This Review is part of a thematic series on **Nuclear Receptors**, which includes the following articles:

- Introduction to the Nuclear Receptor Review Series [*Circ Res.* 2010;106:1557–1558]
- Control of Macrophage Activation and Function by PPARs [*Circ Res.* 2010;106:1559–1569]
- PGC Coactivators in the Developing and Diseased Heart [*Circ Res.* 2010;107:825–838]

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**The PPAR-RXR Transcriptional Complex in the Vasculature: Energy in the Balance**

Estradiol Receptor Signaling and Cardiovascular Disease

*Daniel P. Kelly, Guest Editor*

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**The PPAR-RXR Transcriptional Complex in the Vasculature**

**Energy in the Balance**

*Jorge Plutzky*

**Abstract:** The peroxisome proliferator-activated receptors (PPARs) and the retinoid X receptors (RXRs) are ligand-activated transcription factors that coordinately regulate gene expression. This PPAR-RXR transcriptional complex plays a critical role in energy balance, including triglyceride metabolism, fatty acid handling and storage, and glucose homeostasis: processes whose dysregulation characterize obesity, diabetes, and atherosclerosis. PPARs and RXRs are also involved directly in inflammatory and vascular responses in endothelial and vascular smooth muscle cells. New insights into fundamental aspects of PPAR and RXR biology, and their actions in the vasculature, continue to appear. Although RXRs are obligate heterodimeric partners for PPAR action, the part that RXRs, and their endogenous retinoid mediators, exert in the vessel wall is less well understood. Biological insights into PPAR-RXRs may help inform interpretation of clinical trials with synthetic PPAR agonists and prospects for future PPAR therapeutics. Importantly, the extensive data establishing a key role for PPARs and RXRs in energy balance, inflammation, and vascular biology stands separately from the clinical experience with any given synthetic PPAR agonist. Both the basic science data and the clinical experience with PPAR agonists identify the need to better understand these important transcriptional regulators. (*Circ Res.* 2011;108:1002-1016.)

**Key Words:** atherosclerosis ■ diabetes ■ PPARs ■ RXRs ■ gene expression

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Energy balance is a fundamental necessity and, as such, is relevant to many disciplines. In physics, energy balance considers energy flow and transformation across different states. Engineering studies energy balance in closed systems, like an automobile, and the distance traveled for the fuel consumed. In geophysics, the energy required to extract a fuel from the earth is set against the energy that fuel yields. For nations and their economies, the energy available is balanced against rates of consumption and cost. Arguably, biology provides the most fundamental context for energy balance: survival, which is predicated on obtaining, using, and storing energy resources. From this perspective, it is striking that the increasingly prevalent chronic diseases facing industrial countries, namely obesity, diabetes, dyslipidemia, and atherosclerosis, are either characterized or defined by abnormalities of energy resources.

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In general, 2 fuels serve as the main energy resources for most organisms, namely glucose and fatty acids (Figure 1). The amount of energy derived from glucose (4 kcal/g) is less than half the energy obtained from the combustion of a fatty acid (9 kcal/g). Fatty acids are an optimal energy resource, combining high energy yields, a close packing structure, and an anhydrous nature that requires less water for storage, making them light and portable. Indeed, a 70 kg man carries ~135 000 kcal of energy in fatty acids in fat versus 80 kcal in glucose and 760 kcal in glycogen.

Given their value in survival, the need for carefully handling fatty acids becomes obvious, as evident in the elaborate system for their transport within triglycerides (three fatty acids attached to a glycerol backbone), circulation in specific lipoproteins, chaperoning by fatty acid binding proteins, release by distinct lipases, and combustion by oxidative enzymes. Likewise, the need to expand storage depots of fatty acids as triglycerides in adipose tissue is critical for survival. At the same time, glucose is also essential for survival, serving as a rapidly accessible source of energy.

The nuclear receptor transcription factors peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) are at the center of the complex network that controls the utilization and storage of energy (Figure 1). Coordinated regulation of transcription by nuclear receptors is a recurring theme in the control of metabolism. For PPARs and RXRs, these coordinated effects may extend to the vasculature, where they are also present and help regulate inflammation and atherosclerosis. Although PPARs are established drug targets for treating dyslipidemia and diabetes, the clinical use of PPAR agonists has been controversial, including unexpected if not untoward clinical effects. RXR agonists are also used clinically, primarily for certain leukemias and skin conditions. The complicated clinical experience with PPAR agonists does not obviate the well-established importance of these nuclear receptors but does define the need to better understand their biology and vascular effects: the subject of this review. The extent of this PPAR-RXR data are beyond the scope of any one review; a Medline search of the terms of “PPAR” and “cardiovascular” in yields 478 primary data publications in 2009 alone. The focus here is on existing evidence for the role of PPARs and RXRs in vascular biology and atherosclerosis, highlighting more recent fundamental advances in their biology. How new insights into PPAR-RXR biology might help inform PPAR

Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>9cRA</td>
<td>9 cis retinoic acid</td>
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<tr>
<td>ADH</td>
<td>alcohol dehydrogenase</td>
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<tr>
<td>ATRA</td>
<td>all trans retinoic acid</td>
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<tr>
<td>BCD02</td>
<td>β-carotene-9,10'-dioxygenase 2</td>
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<td>CDK5</td>
<td>cyclin-dependent kinase 5</td>
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<tr>
<td>EC</td>
<td>endothelial cell</td>
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<tr>
<td>GPIHBP1</td>
<td>glycosylphosphatidylinositol-anchored HDL-binding protein 1</td>
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<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>LDL</td>
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<td>LPL</td>
<td>lipoprotein lipase</td>
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<td>NCoR</td>
<td>nuclear receptor corepressor</td>
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<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
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<td>RA</td>
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<td>RALD</td>
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<td>RALDH</td>
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<td>RXR</td>
<td>retinoid X receptor</td>
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<td>SUMO</td>
<td>small ubiquitin-like modifier</td>
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<td>T2D</td>
<td>thiazolidinedione</td>
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<td>VSMC</td>
<td>vascular smooth muscle cell</td>
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Figure 1. Energy balance in health and disease. Energy balance, which involves the management of energy resources is at the core of organismal survival (center ring, black) and closely related to cardiometabolic disorders (outer ring, red). Fatty acid combustion, as occurs in muscle, yields more than twice the energy as compared with glucose (9 versus 4 kcal/g); the physical properties of fatty acids make them an ideal energy resource, stored as triacylglycerides in adipose tissue (fat). The liver, through lipoprotein generation and export, facilitates transport of fatty acids/triglycerides via the circulation. Increases in energy intake and decreases in energy expenditure challenges this balance. Obesity, diabetes, and atherosclerosis are characterized by abnormalities in these same energy resources, namely hyperglycemia, elevated free fatty acids (FFA), and hypertriglyceridemia. The PPAR-RXR transcriptional complex is a key regulator of proteins central to the handling of energy resources, including transport (lipoprotein metabolism), release (lipases), cellular uptake (fatty acid transporters), energy release (beta oxidation), and storage (adipogenesis). The PPAR-RXR complex is also involved in regulating expression of targets involved in inflammation and atherosclerosis.
provides insights into their biology, followed by a similar discussion regarding RXRs.

**General Biology of the PPAR/RXR Transcriptional Regulatory Complex**

The 3 RXR (α, β, γ) and 3 PPAR (γ, α, β/δ) isotypes, each encoded by separate genes, are members of the steroid hormone branch of the nuclear receptor family. Like most other members of this family, RXRs and PPARs contain 5 main domains: a DNA-binding domain (DBD), which governs the direct interaction with RXR and PPAR response elements in the promoter regions of target genes; a ligand binding domain (LBD), which forms the pocket for direct interaction with specific pharmacological and endogenous nuclear receptor ligands; and 2 activation domains: 1 in the amino terminus (AF-1) and 1 in the carboxy terminus (AF-2). The LBDs of the PPARs are particularly large as compared with other nuclear receptors. For example, the PPARγ LBD is ~3 times larger than that of the estrogen receptor, a nuclear receptor for which estrogen, tamoxifen, and raloxifene are all unique modulators resulting in distinct clinical effects. Such unique LBD–agonist interactions would be expected to be more pronounced with the large LBD of PPARs. Although conservation exists between individual PPARs and RXRs, greater variability is present among the amino acids of their LBDs. This variation likely explains why pharmacological PPAR and RXR agonists interact with specific nuclear receptor partners. Clinical differences between agonists that target the same PPAR isotype may stem from these differences. Such structural specificity is also likely to extend to endogenous PPAR and RXR agonists. The contact between a PPAR or RXR ligand and its cognate receptor’s LBD induces a conformational change in the AF2 domain, facilitating the recruitment of coactivators, like CBP or p300, and release of corepressors, like nuclear receptor corepressor (NCoR) or SMRT. The assembly of these accessory molecules is a key determinant of a nuclear receptor’s transcriptional activity. Despite these common themes in PPAR and RXR biology, each PPAR isotype has a distinct biological role as well as unique actions in the vessel wall. Each PPAR isotype is considered, with a focus on new insights into their biology, followed by a similar discussion regarding RXRs.

**Peroxisome Proliferator-Activated Receptor γ**

Given its relatively recent identification in 1994, the rapid arc of PPARγ data that moved from in vitro reports to animal models to human translational studies and then large scale clinical trials is striking. PPARγ was first identified as a transcription factor integral to adipocyte differentiation. Subsequent work recognized the action of PPARγ in regulating gene expression in other settings including skeletal muscle, where it increases expression of glucose transporters. PPARγ is now known to be expressed in ECs, VSMCs, monocytes/macrophages, and T cells; in these settings, PPARγ regulates multiple target genes relevant to inflammation and atherosclerosis. New PPARγ targets in vascular cells continue to be identified (Figure 2). In VSMCs, PPARγ activation limits cellular proliferation and migration, both of which can be understood as more ‘energy-requiring’ VSMC responses. Various mechanisms are involved in these cellular responses, including cell cycle arrest through retinoblastoma phosphor- ylation, alterations in c-fos, and inhibition of telomerase activity (Figure 2). PPARγ agonists also inhibit matrix remodeling by suppressing matrix metalloproteinases. In human studies, PPARγ agonists decreased VSMC-driven in-stent restenosis. PPARγ agonists also limit oxidative responses and increase nitric oxide. The decrease in blood
pressure seen with PPARγ agonists has been directly linked to PPARγ in VSMCs as well as ECs. Recent data suggests PPARγ agonists may decrease atherosclerosis through VSMC responses, given that PPARγ agonists decrease atherosclerosis in wild-type mice but not those with selective PPARγ deficiency in VSMCs.

In ECs, PPARγ also regulates multiple targets. Although ECs have been considered a low-energy cell type, one endothelial response that may require more energy is angiogenesis. Many studies report PPARγ activation as a modulator of angiogenesis, the direction of these effects varies. PPARγ-mediated angiogenesis may be needed for adipose tissue expansion, extending connections between PPARγ and energy balance to the endothelial organ. Recent studies implicate PPARγ in angiogenesis through endothelial progenitor cell (EPC) formation, although the actual functional effects in vivo remain unclear. PPARγ also regulates redox balance and inflammation, including specific chemokines and chemokine receptors (Figure 2). Interestingly, EPCs, redox balance, and PPARγ activation may be interconnected.

Figure 3. Novel mechanisms for PPARγ-RXR modulation. Novel mechanisms for PPAR-RXR modulation continue to emerge, several of which are highlighted here. CDK5 phosphorylation: High-fat diet (HFD) can promote cleavage of p35, producing p25, a CDK5 activator. CDK5 phosphorylation of PPARγ alters its transcriptional program, for example, having no effect on adipogenesis but decreasing expression of the antinflammatory adipokine adiponectin. PPARγ agonists block CDK5 phosphorylation but not through classic receptor agonism, offering new ways for targeting PPARγ responses. SUMOylation: Through this 3 step posttranslational modification involving ATP and the enzymes PIAS1 and Ubc9, SUMO is attached to proteins and alters their function. PPARγ SUMOylation stabilizes the corepressor NCoR on the promoter regions of inflammatory target genes, thus blocking their transcription. SUMOylation may also occur with PPARα and PPARδ although with differing effects. Phospholipase (PLD)-derived CPA: Similar to evidence that lipases can activate PPARs, new data implicate other enzymes in PPAR modulation. PLD reportedly acts on lysophosphatidyl choline to generate cyclic phosphatidic acid (CPA), a simple, primordial phospholipid that can associate with the PPARγ complex and inhibit its activity by stabilizing the SMRT corepressor. Structurally similar molecules like lysophosphatidic acid reportedly activate PPARγ. Phospholipase A2 action on LDL may also modulate PPAR responses in macrophages.

RAS cleavage is mediated by the enzyme PIAS1 and Ubc9, SUMO is an ATP-dependent enzyme catalyzing the covalent addition of a ubiquitin-like protein SUMO to lysine residues. Sumoylation of specific nuclear receptors can result in transcriptional repression by interacting with the co-repressor complex, thereby facilitating the repression of transcriptional targets.

The evidence that all PPARs, including PPARγ, were present in macrophages was among the first data establishing the relevance of these nuclear receptors to atherosclerosis. PPARγ increases expression of CD36 in monocytes/macrophages, but it does not appear to promote foam cell formation. Interestingly, PPARγ in macrophages may contribute to insulin sensitivity, again implicating PPARs as links between metabolism and atherosclerosis. Large-scale evaluation of transcriptional patterns suggests that differences between PPARγ responses in macrophages and adipocytes are determined by cell-specific transcription factors, chromatin accessibility, and chromatin remodeling proteins. In macrophages, PPARγ-mediated induction of diacylglycerol transferase 1 (DGAT1), and the subsequent increased ability to store fatty acids in triglycerides, decreased inflammation. This data are consistent with the concept that fatty acid storage may protect against adverse metabolic and vascular consequences of obesity.

New findings continue to emerge regarding basic PPAR biology, including new insights into how PPAR activation is modulated (Figure 3). The classic model of PPAR action invokes the ligand-triggered assembly of a transcriptional complex that binds to PPAR response elements in specific target genes, inducing their expression. This model however does not account for PPAR-mediated target gene repression, as seen with PPAR inhibition of certain inflammatory genes. The induction of inflammatory transcriptional responses typically requires clearance of transcriptional coactivators, like the NCoR protein, from proinflammatory mediator promoters like nuclear factor κB and activator protein-1.

Recent work suggests that PPARγ agonists repress inflammatory target gene expression by preventing NCoR clearance in vivo and repress PPARγ-RXR activity, decreasing adipogenesis. RALDHs have also been implicated in T-cell responses.
through post-translational modification of PPARγ by the small ubiquitin-related modifier (SUMO) protein, as reported in macrophages (Figure 3).\textsuperscript{36} SUMOylation, the process in which SUMO is reversibly but covalently attached to specific target proteins, has diverse cellular effects, including transcriptional repression.\textsuperscript{37} SUMOylation involves 3 sequential processes: ATP-dependent activation of SUMO (E1 step); conjugation of SUMO to the target protein by the E2 ligase Ubc9 (E2 step); SUMO ligation to the target protein by various E3 ligases, including PIAS1 (protein inhibitors of activated STAT1) (E3 step). Interestingly, this model offers a way in which the antiinflammatory effects of PPARγ agonists may be distinct from their PPARγ LBD affinity. PPARγ SUMOylation has been implicated in other vascular responses including restenosis.\textsuperscript{38} SUMOylation has also been invoked for the effects of PPARα and PPARδ although with different effects.\textsuperscript{39–41} Recent evidence also suggests cyclic phosphatidic acid, generated by phospholipase D, stabilizes the SMRT corepressor on the PPARγ complex (Figure 3).\textsuperscript{42} In addition to SUMOylation and changes in the assembly of accessory molecules, phosphorylation may also contribute to PPAR-mediated transrepression of target genes and modulation of PPAR activity.\textsuperscript{43,44}

Another recent fundamental observation relevant to the control of PPARγ activity was the finding that CDK5 (cyclin-dependent kinase 5), after being activated by p35 cleavage into p25, phosphorylates PPARγ, resulting in decreased expression of a specific subset of PPARγ-regulated target genes (Figure 3).\textsuperscript{45} These changes in PPARγ through CDK5 occur in the absence of any effects on adipogenesis, a canonical PPARγ response.\textsuperscript{46} PPARγ agonists, including thiazolidinediones (TZDs), block CDK5-mediated phosphorylation of PPARγ, suggesting CDK5 as a unique distal mediator of PPARγ effects. This data suggests a new strategy for targeting PPARγ for therapeutic benefit, with particular relevance to the vasculature, given that CDK5 activation may help regulate inflammatory cytokine production.

**Peroxisome Proliferator-Activated Receptor α**

PPARα was the first PPAR identified.\textsuperscript{40} Certain industrial compounds were found to increase peroxisome biogenesis ("proliferate") in livers of mice, effects that were subsequently linked to PPARα activation. Although peroxisome proliferation does not occur in humans, fibrate drugs, which lower triglycerides and raise high-density lipoprotein (HDL), proliferation does not occur in humans, fibrate drugs, which also suppress activator protein-1, were initially developed in an attempt to inhibit fibroblast growth. However, the liver is a major site of PPARγ expression, whereas muscle and adipose tissue express primarily PPARα, making PPARα a major target of thiazolidinediones (TZDs). PPARα activation regulates gene expression involved in cellular fuel metabolism, including lipid and glucose utilization, making it like others PPARs integral in energy balance.\textsuperscript{55} An inverse relationship has been demonstrated for PPARδ and adiposity. Particularly intriguing to discussions regarding integration of PPARs and retinoid effects is recent work suggesting specific transport proteins may help direct retinoic acid to either the retinoic acid receptor (RAR) or PPARδ, with inhibitory effects on obesity and insulin resistance.\textsuperscript{56,57} In muscle, PPARδ activation regulates oxidative metabolism and improves insulin sensitivity. In the liver, PPARδ helps regulate fatty acid handling and oxidation.\textsuperscript{46} Consistent with this, fasting induces PPARα expression, whereas exercise increases expression of LPL. PPARα activation also induces expression of apolipoprotein A1, a major constituent of HDL.

PPARα activation limits inflammatory responses in different vascular and inflammatory cells (Figure 3). In ECs, PPARα activated by synthetic agonists or LPL-mediated release of natural ligands inhibits adhesion molecule expression.\textsuperscript{46,47} PPARα activation also limits inflammation in VSMCs and other settings that can indirectly influence atherosclerosis, such as the liver. PPARs repress inflammation by inhibiting key proximal inflammatory mediators including NF-κB and activator protein-1. In VSMCs, PPARα also limits cellular proliferation by targeting the cyclin-dependent kinase inhibitor and tumor suppressor p16INK4a, resulting in inhibition of retinoblastoma protein phosphorylation, decreased G1 to S phase transition, and less intimal hyperplasia in vivo.\textsuperscript{48,49} In macrophages, in addition to limiting inflammatory changes, PPARα decreases coagulation proteins, eg, tissue factor, while promoting cholesterol efflux and reverse cholesterol transport.\textsuperscript{50} LDL receptor-deficient mice that also lack macrophage PPARα developed more atherosclerosis.\textsuperscript{50} Taken together, the results of most studies indicate that PPARα exerts protective effects on the vasculature.

As with PPARγ, some of the most dramatic progress in regards to PPARα is related to new fundamental insights into its basic biology. Semenkovich and colleagues recently reported that 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0/18:1-GPC) serves as an endogenous PPARα ligand.\textsuperscript{51} Repression of phosphatidylcholine synthesis resulted in less PPARα target gene expression, whereas infusion of this specific phosphocholine induced PPARα gene expression but had little effect on PPARδ or PPARγ responses.\textsuperscript{53} PPARα may also be regulated via SUMOylation.\textsuperscript{54}

**Peroxisome Proliferator-Activated Receptor δ**

PPARδ was the second PPAR identified and thus is also called PPARβ or PPARγ/β.\textsuperscript{4,53} PPARα, widely expressed in many cells and tissues, had been the least studied and most poorly understood PPAR family member, perhaps stemming from the lack of a synthetic PPARδ agonist in clinical use. Recent findings have renewed interest into PPARδ actions, including those relevant to inflammation and atherosclerosis. PPARδ's regulation of fatty acid oxidation overlaps to some extent with PPARα, however, studies in the myocardium establish distinct biological functions for these 2 receptors.\textsuperscript{54} PPARδ regulates gene expression involved in cellular fuel metabolism, including lipid and glucose utilization, making it like others PPARs integral in energy balance.\textsuperscript{55} An inverse relationship has been demonstrated for PPARδ and adiposity. Particularly intriguing to discussions regarding integration of PPARs and retinoid effects is recent work suggesting specific transport proteins may help direct retinoic acid to either the retinoic acid receptor (RAR) or PPARδ, with inhibitory effects on obesity and insulin resistance.\textsuperscript{56,57} In muscle, PPARδ activation regulates oxidative metabolism and improves insulin sensitivity. In the liver, PPARδ helps regulate
Various aspects of lipid metabolism, including induction of HDL components. Indeed, the significant increases in HDL levels seen with PPARδ agonists have suggested its therapeutic potential for atherosclerosis as have studies that demonstrate direct PPARδ effects in inflammatory and vascular cells.

Several studies have shown that PPARδ activation represses inflammation. For example, the PPARδ-specific agonist GW0742 was found to inhibit lipopolysaccharide (LPS)-induced expression of proinflammatory targets, including cyclooxygenase (COX)-2 and inducible nitric oxide synthase. The diverse responses seen with PPARδ in atherosclerosis are noteworthy. In one set of studies, a synthetic PPARδ agonist decreased atherosclerotic lesion size in female low-density lipoprotein (LDL) receptor–deficient mice with moderate hypercholesterolemia, whereas in another report, a different PPARδ agonist had no effect on atherosclerosis despite inhibiting inflammatory target expression. PPARδ agonists limited atherosclerosis in ApoE-deficient mice as well as in an angiotensin II–accelerated model.

Perhaps in keeping with PPARδ’s apparent complex biology, Lee et al found that transplantation of PPARδ-deficient bone marrow into LDLR-deficient mice led to less atherosclerosis, not more, suggesting that PPARδ in bone marrow–derived cells, like macrophages, might promote atherosclerosis. In contrast, however, these investigators found a synthetic PPARδ agonist reduced inflammation, suggesting a fundamental paradox in which PPARδ promotes atherosclerosis but a PPARδ agonist limits it. In reconciling these findings, additional data suggested to these investigators that unliganded PPARδ sequestered BCL6, a repressor of inflammation, whereas in the presence of bound ligand, BCL6 is released from PPARδ and able to limit inflammation.

Subsequent studies support BCL6 involvement in PPARδ-mediated inhibition of inflammation. This BCL6 data, in which control of an accessory protein is critical for specific responses, is reminiscent of the PPARγ SUMOylation effects noted earlier. SUMOylation may also influence transcriptional regulation by PPARδ by altering activity of the KLF5 (Krüppel-like factor 5) transcription factor. PPARδ reportedly takes part in apoptotic cell clearance and release of antiinflammatory cytokines.

PPARδ is also implicated in EC and VSMC responses (Figure 3). In ECs, PPARδ activation reportedly limits adhesion molecule expression through various mechanisms, including protection against oxidative stress, inducing expression of target genes like thioredoxin and catalase. Interestingly, decreasing PPARδ expression increased the effects seen, reminiscent of the BCL6 effects discussed earlier. Endothelial COX-2 may help clear endocannabinoid metabolites, leading to prostacyclin synthase-mediated activation of PPARδ, which then represses expression of the procoagulant tissue factor. In contrast, PPARδ agonists induce the proinflammatory chemokine interleukin 8 in unstimulated ECs. PPARδ may protect the endothelium from shear stress, an effect that may involve PGC1 as a PPARδ accessory molecule.

PPARδ was found to limit apoptosis through the action of prostacyclin (also known as prostaglandin I2 or PG12) by inducing 14-3-3α expression. Several studies implicate PPARδ in promoting angiogenesis, including release of PG12 and subsequent PPARδ activation. PPARδ may promote tumor growth by stimulating angiogenesis. Together, these multiple effects on lipid metabolism, insulin sensitivity, inflammation, atherosclerosis, and diabetes have suggested PPARδ activation as a therapeutic approach to insulin resistant/metabolic syndrome-type patients where so many of these pathogenic pathways converge. It remains unclear whether some untoward side effects have prevented more advanced clinical development of PPARδ-activating agents. Ongoing research may yet define how to better target this important mediator.

Retinoid Receptors and Retinoid Mediators

A central and perhaps somewhat overlooked factor in determining PPAR responses is RXR activity. Because PPARs are obligate RXR heterodimeric partners, the biology of RXR (including its ligands, expression levels, accessory molecules) all become important determinants of not only RXR but also PPAR responses. Many aspects of RXR biology—including genetic variation, how specific retinoid molecules modulate its activity, the enzymes involved in generating retinoid modulators, the binding proteins that handle retinoid transport and delivery, and even dietary aspects of retinoid intake—may influence RXR responses and functional PPAR effects, including those seen with PPAR agonists. Despite this, the role of RXRs in vascular responses and atherosclerosis remains poorly understood. Ultimately, it is the PPAR-RXR transcriptional complex, with their respective ligands, modulators, and accessory molecules, as well as their integrated signaling, that must be understood.

Retinoid receptors include both RXRs and RARs, both of which have α, β, and γ forms. RA receptor-related orphan receptors (ROs) are closely related to RARs across evolution. The focus of the discussion here is more on RXR, given its relationship with PPARs. However RARs, as also obligate RXR heterodimeric partners with vascular effects are relevant to this discussion and provide additional context for understanding RXRs. In general, more functional overlap exists among individual RXRs and RARs than among PPAR isotypes. In addition to PPARs and RARs, RXRs are also an obligatory heterodimeric partner for other subclass I nuclear receptors, including the vitamin D receptor, thyroid hormone receptors, the farnesoid X receptor (FXR), and the liver X receptor (LXR). An inherent challenge in RXR studies is determining whether RXR itself or one of its partners is responsible for the effects seen. In the absence of ligand, RXR is maintained in transcriptionally inactive tetramers that rapidly dissociate on ligand binding. RXRα inactivation is embryonically lethal with hypoplastic cardiac ventricular development and ocular malformations, defects also found in vitamin A deficiency models. Compound RXR-deficient mice indicate that one RXRα copy can perform most RXR functions. Studies in cultured VSMCs and
Figure 4. The retinoid axis and its role in vascular responses. A, Retinoid metabolism. Like certain essential fatty acids, retinol (vitamin A) and its biologically active metabolites cannot be synthesized by higher-order animals but rather must be obtained from the diet either in a preformed state or as derived from dietary precursors. The retinoid system, with its metabolizing enzymes, binding proteins, metabolites, and nuclear receptors, each with their own distinct actions and effects, is highly regulated, tightly controlled, and of diverse functional importance. In general, retinol is converted by alcohol dehydrogenases (ADHs) to retinaldehyde (RALD), which is then converted by RALDHs to retinoic acids (RAs). ATRA, present in vivo, activates the RAR; 9-cis RA, which has not been demonstrated in vivo, activates RXR in vitro. RXR is an obligate heterodimeric partner for PPARγ. Despite prior extensive study in areas like adipogenesis, new aspects of retinoid metabolism relevant to obesity, diabetes, and dyslipidemia continue to be identified, with potential relevance to atherosclerosis. β-Carotene-15,15-monooxygenase (BCMO1) cleaves β-carotene symmetrically to RALD; BCMO1-deficient
RXRs and RARs are activated by metabolites of vitamin A, an essential fat-soluble vitamin also known as retinol (Figure 4A). Different forms of retinol, including ones with or without biological activity, are called “retinoids.” Natural retinoids derive mainly from dietary sources because they cannot be generated in vivo de novo by higher order animals. These usually enter as retinyl esters that then undergo enzymatic cleavage. Various synthetic molecules that can exert biological retinoid activity are also considered retinoids. In terms of naturally occurring molecules, retinol is metabolized to retinaldehyde (RALD) through enzymatic action of alcohol dehydrogenases (ADHs) (Figure 4A). Short-chain dehydrogenase/reductases may also convert retinol to Rald. Rald, which is essential for vision, is metabolized to retinoic acid through the action of retinaldehyde dehydrogenases (RALDH1 to -4). Retinoic acid occurs in all trans (ATRA) or 9 cis retinoic acid (9cRA) forms. ATRA is an established endogenous ligand for RAR, and has been demonstrated in vivo. 9cRA activates RXR in vitro, although it has never been demonstrated in vivo and unlike ATRA, cannot rescue retinoid-deficient embryos. Interestingly, ATRA, through its action on RAR, represses adipogenesis, whereas 9cRA, through RXR, promotes adipocyte differentiation, underscoring how specific retinoids direct distinct cellular responses. Moreover, RA may also activate PPARδ, with transport proteins helping determine which nuclear receptor is targeted.

Carotenoids, photosynthetically derived molecules that give many fruits and vegetables their red, yellow and purple color, can be cleaved to form retinoids, with β-carotene being a familiar example. Specific enzymes can cleave β-carotene either symmetrically, forming retinoid acid, or asymmetrically, yielding apocarotenals (Figure 4A). BCDO2 (β-carotene 9′,10′-dioxygenase 2), which cleaves β-carotene eccentrically, increases oxidative stress. Interestingly, BCDO2 arises in screens for genes that promote atherosclerosis in mice, whereas apo-apocarotenals have been linked with PPAR-RXR repression and increased endothelial inflammation.

Retinoids and the elaborate system controlling their generation, metabolism, transport, and subsequent nuclear receptor activation may influence atherosclerosis indirectly, for example through actions on adipogenesis and inflammation. New data link β-carotene and vitamin A–derived molecules with altered adipogenesis in vitro and adiposity in vivo. We reported that specific asymmetrical β-carotene cleavage products known as apocarotenals can inhibit adipogenesis, whereas others report similar effects with other β-carotene metabolites (Figure 4A). Other specific components of the retinoid axis can modulate lipid metabolism and adipogenesis. Retinol saturation (RetSat), a PPARγ-regulated target gene that generates dihydrometabolins, promotes adipogenesis (Figure 4A). Deficiency of RALDH1 protects mice from diet-induced obesity and subsequent diabetes. This lean phenotype was directly associated with increased levels of Rald in fat. In contrast, mice lacking RDH1 have increased adiposity. Specific proteins involved in intracellular and extracellular retinoid handling also influence metabolism and vascular biology. Increased retinol binding protein 4, associated with diabetes in mice and humans, may alter vascular responses. Retinoids play a very well established role in inflammatory cells, determining differentiation and functional activity in specific T cell subtypes, B cells, and monocytes, providing another way in which these molecules may influence atherosclerosis.

Retinoids and retinoid activated nuclear receptors can also directly effect vascular cells (Figure 4B). This is quite apparent in development, with deficiency of retinoids or retinoid receptors altering cardiac structures. Both RA deficiency and excess can exert teratogenic effects, suggesting tight control of retinoid handling during embryogenesis. Consistent with this developmental role, retinoids and retinoid receptors exert fundamental effects on cellular differentiation, proliferation, and matrix remodeling. RA, through both RAR and RXR, can regulate VSMC responses (Figure 4B). RA may promote VSMC differentiation through the microRNA Mir10-a. In general, RA appears to limit VSMC proliferation. Retinoid effects have also been reported on matrix metalloproteinase activity and plasminogen activator levels. Retinoids have been implicated in pulmonary hypertension and responses to injury. Retinoids are also involved in EC biology (Figure 4B), including mediating decreases in thromboxane receptor expression and glucose-induced oxidative stress. Apo-14′-carotenal, but not other apocarotenoids, increased endothelial adhesion molecule expression. Despite many provocative leads, more studies are needed on how retinoids and retinoid receptors modulate vascular biology. Some RA effects may be through RAR as opposed to RXR, both of which have important effects in the vasculature (Figure 4B). Retinoids regulate many genes relevant to atherosclerosis and arterial injury, including PPARγ itself. The findings in some retinoid/retinoid receptor papers are variable, if not contradictory, which may reflect the complexity, sensitivity, and importance of retinoid metabolism. Many studies rely solely on retinoid stimulation without considering retinoid receptor involvement, whereas others do not designate which form of RA was used for cell stimulation. Despite these issues, the relevance of retinoids and retinoid receptors to atherosclerosis is apparent. For example, low retinol levels have been

**Figure 4.** (continued), mice have increased adiposity. Asymmetrical β-carotene cleavage, which occurs through BCDO2, can yield apo-apocarotenals that undergo further metabolism into varying chain lengths. β-Apo-14′-carotenol can repress adipogenesis in vitro. Retinol saturation (RetSat) converts retinol into specific dihydroretinoids. All-trans,13,14-dihydroretinol can activate RAR; mice lacking RetSat have impaired adipogenesis. Both BCMO1 and RetSat are PPARγ-regulated. RALDH1-deficient mice are protected against diet-induced obesity. RBP4 (retinol-binding protein 4) may promote insulin resistance in mice and humans (see references in the text). Retinoid action in ECs and SMCs. Many components of the retinoid axis are involved in EC and responses to injury. These investigations vary in terms of their testing of a given retinoid(s), specific retinoid receptor(s), or both. See references in the text.
linked to increased atherosclerosis. Clinical trials with retinoid or carotene supplementation, usually administered for potential antioxidant effects, have also been confusing and contradictory, underscoring the need for more investigation.

Clinical Experience With PPAR Agonists
The rapid development of the PPAR field is notable, with the existence of large clinical cardiovascular (CV) trial data with PPAR agonists already available. As such, a review on the PPAR-RXR complex in vascular biology and atherosclerosis requires some consideration of the PPAR agonist clinical trials. Although a complete discussion of these issues is beyond the scope of this review, and has been considered in many recent publications, specific aspects that relate to PPAR biology and their role in vascular and inflammatory cells will be considered. Such a discussion should begin by reinforcing the critical distinction between nuclear receptor biology, and what that nuclear receptor does in vivo under physiological conditions, and the effects of any given therapeutic agonist that targets that nuclear receptor. For PPARγ and then PPARα, a brief summary of the major clinical trials will be followed by a consideration of how basic science may inform those results and influence future prospects for novel PPAR agents.

PPARγ agonists in present clinical use are the TZDs pioglitazone and rosiglitazone. The effect of pioglitazone on cardiovascular (CV) events was investigated in PROactive, a study designed to match glucose control between the TZD and placebo arms to test possible glucose-independent CV benefits. Given that no prior large prospective T2D study had shown CV benefit, a large, and perhaps ill-conceived, multiple component primary end point was chosen. Some of the end points included ones known to be refractory to other proven CV therapeutics, like peripheral arterial disease revascularization, and more subjective ones like elective coronary revascularization. A nonstatistically significant trend toward benefit was seen with pioglitazone (10% risk reduction). A more standard, objective secondary end point of myocardial infarction, stroke, and CV mortality was reduced 16% \( (P<0.05) \). Congestive heart failure was increased with pioglitazone, a known reversible issue with TZDs that is thought to stem from fluid retention not structural myocardial changes. It remains intriguing to wonder how existing antidiabetic therapy and PPAR drug development would differ if the primary and secondary end point of PROactive had been reversed. Additional surrogate marker data supports the PROactive findings. Pioglitazone decreased plaque volume in human coronaries as seen on intravascular coronary ultrasound and on carotid intimal medial thickness. These improvements may have derived from pioglitazone-mediated increases in HDL. The effects of rosiglitazone on CV disease have been more controversial, perhaps in part because no large prospective study on CV outcomes was undertaken with this agent. Several metaanalyses of rosiglitazone data, including one by its manufacturer, suggest a possible increased risk of CV events, although the magnitude of this risk has been debated. TZD use has also been limited by the increase in body weight seen with these agents as well as the increased risk of bone fractures seen in some patients (mostly female).

Basic science studies offer some potential insight into the clinical trials with PPARγ agonists. First, structurally distinct PPARγ agonists may have differing transcriptional effects, resulting in different clinical responses. Indeed, neither rosiglitazone nor pioglitazone cause the irreversible liver failure seen with troglitazone. Clinical differences between pioglitazone and rosiglitazone may derive from how different PPARγ agonists bind to the large and PPARγ LBD, resulting in differences in accessory molecule recruitment and/or release. Consistent with this, transcriptional profiling studies reveal that the target genes regulated by different PPARγ agonists are incompletely overlapping, which could underlie different clinical responses. For example, pioglitazone lowers triglycerides, whereas rosiglitazone does not. Although we reported that PPARα but not PPARγ agonists could repress endothelial vascular cell adhesion molecule (VCAM1), rosiglitazone was the only TZD used in those studies. Subsequent studies showed that pioglitazone repressed VCAM1 expression both in vitro and in vivo, but not in PPARα-deficient ECs or mice. These data suggest that pioglitazone, or one of its several active metabolites, may crosstalk to PPARα, whose activation lowers triglycerides levels. Different PPARγ agonists may also have distinct effects on other novel mechanisms of PPAR action such as SUMOylation and CDK5 phosphorylation discussed earlier. Defining PPARγ agonists on the basis of their structural presence of a TZD ring may have limited functional relevance.

Prospective clinical trials with PPARα-activating fibrates are also available. In fact, fibrates, which are used clinically to lower triglycerides and raise HDL, were in use even before PPARs had been identified. In the Veteran Administration-HDL Intervention Trial (VA-HIT), the impact of gemfibrozil on CV events in patients with established heart disease was studied absent any concomitant statin use. Patients receiving gemfibrozil had a statistically significant 22% relative risk reduction in CV events as compared with those on placebo. The benefit in VA-HIT appeared driven by the T2D patient subgroup. This finding prompted interest in the Fenoibrate Intervention and Event Lowering in Diabetes (FIELD) trial, a study testing fenofibrate in approximately 9800 subjects with T2D. Although fenofibrate reduced some secondary CV end points, the primary end point in FIELD did not significantly differ between groups. FIELD results may have been influenced by the disproportionate drop-in rate of statin therapy in the placebo group, thus reducing their CV risk, and perhaps obscuring any potential fenofibrate benefit. The Action to Control Cardiovascular Risk in Diabetes (ACCORD)-Lipid arm study reported that combining a statin and fenofibrate was no better in decreasing CV events than a statin alone. However, as suggested in other fibrate trials, a benefit was seen in the subgroup with more significantly elevated triglyceride levels and lower HDL levels. Indeed, one criticism of the fibrate dataset...
is the absence of a trial focused solely on patients with more significantly elevated triglycerides and lower HDL levels, which is where fibrates would be most appropriately used.

**Ongoing PPAR Drug Development**

The concerns over rosiglitazone, the PPAR agonists abandoned during development, known TZD side effects, and disappointing PPAR clinical trials have all contributed to decreased development of new PPAR therapeutics despite prior intense interest in this area. One issue may have been the pursuit of much more potent PPAR-activating agents, which may also carry greater toxicity. Indeed, naturally occurring PPAR activators appear to be much less avid agonists than most of the synthetic PPAR agonists. Other factors have maintained interest in the prospects of therapeutic benefits through PPAR mechanisms, including the ongoing emergence of strong preclinical data for PPAR benefits on inflammation and atherosclerosis, favorable signals in smaller human studies and in subgroup analyses of clinical trials, the encouraging CV signal seen in PROactive, the impressive durability of antidiabetic effects with TZDs, hypertriglyceridemia and low HDL as contributors to residual risk in statin-treated patients, and the identification of novel PPAR mechanisms that might be targeted.

The concept of selective PPAR and RXR modulation, as undertaken with selective estrogen modulators, argues that with the appropriate chemical structure, a given PPAR agonist might have clinical benefit without adverse effects.

Many different structures of PPARγ agonists have been developed, including for example non-TZD agents (n-TZDs). The question remains whether a PPARγ agonist might exist that retains insulin sensitizing and antiinflammatory effects without weight gain, fluid retention, and/or bone fractures, as has been suggested for some lead compounds.

Other new therapeutic leads are coming from identification of distal targets of PPARγ activation. For example, pioglitazone was found to bind to and stabilize a mitochondrial protein known as mitoNEET. Perhaps mitoNEET stabilization could contribute to some of the beneficial effects of pioglitazone.

Similarly, perhaps CDK5 or SUMOylation might offer new options for ways to screen for new therapeutics. An alternative strategy is to base PPAR therapeutics on naturally-occurring molecules. Attempts have also been made to identify novel PPARα agonists. At least one PPARα agonist LY518674, with a PPARα binding as much as 10,000 times greater than the existing fibrates, was tested and abandoned.

No PPARδ agonist has ever reached approval although PPARδ-mediated increases in HDL remain appealing.

Considerable prior interest focused on designing molecules that might target 2 or even all 3 PPAR isotypes, thus combing their clinical effects. Aleglitazar is a dual PPARα/γ agonist in late-stage development. This intriguing combination would be both an insulin sensitizer, as well as a triglyceride-lowering/HDL-raising agent, in addition to the potential vascular and inflammatory effects reviewed above. At least one clinical study supports these possibilities. A large prospective CV trial among patients with diabetes presenting with acute coronary syndromes is underway with aleglitazar (clinicaltrials.gov NCT01042769). Other dual PPAR agonists have not gone forward as a result of toxicity. The appropriate balance of PPARα versus PPARγ activation has been discussed as a potential variable that might influence clinical effects and safety.

**Conclusion**

The epidemic of diabetes, obesity, and their CV complications can be understood in terms of energy imbalance, with unprecedented access to calories coupled to declining rates of exertion, both to obtain nutrients and in activities of daily living. Through coordinated control of gene expression, PPARs, RXRs, and their joint action regulate key aspects of energy storage and utilization while also modulating inflammation and vascular responses. The biological complexity of these transcription factors has made the need for further study apparent. Significant progress in this area will require attention to the nature of the receptors themselves and their physiological function as opposed to the action of any given synthetic agonist. Additional insight is needed into endogenous PPAR and RXR ligands, how these molecules are formed, and how they are delivered to the nucleus. Such information may help uncover mechanisms that protect against cardiometabolic problems, pharmacogenomic issues that limit drug responses, and better templates for drug development. Little is known about modulation of PPAR and RXR activation, for example, through posttranslational modification or signal termination. Additional data are needed regarding RXR and its contribution to vascular and inflammatory responses. Burgeoning insight into accessory molecules will need to be applied to vascular cells. Although the value in better understanding the PPAR-RXR complex remains independent of any present or future drug therapy, in the absence of this foundation and framework, the risk of chasing individual PPAR/RXR drugs with less favorable effects will remain. Although the complexity of both PPARs and RXRs can be daunting, it is also consistent with their importance in helping govern energy balance and support organismal survival for millennia. In present times, we now know atherosclerosis and its complications are also in that balance and influenced by PPAR-RXR biology.

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