.
92. Bell-Parikh, L. C., T. Ide, J. A. Lawson, P. McNamara, M. Reilly, and G. A. FitzGerald. 2003. Biosynthesis of 15-deoxy-delta12,14-PGJ2 and the ligation of PPARgamma. *J. Clin. Invest.* **112:** 945–955.
93. Huang, J. T., J. S. Welch, M. Ricote, C. J. Binder, T. M. Willson, C. Kelly, J. L. Witztum, C. D. Funk, D. Conrad, and C. K. Glass. 1999. Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. *Nature.* **400:** 378–382.
94. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **8:** 1224–1234.
95. Zhu, Y., C. Qi, J. R. Korenberg, X. N. Chen, D. Noya, M. S. Rao, and J. K. Reddy. 1995. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc. Natl. Acad. Sci. USA.* **92:** 7921–7925.
96. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell.* **79:** 1147–1156.
97. Barak, Y., M. C. Nelson, E. S. Ong, Y. Z. Jones, P. Ruiz-Lozano, K. R. Chien, A. Koder, and R. M. Evans. 1999. PPARγ is required for placental, cardiac, and adipose tissue development. *Mol. Cell.* **4:** 585–595.
98. Kubota, N., Y. Terauchi, H. Miki, H. Tamemoto, T. Yamauchi, K. Komeda, S. Satoh, R. Nakano, C. Ishii, T. Sugiyama, K. Eto, Y. Tsubamaoto, A. Okuno, K. Murakami, H. Sekihara, G. Hasegawa, M. Naito, Y. Toyoshima, S. Tanaka, K. Shiota, T. Kitamura, T. Fujita, O. Ezaki, S. Aizawa, R. Nagai, K. Tobe, S. Kimura, and T. Kadowaki. 1999. PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell.* **4:** 597–609.
99. Rosen, E. D., P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. A. Milstone, B. M. Spiegelman, and R. M. Mortensen. 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. *Mol. Cell.* **4:** 611–617.
100. Lehmann, J. M., L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPARgamma). *J. Biol. Chem.* **270:** 12953–12956.
101. Gurnell, M., D. B. Savage, V. K. Chatterjee, and S. O'Rahilly. 2003. The metabolic syndrome: peroxisome proliferator-activated receptor gamma and its therapeutic modulation. *J. Clin. Endocrinol. Metab.* **88:** 2412–2421.
102. He, W., Y. Barak, A. Hevener, P. Olson, D. Liao, J. Le, M. Nelson, E. Ong, J. M. Olefsky, and R. M. Evans. 2003. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl. Acad. Sci. USA.* **100:** 15712–15717.
103. Hevener, A. L., W. He, Y. Barak, J. Le, G. Bandyopadhyay, P. Olson, J. Wilkes, R. M. Evans, and J. Olefsky. 2003. Muscle-specific PPARg deletion causes insulin resistance. *Nat. Med.* **9:** 1491–1497.
104. Norris, A. W., L. Chen, S. J. Fisher, I. Szanto, M. Ristow, A. C. Jozsi, M. F. Hirshman, E. D. Rosen, L. J. Goodyear, F. J. Gonzalez, B. M. Spiegelman, and C. R. Kahn. 2003. Muscle-specific PPARgamma-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *J. Clin. Invest.* **112:** 608–618.
105. Gavrilova, O., M. Haluzik, K. Matsusue, J. J. Cutson, L. Johnson, K. R. Dietz, C. J. Nicol, C. Vinson, F. J. Gonzalez, and M. L. Reitman. 2003. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J. Biol. Chem.* **278:** 34268–34276.
106. Wu, Z., Y. Xie, R. F. Morrison, N. L. Bucher, and S. R. Farmer. 1998. PPARgamma induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPalpha during the conversion of 3T3 fibroblasts into adipocytes. *J. Clin. Invest.* **101:** 22–32.
107. Lefebvre, A-M., J. Peinado-Onsurbe, I. Leitersdorf, M. R. Briggs, J. R. Paterniti, J-C. Fruchart, C. Fievet, J. Auwerx, and B. Staels. 1997. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler. Thromb.* **17:** 1756–1764.
108. Brun, R. P., P. Tontonoz, B. M. Forman, R. Ellis, J. Chen, R. M. Evans, and B. M. Spiegelman. 1996. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev.* **10:** 974–984.
109. Maeda, N., M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida, H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura, and Y. Matsuzawa. 2001. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes.* **50:** 2094–2099.
110. Yamauchi, T., J. Kamon, H. Waki, K. Murakami, K. Motojima, K. Komeda, T. Ide, N. Kubota, Y. Terauchi, K. Tobe, H. Miki, A. Tsuchida, Y. Akanuma, R. Nagai, S. Kimura, and T. Kadowaki. 2001. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J. Biol. Chem.* **276:** 41245–41254.
111. Dandona, P., A. Aljada, and A. Bandyopadhyay. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* **25:** 4–7.
112. Daynes, R. A., and D. C. Jones. 2002. Emerging roles of PPARs in inflammation and immunity. *Nat. Rev. Immunol.* **2:** 748–759.
113. Peraldi, P., M. Xu, and B. M. Spiegelman. 1997. Thiazolidinediones block tumor necrosis factor-alpha-induced inhibition of insulin signaling. *J. Clin. Invest.* **100:** 1863–1869.
114. Haffner, S. M., A. S. Greenberg, W. M. Weston, H. Chen, K. Williams, and M. I. Freed. 2002. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation.* **106:** 679–684.
115. Jiang, C., A. T. Ting, and B. Seed. 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature.* **391:** 82–86.
116. Ricote, M., A. F. Valledor, and C. K. Glass. 2004. Decoding transcriptional programs regulated by PPARs and LXRs in the macrophage: effects on lipid homeostasis, inflammation, and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **24:** 230–239.
117. Chawla, A., Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans. 2001. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat. Med.* **7:** 48–52.
118. Straus, D. S., G. Pascual, M. Li, J. Welch, M. Ricote, C. H. Hsiang, L. L. Sengchanthalansgsy, G. Ghosh, and C. K. Glass. 2000. 15-Deoxy-delta^{12,14}-prostaglandin J_2 inhibits multiple steps in the NF-kB signaling pathway. *Proc. Natl. Acad. Sci. USA.* **97:** 4844–4849.
119. Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, and M. G. Santoro. 2000. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature.* **403:** 103–108.
120. Welch, J. S., M. Ricote, T. E. Akiyama, F. J. Gonzalez, and C. K. Glass. 2003. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc. Natl. Acad. Sci. USA.* **100:** 6712–6717.
121. Wellen, K. E., and G. S. Hotamisligil. 2003. Obesity-induced inflammatory changes in adipose tissue. *J. Clin. Invest.* **112:** 1785–1788.
122. Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A. Tartaglia, and H. Chen. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **112:** 1821–1830.
123. Tontonoz, P., L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans. 1998. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* **93:** 241–252.
124. Febbraio, M., D. P. Hajjar, and R. L. Silverstein. 2001. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J. Clin. Invest.* **108:** 785–791.
125. Liang, C. P., S. Han, H. Okamoto, R. Carnemolla, I. Tabas, D. Accili, and A. R. Tall. 2004. Increased CD36 protein as a response to defective insulin signaling in macrophages. *J. Clin. Invest.* **113:** 764–773.
126. Chawla, A., W. A. Boisvert, C. H. Lee, B. A. Laffitte, Y. Barak, S. B. Joseph, D. Liao, L. Nagy, P. A. Edwards, L. K. Curtiss, R. M. Evans, and P. Tontonoz. 2001. A PPAR gamma-LXR-ABCA1 pathway in

macrophages is involved in cholesterol efflux and atherogenesis. *Mol. Cell.* **7:** 161–171.
127. Szanto, A., S. Benko, I. Szatmari, B. L. Balint, I. Furtos, R. Ruhl, S. Molnar, L. Csiba, R. Garuti, S. Calandra, H. Larsson, U. Diczfalusy, and L. Nagy. 2004. Transcriptional regulation of human CYP27 integrates retinoid, peroxisome proliferator-activated receptor, and liver X receptor signaling in macrophages. *Mol. Cell. Biol.* **24:** 8154–8166.
128. Minamikawa, J., S. Tanaka, M. Yamauchi, D. Inoue, and H. Koshiyama. 1998. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes. *J. Clin. Endocrinol. Metab.* **83:** 1818–1820.
129. Koshiyama, H., D. Shimono, N. Kuwamura, J. Minamikawa, and Y. Nakamura. 2001. Inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes. *J. Clin. Endocrinol. Metab.* **86:** 3452–3456.
130. Li, A., K. Brown, M. Silvestre, T. Willson, W. Palinski, and C. Glass. 2000. Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J. CIin. Invest.* **106:** 523–531.
131. Collins, A. R., W. P. Meehan, U. Kintscher, S. Jackson, S. Wakino, G. Noh, W. Palinski, W. A. Hsueh, and R. E. Law. 2001. Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and nondiabetic low density lipoprotein receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **21:** 365–371.
132. Chen, Z., S. Ishibashi, S. Perrey, J. Osuga, T. Gotoda, T. Kitamine, Y. Tamura, H. Okazaki, N. Yahagi, Y. Iizuka, F. Shionoiri, K. Ohashi, K. Harada, H. Shimano, R. Nagai, and N. Yamada. 2001. Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression and HDL. *Arterioscler. Thromb. Vasc. Biol.* **21:** 372–377.
133. Yagu, H., T. Kitamine, J. Osuga, R. Tozawa, Z. Chen, Y. Kaji, T. Oka, S. Perrey, Y. Tamura, K. Ohyashi, H. Okazaki, N. Yahagi, F. Shionoiri, Y. Iizuka, K. Harada, H. Shimano, H. Yamashita, T. Gotoda, N. Yamada, and S. Ishibashi. 2000. Absence of ACAT-1 at tenuates atherosclerosis but causes dry eye and cutaneous xanthomatosis in mice with congenital hyperlipidemia. *J. Biol. Chem.* **275:** 21324–21330.
134. Fazio, S., A. S. Major, L. L. Swift, L. A. Gleaves, M. Accad, M. F. Linton, and R. V. Farese, Jr. 2001. Increased atherosclerosis in LDL receptor-null mice lacking ACAT1 in macrophages. *J. Clin. Invest.* **107:** 163–171.
135. Kusunoki, J., D. K. Hansoty, K. Aragane, J. T. Fallon, J. J. Badimon, and E. A. Fisher. 2001. Acyl-CoA:cholesterol acyltransferase inhibition reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation.* **103:** 2604–2609.
136. Sauerberg, P., I. Pettersson, L. Jeppesen, P. S. Bury, J. P. Mogensen, K. Wassermann, C. L. Brand, J. Sturis, H. F. Woldike, J. Fleckner, A. S. Andersen, S. B. Mortensen, L. A. Svensson, H. B. Rasmussen, S. V. Lehmann, Z. Polivka, K. Sindelar, V. Panajotova, L. Ynddal, and E. M. Wulff. 2002. Novel tricyclic-alpha-alkyloxyphenylpropionic acids: dual PPARalpha/gamma agonists with hypolipidemic and antidiabetic activity. *J. Med. Chem.* **45:** 789–804.
137. Ye, J. M., M. A. Iglesias, D. G. Watson, B. Ellis, L. Wood, P. B. Jensen, R. V. Sorensen, P. J. Larsen, G. J. Cooney, K. Wassermann, and E. W. Kraegen. 2003. PPARalpha/gamma ragaglitazar eliminates fatty liver and enhances insulin action in fat-fed rats in the absence of hepatomegaly. *Am. J. Physiol. Endocrinol. Metab.* **284:** E531–E540.
138. Zuckerman, S. H., R. F. Kauffman, and G. F. Evans. 2002. Peroxisome proliferator-activated receptor alpha,gamma coagonist LY465608 inhibits macrophage activation and atherosclerosis in apolipoprotein E knockout mice. *Lipids.* **37:** 487–494.
139. Kliewer, S. A., B. M. Forman, B. Blumberg, E. S. Ong, U. Borgmeyer, D. J. Mangelsdorf, K. Umesono, and R. M. Evans. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA.* **91:** 7355–7359.
140. Wang, Y. X., C. H. Lee, S. Tiep, R. T. Yu, J. Ham, H. Kang, and R. M. Evans. 2003. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell.* **113:** 159–170.
141. Oliver, W. R., Jr., J. L. Shenk, M. R. Snaith, C. S. Russell, K. D. Plunket, N. L. Bodkin, M. C. Lewis, D. A. Winegar, M. L. Sznaidman, M. H. Lambert, H. E. Xu, D. D. Sternbach, S. A. Kliewer, B. C. Hansen, and T. M. Willson. 2001. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc. Natl. Acad. Sci. USA.* **98:** 5306–5311.
142. Michalik, L., B. Desvergne, N. S. Tan, S. Basu-Modak, P. Escher, J. Rieusset, J. M. Peters, G. Kaya, F. J. Gonzalez, J. Zakany, D. Metzger, P. Chambon, D. Duboule, and W. Wahli. 2001. Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J. Cell Biol.* **154:** 799–814.
143. Tan, N. S., L. Michalik, N. Noy, R. Yasmin, C. Pacot, M. Heim, B. Fluhmann, B. Desvergne, and W. Wahli. 2001. Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes Dev.* **15:** 3263–3277.
144. Vosper, H., L. Patel, T. L. Graham, G. A. Khoudoli, A. Hill, C. H. Macphee, I. Pinto, S. A. Smith, K. E. Suckling, C. R. Wolf, and C. N. Palmer. 2001. The peroxisome proliferator-activated receptor delta promotes lipid accumulation in human macrophages. *J. Biol. Chem.* **276:** 44258–44265.
145. Chawla, A., C. H. Lee, Y. Barak, W. He, J. Rosenfeld, D. Liao, J. Han, H. Kang, and R. M. Evans. 2003. PPARdelta is a very low-density lipoprotein sensor in macrophages. *Proc. Natl. Acad. Sci. USA.* **100:** 1268–1273.
146. Rival, Y., N. Beneteau, T. Taillandier, M. Pezet, E. Dupont-Passelaigue, J. F. Patoiseau, D. Junquero, F. C. Colpaert, and A. Delhon. 2002. PPARalpha and PPARdelta activators inhibit cytokine-induced nuclear translocation of NF-kappaB and expression of VCAM-1 in EAhy926 endothelial cells. *Eur. J. Pharmacol.* **435:** 143–151.
147. Lee, C. H., A. Chawla, N. Urbiztondo, D. Liao, W. A. Boisvert, and R. M. Evans. 2003. Transcriptional repression of atherogenic inflammation: modulation by PPARdelta. *Science.* **302:** 453–457.