

## Peroxisome Proliferator–Activated Receptors as Transcriptional Nodal Points and Therapeutic Targets

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**Abstract**—Peroxisome proliferator–activated receptors (PPARs) are ligand-activated transcription factors involved in the transcriptional regulation of key metabolic pathways such as lipid metabolism, adipogenesis, and insulin sensitivity. More recent work implicates all 3 PPAR isotypes ( $\alpha$ ,  $\gamma$ , and  $\delta$ , also known as  $\beta$  or  $\beta/\delta$ ) in inflammatory and atherosclerotic pathways. Because these nuclear receptors are activated by extracellular signals and control multiple gene targets, PPARs can be seen as nodes that control multiple inputs and outputs involved in energy balance, providing insight into how metabolism and the vasculature may be integrated. The ongoing clinical use of fibrates, which activate PPAR $\alpha$ , and thiazolidinediones, which activate PPAR $\gamma$ , establishes these receptors as viable drug targets, whereas considerable in vitro animal model and human surrogate marker studies suggest that PPAR activation may limit inflammation and atherosclerosis. Together, these various observations have stimulated intense interest in PPARs as therapeutic targets and led to large-scale cardiovascular end-point trials with PPAR agonists. The first of these studies has generated mixed results that require careful review, especially in anticipation of additional clinical trial data and ongoing attempts to develop novel PPAR modulators. Such analysis of the existing PPAR data, the appropriate use of currently approved PPAR agonists, and continued progress in PPAR therapeutics will be predicated on a better understanding of PPAR biology. (*Circulation*. 2007;115:518-533.)

**Key Words:** atherosclerosis ■ diabetes mellitus ■ inflammation ■ metabolism ■ lipoproteins  
■ peroxisome proliferator-activated receptors ■ transcription

Our view of the blood vessel as an organ that integrates mechanical and chemical signals into coordinated responses involving the endothelium, smooth muscle cells, lymphocytes, and monocytes/macrophages<sup>1</sup> raises a fundamental question: How does such integration occur? Physiologically, what mechanisms synchronize these systems to regulate vascular tone and hemostasis and adaptive responses in the circulation? Pathologically, how do cardiovascular risk factors perturb this balance and foster a chronic inflammatory state? Therapeutically, does this orchestrated view of the vasculature suggest targets that might address underlying “syndromes” rather than individual risk parameters? Peroxisome proliferator–activated receptors (PPARs) have been implicated in the answers to all of these questions, thus explaining the attention focused on these steroid hormone nuclear receptors.<sup>2–4</sup> The ongoing clinical use of synthetic PPAR agonists, eg, insulin-sensitizing thiazolidinediones (TZDs) and lipid-lowering fibrates, and the evidence that PPAR activation also may limit inflammation and atherosclerosis have only heightened this interest and the pursuit of novel PPAR agonists. At the same time, the untoward effects and recent mixed clinical cardiovascular trial results seen with synthetic PPAR agonists have prompted questions about PPARs, their mechanisms of action, and the future of PPAR therapeutics. Together, these issues make the science of PPARs and PPAR agonists of obvious importance to the clinician. Here, we overview the clinically relevant aspects of PPAR biology

before considering each PPAR isoform and the existing data for PPAR involvement in vascular responses in relevant cell types. This information provides a basis for considering the existing and emerging clinical data in this rapidly evolving field.

### PPAR Biology

The cumbersome term peroxisome proliferator–activated receptor derives from early observations in rodents that certain industrial compounds could cause peroxisomes, subcellular organelles involved in fatty acid  $\beta$ -oxidation and detoxification steps, to increase (proliferate) in size and number.<sup>5,6</sup> Subsequently, these compounds, including fibrates, were found to bind to certain recently identified nuclear receptors; hence, the term arose.<sup>7</sup> PPAR agonists are not known to induce peroxisome proliferation in primates or humans, making the term PPARs archaic as well.<sup>8</sup>

Like other steroid hormone nuclear receptors, PPARs contain 5 modular domains: a ligand-binding domain (LBD) in which the specific PPAR agonist binds; a transactivating domain (activation function 2), which, in response to ligand binding, undergoes a permissive conformational change required for transcriptional activation; and a DNA-binding domain, which interacts with specific PPAR response elements (PPRE) in the promoter region of PPAR-regulated target genes (Figure 1).<sup>9</sup> Three PPAR isotypes have been identified: PPAR $\gamma$ , PPAR $\alpha$ , and PPAR $\delta$  (also known as

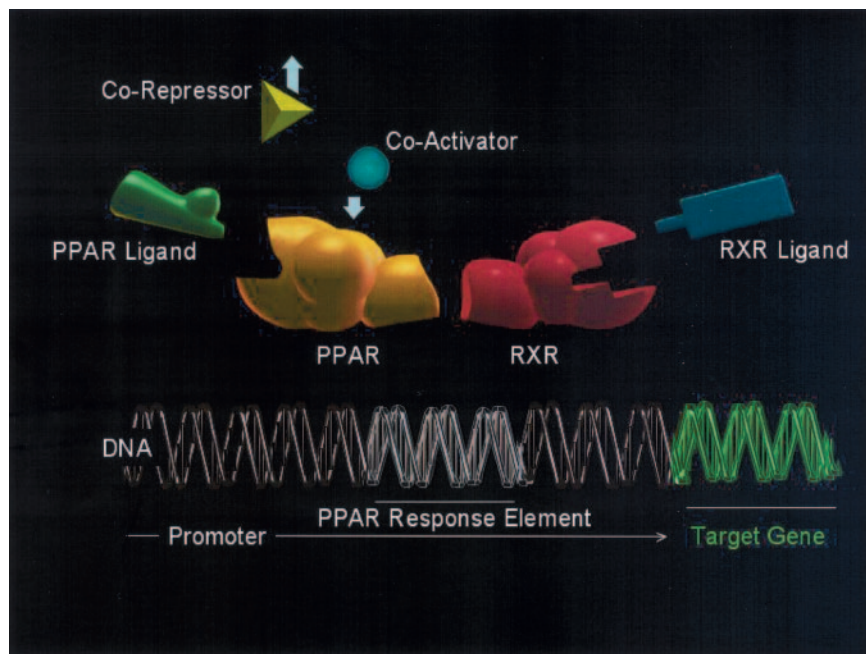
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**Figure 1.** PPAR mechanisms of transcriptional regulation. PPARs, members of the steroid hormone nuclear receptor family, are ligand-activated transcription factors that regulate key pathways of metabolism. Central elements in the PPAR-mediated transcriptional pathway are shown (not drawn to scale). All 3 PPAR isotypes ( $\alpha$ ,  $\gamma$ , and  $\beta/\delta$ , also known as  $\beta$  or  $\delta$ ) contain 5 conserved domains. In response to a cognate ligand, a conformational change occurs in the activation function 2 (AF2) domain, which facilitates recruitment of coactivators and release of corepressors. Corepressors, like nuclear corepressor and SMRT, and coactivators, such as cAMP response element-binding protein and p300, are large families of molecules that help determine PPAR responses. PPAR transcription requires formation of a heterodimeric complex with RXR, another nuclear receptor with its own ligand, purportedly 9 *cis*-retinoic acid. Together, the activated RXR-PPAR complex interacts with specific PPREs (direct repeat 1) in the promoter regions of positively regulated target genes. PPAR activation also

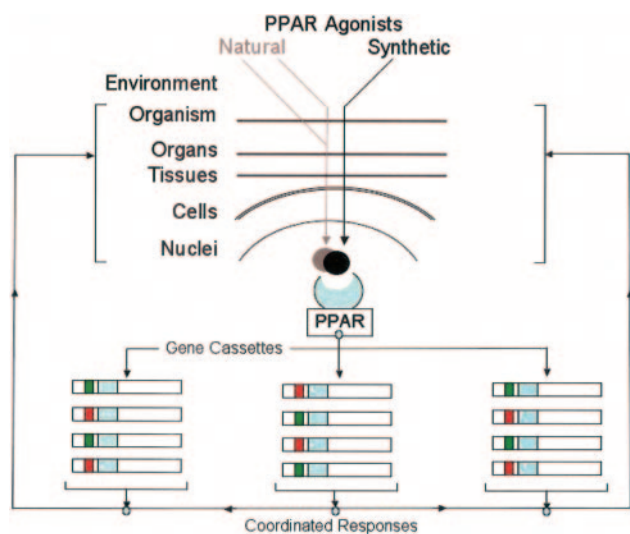
can repress the expression of target genes through mechanisms that are less well defined. Because each PPAR isotype controls the expression of multiple genes, PPAR activation by a given upstream signal, whether an agonist or natural ligand, can control the expression of entire cassettes of genes, not unlike a node in electrical and computer networks (Figure 2, Table 1). Although attention has focused on how specific PPAR ligands determine distal PPAR responses, each of the elements represented here—ligand, PPAR, coactivators/corepressors, direct repeat 1 sequence, RXR—can influence PPAR activation and the subsequent biological and clinical effects stimulated by a given agonist.

PPAR $\beta$  or PPAR $\beta/\delta$ , as used here). Despite unique attributes of each PPAR isotype, these receptors also share a common biology. PPAR activation is initiated by the binding of a cognate ligand to the LBD of a specific PPAR isotype (Figure 1). Ligand binding and activation function 2 movement allow PPAR heterodimerization with the retinoid X receptor (RXR), another nuclear receptor activated by its own ligand (purportedly 9 *cis*-retinoic acid), which is required for transcriptional PPAR activity.<sup>10,11</sup> RXR also can dimerize with itself or other specific nuclear receptor partners. Through their respective DNA binding domains, the PPAR/RXR complex binds to DNA at sequence-specific regions in gene promoters known as PPREs, which consist of direct repeats of DNA separated by a single nucleotide (direct repeat 1).

Transcriptional PPAR responses also depend heavily on ligand-induced recruitment or release of small accessory molecules known as coactivators and corepressors, respectively. These cofactors, a large, diverse family involving multiple members such as nuclear corepressor, PPAR-binding protein, PPAR $\gamma$  coactivator, and cAMP response element-binding protein are critical determinants of the cellular PPAR response.<sup>12–15</sup> This multiprotein complex induces transcription by chromatin remodeling and interaction with the basal transcriptional machinery.<sup>16,17</sup> In contrast to the positive regulation of target genes described earlier, PPAR activation also can repress transcription. This is a common but less-well-understood theme in PPAR-mediated repression of inflammation. Recent advances suggest that PPAR $\gamma$ -mediated “transrepression” may involve stabilization of corepressor recruitment after posttranslational PPAR modification by sumoylation.<sup>18</sup> PPAR responses also are regu-

lated by phosphorylation, as has been evident in vascular cell responses.<sup>19,20</sup>

Even this brief overview of PPAR biology identifies multiple levels of control—ligand, PPAR, accessory molecules, promoter regions—contributing to the specific, nonredundant roles of each PPAR isotype. Indeed, each PPAR isotype is encoded by separate genes, has distinct tissue distributions, binds specific ligands, and can regulate unique target genes. The nonoverlapping clinical effects seen with synthetic PPAR agonists also support biological differences among PPAR isotypes, a facet being exploited in the development of novel PPAR agonists, including agents that can target >1 isoform. Understanding these differences among PPARs and their agonists may prove critical for further progress in harnessing PPARs for therapeutic purposes. The greatest divergence in structural homology among PPAR isotypes is localized to the LBD, providing a physical basis for specific ligand-PPAR isotype interaction.<sup>6</sup> Moreover, PPARs possess particularly large LBDs, even compared with other nuclear receptors. By contacting the LBD in different ways, specific PPAR agonists can induce unique responses through distinct receptor conformational changes and subsequent accessory molecule release or recruitment, hence the notion of PPAR modulators. Through these coordinated, complex mechanisms, PPAR activation results in transcriptional regulation. Because PPARs are activated by extracellular signals (discussed further below) and can positively or negatively regulate entire gene cassettes in different pathways, PPAR activation fits classic definitions of a network nodal point,<sup>21</sup> in this case controlling cellular, tissue, organ, and organism responses (Figure 2 and Table 1).



**Figure 2.** PPARs as nodal points of transcription in metabolism. A schematic of how PPARs can function as nodal points integrating networks of transcriptional responses is shown. By controlling the expression of multiple gene cassettes in different pathways, PPARs can mediate a coordinated, programmed response to a specific stimulus. Because these stimuli can derive from outside the cell, PPARs provide a mechanism through which the nucleus of a cell in a given tissue/organ can “communicate” with intracellular, extracellular, and even environmental inputs and respond by altering gene expression. One such environmental input may be diet, with evidence for PPARs as a mechanism for the nutritional control of gene expression. PPAR activation can either induce (green box) or repress (red box) target genes, although so-called transrepression remains incompletely understood and apparently independent of a specific, direct PPAR-PPRE interaction. PPAR-regulated cassettes can be specific to each PPAR isotype and include lipid metabolism, energy balance (adipogenesis, glucose homeostasis, insulin sensitivity, fatty acid oxidation), and possibly inflammation and atherosclerosis (see Table 1). Importantly, as suggested here, the overlap between responses induced by synthetic PPAR agonists and the still-elusive endogenous PPAR agonists remains unclear and may in fact be distinct.

The serendipitous but invaluable discovery of chemically synthesized compounds that bind to and activate PPARs and the current therapeutic use of these agents have, in many ways, defined the PPAR field. The insulin-sensitizing TZDs (pioglitazone, rosiglitazone) are PPAR $\gamma$  activators that increase sensitivity to insulin and are used to treat diabetes mellitus.<sup>22,23</sup> Triglyceride-lowering/high-density lipoprotein (HDL)-raising fibrates (gemfibrozil, fenofibrate) are PPAR $\alpha$  agonists used clinically to treat dyslipidemia.<sup>24,25</sup> Responses to PPAR agonists are often equated to the biology of a given PPAR isotype, but the variability in the effects of synthetic PPAR agonists identifies the flaws in such assumptions. This distinction between the receptor and its agonists is particularly important given the variable effects of PPAR agonists discussed further below.

The role of PPARs *in vivo* under physiological conditions has remained more difficult to define completely, including fundamental unanswered questions regarding the identity of endogenous PPAR ligands. Early seminal studies revealed that certain polyunsaturated fatty acids such as linoleic and linolenic acid could bind to all PPARs.<sup>24,26–28</sup> Although these observations provided a major advance in the field, these data were limited largely to *in vitro* findings that required high

**TABLE 1. PPAR Activation Controls Distinct Pathways Through the Regulation of Specific Gene Cassettes**

PPAR- $\alpha$ target pathways	
Fatty acid oxidation	
Acyl coenzyme A oxidase	
Carnitine palmitoyl transferase 1	
Medium-chain acyl coenzyme A dehydrogenase	
Fatty acid transport protein	
Lipid metabolism	
LPL	
Apolipoprotein CIII	
Angiotensin-like protein 4 (FIAF)	
ApoA1	
Inflammation/vascular	
Vascular cell adhesion molecule-1	
COX-1	
Interleukin-6	
CCATT/enhancer binding protein	
Tissue factor	
PPAR- $\gamma$ target pathways	
Adipogenesis	
AP2	
Adiponectin	
Adipsin	
CCATT/enhancer binding protein $\alpha$	
Lipid metabolism	
LPL	
ABCA1	
ABCG1	
Fatty acid binding protein 4	
Glucose control	
GLUT4	
Thioredoxin-binding protein-2	
Resistin	
Inflammation/vascular	
Interferon- $\gamma$	
Chemokines (Mig, iTAC, IP10)	
Chemokine receptors (CCR2)	
Tumor necrosis factor- $\alpha$	
MMP-9	

fatty acid concentrations, offered little information regarding selective PPAR isotype activation, and left the connections between endogenous lipid metabolism and PPAR responses unresolved. More recently, specific pathways of lipid metabolism that can generate differential PPAR activation have been identified. Lipoprotein lipase (LPL), a key enzyme in triglyceride metabolism, can hydrolyze triglyceride-rich lipoproteins, like very-low-density lipoproteins, to generate PPAR ligands.<sup>29,30</sup> More recent work extends this model to other lipoprotein substrates and lipases.<sup>31</sup> For example, HDL hydrolysis by endothelial lipase can activate PPARs through a pathway that is distinct from the PPAR effects of LPL.<sup>32</sup>



Other specific PPAR activators have been reported, including 15 d-prostaglandin J2,<sup>33</sup> oxidized linoleic acid,<sup>34</sup> leukotrienes,<sup>35</sup> and lysophosphatidic acid.<sup>36</sup> Some of these molecules clearly have PPAR-independent effects<sup>37</sup>; their physiological relevance remains to be determined.

Building upon the extensive data establishing PPARs as key mediators in metabolism arose evidence for PPAR expression in essentially all major vascular and inflammatory cells. All 3 PPAR isotypes are now reported to have functional effects in the vessel wall, including the modulation of cell signaling, lipid homeostasis, and inflammation.<sup>38</sup> The functional role of individual PPARs, and thus their therapeutic potential as drug targets, can be deconstructed by categorizing each isotype according to its expression pattern, the target genes it regulates, and the biological effects seen after its activation. Following this overview of basic PPAR mechanisms, the preclinical and clinical evidence for how each PPAR might modulate vascular responses provides a framework for considering recent clinical trials with synthetic PPAR agonists.

### PPAR $\alpha$ : A Central Regulator of Fatty Acid Metabolism

PPAR $\alpha$ , the first PPAR cloned, plays an important role in regulating the  $\beta$ -oxidation of fatty acids, a major source of cellular energy.<sup>7</sup> Consistent with this, PPAR $\alpha$  is expressed primarily in metabolically active, energy-requiring tissues, including liver, heart, skeletal muscle, and kidney.<sup>39</sup> PPAR $\alpha$  target genes include multiple proteins essential for fatty acid uptake, intracellular transport, and  $\beta$ -oxidation, including fatty acid transport protein, fatty acid translocase, long-chain fatty acid acetyl-coenzyme A synthase, and carnitine palmitoyl transferase I.<sup>40</sup> PPAR $\alpha$  activation induces expression of LPL, which hydrolyzes triglyceride rich lipoproteins, the major source of circulating fatty acids. PPAR $\alpha$  also represses apolipoprotein (Apo) CIII expression, which is an endogenous inhibitor of LPL activity.<sup>41,42</sup> PPAR $\alpha$  activation increases transcription of the major HDL apolipoproteins, ApoAI and ApoAII.<sup>43,44</sup> Given these biological effects, the clinical response to fibrates—lowering triglycerides, the major source of circulating fatty acids, and raising HDL, the major apolipoprotein of which is AI—can be understood as consequences of PPAR $\alpha$  activation. Of note, fasting potently induces PPAR $\alpha$  expression, underscoring the importance of this transcription factor in energy balance.<sup>45</sup>

The viable but metabolically perturbed PPAR $\alpha$ -deficient mouse model has provided many insights into PPAR $\alpha$  biology.<sup>46</sup> Mice lacking PPAR $\alpha$  have elevated free fatty acid levels and fatty livers, consequences of their inability to combust fatty acids. Not surprisingly, these mice are hypoglycemic as a result of their reliance on glucose as an energy resource and the lack of fatty acid-derived carbon chains for gluconeogenesis.<sup>46</sup> PPAR $\alpha$  agonists fail to induce peroxisomal proliferation in PPAR $\alpha$ -deficient mice, a genetic proof of principle, although not specifically relevant to human biology. This is one of several examples of biological divergence in PPAR biology between mice and humans. Species-specific differences are clinically relevant, given some of the animal toxicity observed with PPAR agonists and how such data can influence drug development.<sup>47</sup> PPAR $\alpha$  effects also may vary, depending on different tissue locations.

Although PPAR $\alpha$  agonists have been suggested to decrease weight,<sup>48–50</sup> recent data with PPAR $\alpha$  overexpression in mouse muscle suggest that PPAR $\alpha$  may promote obesity-related diabetes.<sup>51</sup> The relationship of these findings to PPAR $\alpha$  in humans is not clear. Although much remains to be understood, PPAR $\alpha$  clearly is a molecular sensor of the metabolic milieu and a functional nodal point coordinating the transcriptional regulation of energy balance and lipid metabolism. The role of metabolism and energy balance in determining vascular responses makes the potential importance of PPAR $\alpha$  in the vasculature clear.

## PPAR $\alpha$ in the Vasculature

### Vascular Endothelium

The demonstration of PPAR $\alpha$  in the endothelium raised the possibility that PPAR agonists of either synthetic or natural origin might directly modulate endothelial responses.<sup>52,53</sup> Moreover, PPAR $\alpha$  activation by synthetic agonists or certain fatty acids could repress endothelial inflammatory responses, including vascular cell adhesion molecule-1 expression, an early atherogenic step.<sup>54–56</sup> The prospect that fibrates might repress vascular cell adhesion molecule-1 expression through PPAR $\alpha$  activation is bolstered by the failure of synthetic PPAR $\alpha$  agonists to have this effect in endothelial cells isolated from PPAR $\alpha$ -deficient mice.<sup>29,54</sup> Decreased leukocyte adhesion through certain omega-3 fatty acids also requires PPAR $\alpha$  in vivo, at least in some experimental settings.<sup>56</sup> Interestingly, in the genetic absence of PPAR $\alpha$ , basal endothelial vascular cell adhesion molecule-1 expression is increased, one of several lines of evidence suggesting PPAR $\alpha$  functions as a “brake” on inflammation.<sup>29,31</sup> This phenomenon also has been observed in other cell types, including hepatocytes.<sup>57</sup> Of note, lipolytic mechanisms of PPAR activation, like very-low-density lipoprotein hydrolysis by LPL, also can repress vascular cell adhesion molecule-1 expression in a PPAR $\alpha$ -dependent manner.<sup>29,32</sup> These data suggest mechanisms active under physiological conditions that may replicate the effects of synthetic PPAR $\alpha$  agonists and help to explain the vascular protection seen among individuals with intact or supraphysiological LPL activity.<sup>58,59</sup> Multiple other endothelial PPAR $\alpha$ -regulated targets exist, including enzymes involved in redox responses and nitric oxide signaling.<sup>60–62</sup> Mechanistically, many of these PPAR $\alpha$  targets are repressed through inhibition of the critical proinflammatory mediator nuclear factor- $\kappa$ B.<sup>63</sup> PPAR $\alpha$  activation may increase the transcription of I $\kappa$ B, which functions as a cytoplasmic nuclear factor- $\kappa$ B inhibitor, or may directly interfere with nuclear factor- $\kappa$ B assembly.<sup>64,65</sup> PPAR $\alpha$  also is implicated as a feedback mechanism for limiting inflammation and oxidation.<sup>63</sup>

### Vascular Smooth Muscle

PPAR $\alpha$  expression in vascular smooth muscle cells (VSMCs) raises similar issues regarding direct PPAR activation, in this case, in a cellular setting relevant to hypertension, atherosclerosis, and restenosis after coronary intervention. PPAR $\alpha$  activation reportedly inhibits interleukin-1-stimulated secretion of interleukin-6 by human aortic smooth muscle cells.<sup>66</sup> Consistent with this, synthetic PPAR $\alpha$  agonists decrease circulating levels of inflammatory markers and mediators,

including interleukin-6, and C-reactive protein.<sup>66</sup> Likewise, lipopolysaccharide-stimulated interleukin-6 levels from the aortas of PPAR $\alpha$ -deficient mice are 4-fold higher than control mice.<sup>67</sup> Fenofibrate pretreatment suppresses this interleukin-6 induction but only in wild-type, not PPAR $\alpha$ -deficient, mice. In addition to modulating inflammatory cytokine signaling, PPAR $\alpha$  regulates VSMC proliferation and migration in vitro.<sup>68,69</sup> This effect occurs, in part, through the upregulation of p16<sup>INK4a</sup>, a cyclin-dependent kinase inhibitor that blocks cell cycle progression.<sup>70</sup> In a murine model of vascular injury, the reduction in intimal smooth muscle cell proliferation noted with PPAR $\alpha$  agonist pretreatment correlated with p16<sup>INK4a</sup> induction.<sup>70</sup> This VSMC effect was absent in PPAR $\alpha$ -deficient mice. In contrast, other reports implicate PPAR $\alpha$  agonists and PPAR $\alpha$  activation in hypertensive responses, including humans.<sup>71,72</sup> In general, hypertension has not been noted in fibrate clinical trials, but these observations require further investigation, especially because molecules with more potent PPAR $\alpha$  activity are in development.<sup>73,74</sup> Not surprisingly, given its role in energy balance, PPAR $\alpha$  also is expressed in other myocytes, including the myocardium. Overexpression of PPAR $\alpha$  in murine heart and the resultant increased fatty acid oxidation in this tissue replicate diabetic cardiomyopathy.<sup>75,76</sup> Similar myocardial toxicities have not been specifically described in humans treated with synthetic PPAR $\alpha$  agonists but need to be considered as more data sets from clinical trials become available.

### Monocytes/Macrophages/Lymphocytes

PPAR $\alpha$  is expressed in inflammatory cells integral to atherosclerosis like monocytes, macrophages, and lymphocytes.<sup>38</sup> Of note, unlike human macrophages, mice macrophages lack PPAR $\alpha$  expression,<sup>77</sup> highlighting the potential complexities in preclinical PPAR studies. Presumably, results from experiments testing PPAR $\alpha$  activation in murine macrophages, including responses to PPAR $\alpha$  agonists, reflect PPAR $\alpha$ -independent effects. In human macrophages, PPAR $\alpha$  activation induces expression of the cholesterol efflux protein ABCA1, increasing cholesterol efflux.<sup>78</sup> Macrophage production of the potent procoagulant tissue factor, a contributor to plaque thrombogenicity, is repressed by PPAR $\alpha$  agonists.<sup>53,79</sup> In T lymphocytes, PPAR $\alpha$  activation limits proximal signals in the inflammatory cascade, including expression of interferon- $\gamma$  and tumor necrosis factor- $\alpha$ .<sup>80</sup> T lymphocytes isolated from PPAR $\alpha$ -deficient mice demonstrate enhanced expression of interferon- $\gamma$ . This effect may be mediated indirectly through the dysregulation of another transcription factor named T-bet, which controls cytokine production in lymphocytes.<sup>81</sup>

### PPAR $\alpha$ in Inflammation and Atherosclerosis In Vivo

Despite these extensive data for PPAR $\alpha$  action in vascular and inflammatory cells, the benefits of PPAR $\alpha$  agonists on inflammation and atherosclerosis require demonstration in vivo if they are to be considered clinically relevant. In vivo PPAR agonist studies are challenging because of responses that may be specific to a given drug, differences among species, untoward and/or unexpected PPAR-dependent and -independent effects, the known impact of PPAR agonist

concentrations on receptor-independent responses, and finally the inherent difficulty in proving that an observed drug response derives from direct nuclear receptor activation and not some other effect, eg, metabolic improvements.

PPAR $\alpha$  agonists have been tested extensively in mice in vivo, including in the context of atherosclerosis. However, the lack of PPAR $\alpha$  expression in murine macrophages and the unique murine phenomenon of peroxisome proliferation may color such data and limit extrapolation to humans. In an early in vivo PPAR $\alpha$  study, PPAR $\alpha$ -deficient mice crossed with the ApoE-deficient mouse atherosclerosis model developed fewer atherosclerotic lesions, not the increase predicted by the other antiinflammatory and antiatherosclerotic data reported with these agents.<sup>82</sup> The explanation for these findings remains unclear. Fibrate treatment of ApoE-deficient mice modestly improved the cholesterol content of the aorta without altering lesion size. When the human ApoAI gene, a known PPAR $\alpha$  target, was overexpressed in ApoE-null mice, significant reductions in atherosclerosis were seen with fibrate therapy.<sup>83</sup> In other work, fenofibrate (but not PPAR $\gamma$  agonists) decreased atherosclerotic lesions in a nondiabetic dyslipidemic mouse model in which human ApoE2 has been inserted into ApoE-deficient mice.<sup>84</sup> These observations underscore the potential species-specific differences that may influence PPAR responses. In another study, PPAR $\alpha$  agonist treatment of low-density lipoprotein (LDL) receptor-deficient mice decreased atherosclerosis by 50% in the aortic arch and 90% in the descending thoracic and abdominal aortas.<sup>85</sup> In this model, inflammatory gene expression in these aortas was decreased, including targets such as vascular cell adhesion molecule-1, intracellular adhesion molecule-1, tumor necrosis factor- $\alpha$ , and monocyte chemoattractant protein-1.<sup>85</sup> In addition, PPAR $\alpha$  agonist pretreatment reduced cholesterol accumulation in peritoneal macrophage foam cells in LDL receptor-deficient mice but not when PPAR $\alpha$  also was absent.<sup>85</sup> In these experiments, PPAR $\alpha$  agonist treatment also was associated with modestly reduced total cholesterol, LDL, very-low-density lipoprotein, HDL, and insulin levels and less weight gain and adiposity despite similar food intake. Elevated interleukin-6 levels were noted in PPAR $\alpha$ -deficient mice and were not suppressed by fenofibrate. This provides further evidence that PPAR $\alpha$  may limit inflammation under basal conditions. These decreases in atherosclerosis seen with PPAR $\alpha$  agonists also could be influenced by PPAR $\alpha$  expression in other relevant cell types like T lymphocytes, VSMCs, and endothelial cells.<sup>63</sup>

In humans, surrogate marker studies with PPAR $\alpha$  agonists have largely, but not uniformly, supported possible atherosclerotic benefits. In a small group of normal subjects, fenofibrate treatment for 4 weeks decreased interleukin-6 and C-reactive protein plasma levels.<sup>66</sup> In the Bezafibrate Coronary Atherosclerosis Intervention Trial, bezafibrate treatment decreased angiographic evidence of coronary atherosclerosis.<sup>86</sup> More recently, in a group of 300 patients with type 2 diabetes and mixed dyslipidemia but no known coronary disease, treatment with fenofibrate, simvastatin, or both significantly reduced high-sensitivity C-reactive protein levels.<sup>87</sup>

Interestingly, the clinical use of fibrates preceded the identification of PPARs as nuclear receptors. As such, some

**TABLE 2. Prospective Cardiovascular Clinical Trials With Approved PPAR-Activating Drugs**

Study	Intervention	n	Length, y	Primary End Point	Secondary End Point	Comments
PPAR- $\alpha$ , completed						
HHS	Gemfibrozil	4081	5	First CVD event: 34% decrease ( $P<0.02$ )	...	No difference in all-cause mortality
VA-HIT	Gemfibrozil	2531	6	Nonfatal MI+CHD death: 22% decrease in RR ( $P=0.006$ )	CHD death+nonfatal MI+stroke: 24% ( $P<0.001$ )	Significant decreases in CV events among DM patients (24%, $P=0.05$ )
BIP	Bezafibrate	3090	5	Fatal/nonfatal MI+SCD: no significant difference	Total mortality; hospitalization for unstable angina, coronary revascularization+stroke: all nonsignificant	Subgroup analysis: benefit in those with elevated triglycerides
FIELD	Fenofibrate	9795	5	CHD death+nonfatal MI: 11% decrease in RR, nonsignificant.	CV death, MI, stroke, coronary or carotid revascularization, microvascular disease: 24% decrease in nonfatal MI ( $P=0.010$ ), 11% decrease in total CV events ( $P=0.035$ ); increase in CV mortality, not significant	Significant and disproportionate statin drop-in rate in placebo group may have affected negative results; decreased microvascular disease
PPAR- $\alpha$ , in progress						
ACCORD	2 $\times$ 2, Glucose: intensive vs standard lipids (Feno) and BP	10 251	4 to 8	CHD death, nonfatal MI or stroke	Microvascular disease; quality of life, cost-effectiveness	In progress
PPAR- $\gamma$ , completed						
ProACTIVE	Pioglitazone	5238	3	Time to combined CV point: no significant difference	Total mortality+stroke+nonfatal MI: 16% reduction ( $P=0.027$ ); positive effects in subgroups: MI (presented), stroke (in press)	Pio: increased "edema/CHF" (not well adjudicated); CV end point included PVD, elective procedures
DREAM	Rosiglitazone vs ramipril; 2 $\times$ 2 design	5269	3	New DM or death: Rosi, 60%; reduction with ramipril, not significant	Major CV events, renal disease	Rosi: decreased LFTS; increased CHF (14 vs 2); ramipril: trend to normoglycemia, CHF (12 vs 4), CV events pending
PPAR- $\gamma$ , in progress						
BARI-2D	Insulin $\pm$ SU vs TZD $\pm$ Met	2800	5	Total mortality	CV events, quality of life	Testing insulin-sensitizing vs insulin-providing strategy
IRIS	Pio	3136	3	Stroke or MI	Stroke, ACS, mortality, new DM	Non-DM subjects s/p recent stroke; does TZD prevent stroke or MI?
RECORD	Rosi+Met; Rosi+SU; SU+Met	4458	6	Time to combined CV point	Individual CV end points; glucose control	

HHS indicates Helsinki Heart Study; CVD, cardiovascular disease; MI, myocardial infarction; CHD, coronary heart disease; RR, relative risk; CV, cardiovascular; DM, diabetes mellitus; BIP, Bezafibrate Infarction Prevention study; SCD, sudden cardiac death; Feno, fenofibrate; BP, blood pressure; Pio, pioglitazone; PVD, peripheral vascular disease; Rosi, rosiglitazone; LFTS, liver function tests; BARI-2D, Bypass Angioplasty Revascularization Investigation; SU, sulfonylurea; Met, metformin; IRIS, Insulin Resistance After Stroke; ACS, acute coronary syndrome; s/p, status/post; and RECORD, Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes.

clinical fibrate trials can be revisited for clues regarding the impact of presumable PPAR $\alpha$  activation (Table 2). In primary prevention studies, gemfibrozil decreased cardiovascular events in the Helsinki Heart Study, particularly among patients with diabetes, but an increase in noncoronary death rates also was noted.<sup>88</sup> In the Bezafibrate Infarction Prevention trial, only the subgroup with the highest triglyceride levels enjoyed a decrease in clinical cardiovascular events with fibrate therapy.<sup>89</sup> In the Veteran's Administration-HDL Intervention Trial (VA-HIT), a statistically significant decrease in cardiovascular events occurred after treatment with gemfibrozil in this cohort with a history of cardiovascular disease, average LDL levels, and modestly decreased HDL/elevated triglycerides.<sup>90–92</sup> Of note, VA-HIT subjects were not on any 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). The VA-HIT results may have been driven largely by the effect of gemfibrozil in patients with insulin resistance and/or diabetes, a group enriched in this study given the lipid criteria for enrollment.<sup>91,92</sup>

The VA-HIT results helped stimulate considerable anticipation for the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, a large, randomized, placebo-controlled trial testing the effects of fenofibrate on first or recurrent cardiovascular events in patients with type 2 diabetes (Table 2).<sup>93</sup> In FIELD, the primary end point did not achieve a statistically significant difference between treatment groups. Several secondary end points were significantly reduced, including nonfatal myocardial infarction and total cardiovascular events. Somewhat surprising decreases also

were observed in small-vessel disease, namely retinopathy and nephropathy. A statistically insignificant increase in cardiovascular mortality also was noted with fenofibrate, with a hazard ratio of 1.19.

Various factors have been raised as possible contributors to the negative primary end-point results of fenofibrate in the FIELD study. In that trial, although fenofibrate did increase HDL and lower triglycerides, these effects were modest; some might argue that the relatively higher baseline levels (HDL, 42 mg/dL) made this cohort less likely to experience fibrate benefits. A disproportionately higher drop-in rate of statin use occurred in the placebo group compared with the fenofibrate group. This difference likely lowered risk more in the placebo group. This disproportionate use of statins in the placebo arm may have occurred as a result of the modest LDL-lowering treatment effect reported with fenofibrate (but not gemfibrozil). Although these factors may help to explain and guide interpretation of FIELD and future studies with fibrates, they cannot substitute for the FIELD results themselves. The issues this study raises for both clinicians and PPAR investigators remain an intense area of debate and investigation.<sup>94</sup>

The effect of fenofibrate in FIELD may be consistent with what would have been expected for the observed changes in triglyceride and HDL levels, especially given concomitant statin therapy. Although it was a secondary end point, a significant decrease in nonfatal myocardial infarction and a decline in small-vessel disease progression could be of obvious clinical significance to patients with diabetes. Comparing the positive VA-HIT results achieved with gemfibro-



zil, a less potent PPAR $\alpha$  agonist, to the lack of effect on the primary end point seen in FIELD with fenofibrate, a more potent PPAR $\alpha$  agonist, might support PPAR “modulation,” as opposed to more potent activation, as being more clinically efficacious. More avid PPAR binding may not necessarily correlate with greater clinical benefit, especially because PPAR agonists have been defined largely on *in vitro* PPAR responses. At the same time, concomitant statin therapy also would have likely eroded the benefits of gemfibrozil in VA-HIT because other studies establish statin benefits independent of baseline LDL or prior history of cardiovascular disease in patients with diabetes.<sup>95,96</sup> Alternatively, the effects of gemfibrozil also could be independent of PPAR $\alpha$  activation altogether. One also cannot exclude some possible offsetting, untoward effect of fibrates, fibrate-mediated PPAR $\alpha$  activation, or other issues with FIELD that remains obscure. Importantly, FIELD does not establish the impact of fibrate/statin combination therapy on cardiovascular disease. Given the persistent cardiovascular event rate in the on-treatment arm of statin trials, the possibility remains that the combination of a statin plus a fibrate might offer greater cardiovascular risk reduction than a statin alone. This hypothesis requires direct testing; this is being studied in other settings such as the ACCORD (Action to Control Cardio-metabolic Risk in Diabetes) trial (Table 2). Statin/fibrate combination therapy does increase the risk of rhabdomyolysis, although it appears to be less of an issue with fenofibrate than gemfibrozil.<sup>97</sup> One might also look to VA-HIT and FIELD for evidence supporting the potential benefits of fibrates in that small but significant percentage of individuals who are statin intolerant or for possible fibrate benefits on small-vessel disease, which is a major source of diabetic morbidity. Interestingly, the decrease in microvascular disease observed on fibrate therapy could reflect a loss of endogenous PPAR ligand generation,<sup>29</sup> as might be predicted to occur through loss of LPL function in the microvasculature where this lipase is typically found. It is worth noting that PPAR agonist therapies in current use, including fibrates, were identified serendipitously and not based on endogenous PPAR agonists. Insight into the nature of natural PPAR agonists could offer alternative, if not better, drug templates.<sup>98</sup>

### PPAR $\gamma$ : A Key Regulator of Adipogenesis and Insulin Sensitivity

Efforts to define fat-specific transcription factors led to the identification of PPAR $\gamma$  as part of the transcriptional complex for aP2, an adipocyte-restricted intracellular lipid-binding protein.<sup>99,100</sup> The importance of PPAR $\gamma$  in adipocyte differentiation was apparent when PPAR $\gamma$  transfection into fibroblasts was sufficient to direct those cells toward an adipocyte-like phenotype. Considerable evidence has established the importance of PPAR $\gamma$  in fat, including its high levels in adipocytes, the lack of white fat in PPAR $\gamma$ -deficient mice, PPAR $\gamma$  regulation of adipokine expression, PPAR $\gamma$  interaction with other key adipocyte proteins, and the association of a PPAR $\gamma$  dominant-negative polymorphism with lipodystrophy.<sup>2,100,101</sup> In addition to adipogenesis, PPAR $\gamma$  also regulates genes involved in lipid metabolism, including LPL, acyl-coenzyme A synthetase, and aP2, and glucose control such as the glucose transporter GLUT4 and phos-

phoenolpyruvate carboxykinase.<sup>23,102</sup> The discovery via drug screening of TZDs as insulin sensitizers initially lacked an obvious molecular target to account for this effect; this was resolved with the cloning of PPAR $\gamma$  and the subsequent characterization of TZDs as high-affinity PPAR $\gamma$  ligands.<sup>22</sup> Again, reminiscent of a network node, the role of PPAR $\gamma$  in adipocyte differentiation provided a novel and direct link between the clinical action of TZDs and the regulated transcription of gene networks involved in insulin sensitivity and adiposity. Subsequent work extended this involvement to pathways of atherosclerosis.

## PPAR $\gamma$ in the Vasculature

### Vascular Endothelium

PPAR $\gamma$  expression in all major vascular cells, inflammatory cells, and human atherosclerosis itself and the early evidence for small but significant blood pressure-lowering effects of TZDs directed attention toward PPAR $\gamma$  in inflammation, vascular biology, and atherosclerosis.<sup>103</sup> The observation that humans with a dominant-negative PPAR $\gamma$  mutation developed hypertension and insulin resistance further supported this possibility.<sup>104</sup> In the endothelium, PPAR $\gamma$  has been variably reported to repress adhesion molecule expression, evident with certain PPAR $\gamma$  agonists, but not *in vivo*.<sup>54,55,105</sup> PPAR $\gamma$  agonists can repress the expression of certain chemoattractant cytokines (chemokines) in both endothelial cells and colonic epithelium; the latter provides a rationale for studying the effects of TZD on inflammatory bowel disease.<sup>106–109</sup> PPAR $\gamma$  activation also has been implicated in nitric oxide production, although some of these studies were done with 15d-PGJ2, which has known PPAR $\gamma$ -independent effects.<sup>110–113</sup> Long-term endothelial cell exposure to laminar shear stress may generate PPAR $\gamma$  activation, which is of interest because laminar shear stress also induces nitric oxide synthase and other antiinflammatory effects.<sup>114</sup> Alternatively, PPAR $\gamma$  agonists may increase nitric oxide bioavailability in cultured endothelial cells by repressing the NADPH oxidase enzyme complex, with subsequent decreased superoxide anion production.<sup>60,115</sup> Multiple other PPAR $\gamma$  endothelial effects have been reported, although the implications of these findings in humans remain under investigation.<sup>116–118</sup>

### Vascular Smooth Muscle Cells

PPAR $\gamma$  agonists decrease VSMC production of matrix metalloproteinases (MMPs) like MMP-9, matrix-remodeling enzymes implicated in plaque rupture.<sup>119</sup> TZD therapy modestly but consistently decreases systolic blood pressure, which is an effect linked to direct activation of PPAR $\gamma$  in VSMCs. PPAR $\gamma$  activation has been shown to downregulate the angiotensin II type I receptor *in vitro*.<sup>120</sup> Rosiglitazone treatment of rats blunted angiotensin II receptor expression in intact mesenteric and aortic vessels. Other groups have found decreased mitogenic signaling through insulin in VSMCs pretreated with a PPAR $\gamma$  agonist.<sup>121</sup> Interestingly, the possibility that some angiotensin receptor blockers may activate PPAR $\gamma$  has been raised.<sup>122,123</sup> Taken together, these data suggest that PPAR $\gamma$  activation may contribute to maintain VSMCs in a quiescent, differentiated state.

## The Macrophage

PPAR $\gamma$  is expressed in macrophages and foam cells in the lipid core of atherosclerotic lesions in humans.<sup>124,125</sup> Early studies reported that PPAR $\gamma$  agonist treatment could inhibit expression of the scavenger receptor A, MMPs, and cytokine-induced inflammatory gene expression such as inducible nitric oxide synthase, as well as MMP-9.<sup>124,125</sup> PPAR $\gamma$  activation also can induce expression of CD36, a class B scavenger receptor. This protein enhances oxidized LDL uptake into cells.<sup>126</sup> However, subsequent work established that PPAR $\gamma$  is not required for foam cell formation<sup>122,127</sup> and that TZDs in vivo can reverse the increase in CD36 seen in certain mouse models of obesity and atherosclerosis.<sup>128</sup> PPAR $\gamma$  also may decrease cholesterol content in macrophages by increasing cholesterol efflux through ABCA1 and ABCG1 transporters; indeed, macrophages lacking PPAR $\gamma$  have decreased ABCA1 and ABCG1 expression.<sup>129</sup> Direct administration of PPAR $\alpha$  and PPAR $\gamma$  agonists decreases atherosclerotic lesions in several mouse models of atherosclerosis through separate, ABCA1-independent mechanisms.<sup>85</sup> Tissue-restricted PPAR $\gamma$  deletion in mice has provided a valuable research tool that circumvents the lethality of PPAR $\gamma$  deficiency, with evidence for unique effects among PPAR subtypes in limiting murine atherosclerosis.<sup>77,85</sup> Studies in PPAR $\gamma$ -deficient macrophages also have suggested possible PPAR $\gamma$ -independent TZD effects, although at concentrations that may not overlap those found in humans.<sup>126</sup>

## PPAR $\gamma$ in Inflammation and Atherosclerosis In Vivo

Considerable in vivo data with PPAR $\gamma$  agonists now exist. In mice, multiple PPAR $\gamma$  agonists consistently decrease atherosclerosis in various models, including LDL receptor deficiency and after angiotensin infusion.<sup>85,130,131</sup> Expression profiling in mice supports PPAR $\gamma$  agonists as repressors of inflammation.<sup>132</sup> More recently, a high-fat diet induced hypertension in mice lacking endothelial PPAR $\gamma$ ; in these genetically modified animals, rosiglitazone had no blood pressure-lowering effects.<sup>133</sup> Thus, PPAR $\gamma$  may modulate blood pressure responses, but only in conditions of a perturbed metabolic milieu.

In humans, extensive surrogate marker studies with PPAR $\gamma$  agonists also have supported possible vascular benefits of TZDs, including decreased carotid artery intimal medial thickness,<sup>134</sup> improved endothelial reactivity,<sup>135</sup> and lower levels of inflammatory markers and mediators in response to TZDs,<sup>136</sup> even in nondiabetic patients.<sup>137</sup> For example, TZDs decrease C-reactive protein to an even greater extent than reported with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins).<sup>136,138–141</sup> Some of these serum markers modulated by TZDs in humans are the very same targets shown to be similarly regulated in vitro, eg, MMP-9. Both PPAR $\gamma$  agonists in clinical use raise HDL, whereas pioglitazone also has been shown to lower triglycerides.<sup>142</sup> PPAR $\gamma$  agonists reportedly decrease in-stent restenosis in early studies in humans.<sup>143–145</sup> The fat-specific PPAR $\gamma$ -regulated hormone adiponectin may be a target that unites the role of PPAR $\gamma$  in adipocytes and TZD effects on inflammation; extensive preclinical data demonstrate that

adiponectin exerts antiinflammatory effects and is markedly induced in TZD-treated humans.<sup>139–141,146</sup>

Together, these data provided a rationale for clinical trials examining TZD effects on clinical cardiovascular events in humans (Table 2). Several such trials have been undertaken, and data from these studies have begun to emerge. The Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) tested the effects of pioglitazone combined with usual antidiabetic therapy versus active but non-TZD antidiabetic therapy on a combined vascular end point in patients with known vascular disease.<sup>147</sup> A goal in the PROactive study was to achieve similar, matched hemoglobin A1c levels in both the TZD and non-TZD arms, perhaps providing more definitive insight into glucose-independent vascular effects of TZDs. The combined composite primary end point was a broad one: time from randomization to all-cause mortality, nonfatal myocardial infarction (including silent myocardial infarction), stroke, acute coronary syndrome, percutaneous or surgical revascularization on the coronary or peripheral vasculature, and/or amputation above the ankle. A “principal secondary end point” consisting only of the more clinically relevant and objective events of all-cause mortality, nonfatal myocardial infarction, and stroke was added before the unblinding of the data but was not part of the original study design article.<sup>148</sup> Despite the extensive in vitro and in vivo data supporting TZD effects on atherosclerosis, no statistically significant difference was observed in the primary end point between study groups. In contrast, the principal secondary end point as calculated by the investigators was positive, with a 16% decrease in those clinical events ( $P=0.027$ ). Post hoc, prespecified analyses of subgroups have been presented but not yet published; they reveal a statistically significant decrease in myocardial infarction among those with a history of myocardial infarction,<sup>149</sup> whereas recurrent stroke among those with a history of cerebrovascular event was decreased significantly.<sup>150</sup>

The PROactive study has stimulated considerable discussion regarding its clinical implications. Perhaps most fundamental among these issues is whether the lack of a significant effect by pioglitazone on the primary end point in this study arose from flaws in study design or a lack of efficacy of the TZD on cardiovascular outcomes in this cohort. As the authors acknowledge, their assumption that peripheral vascular disease (including leg revascularization) would respond to therapy in the same way as coronary risk reduction may have been erroneous. In fact, prior experience, for example with statins, might have argued against this assumption. Moreover, the timing of revascularization, especially in the periphery, often can be a subjective decision. Other than leg and coronary revascularization, all other outcomes in the composite primary end point were lower in the pioglitazone arm. Another contributor to the negative primary end point may have been the duration of the study, which was completed in 36 months rather than the 48 months originally planned. This time frame may have been too short for some effects to be seen, especially for end points such as stroke. The older age of the population (mean age, 61 years) and their relatively later stage of cardiovascular disease may also have been relevant factors influencing outcomes. Because fatty liver has



been associated with both diabetes and atherosclerotic risk,<sup>151,152</sup> some have argued that the exclusion of patients with liver function abnormalities may have eliminated from the study those patients most likely to benefit from TZDs. Indeed, several clinical studies suggest TZDs may improve fatty liver, eg, by lowering liver function tests.<sup>153–155</sup> Alternatively, given the extent of preclinical and surrogate data suggesting cardiovascular benefit with TZDs, it remains conceivable that PPAR $\gamma$  activation could have untoward effects that offset the predicted benefits. Fortunately, additional data from other studies recently completed or in progress regarding TZD cardiovascular effects should help address this issue.

In the Diabetes Reduction Approaches With Ramipril and Rosiglitazone Medications (DREAM) study, the effects of the TZD rosiglitazone and the angiotensin-converting enzyme inhibitor ramipril on the prevention of diabetes (as part of a combined end point of new diabetes and death) were studied in a 2-by-2 placebo-controlled design.<sup>156</sup> Interestingly, rosiglitazone significantly reduced the progression to diabetes among a cohort with impaired glucose tolerance and/or impaired fasting glucose, whereas ramipril had no effect on this measure (although ramipril did improve regression to normoglycemia).<sup>157</sup> This decrease in progression to diabetes with a TZD, which is consistent with prior studies including women with a history of gestational diabetes and the troglitazone arm of the Diabetes Prevention Program,<sup>158,159</sup> also might be expected to decrease subsequent cardiovascular events over time, although this has not yet been established. Nevertheless, the evidence that a TZD can delay or perhaps even prevent diabetes will now receive further scrutiny and pose challenges for regulatory agencies in deciding whether indications for diabetes prevention are warranted and, if so, pursuant to what definitions and restrictions. Of note, in DREAM, rosiglitazone also significantly lowered liver function tests compared with placebo, consistent with similar observations in smaller trials, as discussed earlier. Ramipril had no such effect on liver tests. Whether these changes in liver function tests reveal possible metabolic, inflammatory, and vascular benefits from a reduction in hepatic fat deposition is a hypothesis that requires further consideration.

One recent intriguing clinical study achieved what the PROactive investigators had set out to obtain, namely matched glucose control (HbA1c) between 2 groups of patients with diabetes treated with either a TZD (pioglitazone) or a sulfonylurea (glimepiride).<sup>139</sup> In the Pioneer study (n=173), the TZD arm demonstrated significantly greater improvements in inflammatory markers (including high-sensitivity C-reactive protein, MMP-9, and monocyte chemoattractant protein-1) than the sulfonylurea-treated group despite equivalent reductions in fasting glucose and HbA1c levels between groups. In a provocative subgroup analysis, patients who had no significant glucose lowering in response to the TZD group still had improved surrogate markers for atherosclerosis. Although limited by the small numbers of patients in these subgroups, such findings continue to raise possible disassociations between TZD effects on inflammation and the vasculature versus its glycemic benefits. Interestingly, small doses of rosiglitazone lower C-reactive protein independently of a glucose effect.<sup>160</sup> Small clinical

studies continue to report dramatic improvement in surrogate markers of atherosclerosis and inflammation in response to pioglitazone and rosiglitazone.<sup>140,141</sup> Together, these data have maintained interest in PPAR $\gamma$  as a therapeutic target while underscoring the need for adequate clinical trials data regarding the impact of TZDs on cardiovascular disease. In the recently published Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone (CHICAGO) study, significant effects of pioglitazone on carotid intimal media thickness (as compared with placebo) were reported in patients nearly matched for glycemic control<sup>160a</sup>; more rosiglitazone and pioglitazone data sets also are anticipated.

In the PROactive study, an increased incidence of congestive heart failure (CHF) was reported in the pioglitazone arm, although these events were not well adjudicated. Prior work has clearly established that TZDs can induce fluid retention, as evident from the modest decrease in hematocrit and volume expansion documented with TZD exposure.<sup>161</sup> The incidence of pedal edema seen with TZD monotherapy is  $\approx$ 3% to 5% compared with 1.2% in placebo groups.<sup>162</sup> When combined with either metformin or sulfonylurea, the incidence of pedal edema with TZDs approaches 7.5%, compared with 2.1% and 2.5% with metformin or sulfonylurea alone, respectively.<sup>163</sup> The risk of pedal edema appears similar with both TZDs in clinical use.<sup>164</sup> Concomitant TZD and insulin use has been associated with a 2- to 3-fold higher rate of edema compared with insulin alone, with rates increasing from 5% to 7% with insulin alone to 13% to 15% with insulin and TZD.<sup>163</sup> The incidence of CHF typically has been in the 1% range in prior TZD antidiabetic trials, most of which excluded patients with class III or IV CHF. A larger observational study found a risk of heart failure of 4.5% among those on a TZD versus 2.6% in those not receiving TZD treatment, with an adjusted odds ratio of edema of 1.6 with TZD treatment. In the Kaiser Permanente Northern California Registry, the incidence of CHF was 0.2% in 24 973 patients treated with a TZD who had no prior history of CHF and 3.5% in the 1964 TZD-treated patients with a history of heart failure. A hazard ratio for CHF was calculated as 1.2 after adjustment for other risk factors.

Some uncertainty has persisted as to the nature of the edema and possible CHF observed with TZD treatment and whether these responses reflect true left-sided heart CHF, an inability to tolerate fluid retention, or the known vasodilating properties of these drugs. A fairly extensive data set, albeit with shorter-term drug exposure, would indicate that TZDs do not adversely effect myocytes or myocardial function,<sup>162</sup> an important point that must be kept in mind when we consider the effects of TZDs on volume status. Indeed, other data suggest that TZDs may provide myocardial protection, eg, during ischemic injury.<sup>165,166</sup> Of note, in the PROactive study, patients receiving pioglitazone had no difference in CHF-related cardiovascular mortality compared with those on placebo.<sup>147</sup> Given the high mortality among patients with diabetes and cardiovascular disease who experience new CHF, this lack of mortality difference in the PROactive study may support the notion that these patients with known cardiovascular disease did not experience CHF because of a decrease in intrinsic myocardial function. The need to better

understand TZD-induced fluid retention is obvious and may allow more patients to receive these agents without concern. Recent studies implicate upregulation of a specific sodium channel in the distal nephron that may provide a PPAR $\gamma$ -mediated mechanism for TZD-induced edema.<sup>167,168</sup> Other mechanisms invoked for TZD-mediated edema include increased sympathetic nervous system activity, altered interstitial ion transport, and altered endothelial permeability.<sup>169–171</sup> Although some patients with diabetes, even absent class III or IV heart failure may not tolerate this volume expansion, this edema is reversible and should not necessarily be equated with myocardial toxicity. In DREAM, a disproportionate number of subjects on rosiglitazone had an adjudicated increase in edema and nonfatal CHF compared with placebo (14% versus 2%;  $P < 0.001$ )<sup>157</sup>; subjects receiving ramipril also had more CHF (12% versus 4%;  $P = \text{NS}$ ),<sup>172</sup> but given the 2-by-2 nature of this study, it is likely some of these patients also were receiving rosiglitazone. Clinically, one could argue that the significant decrease in progression to diabetes or death seen with rosiglitazone in hundreds of patients would well offset the possible increase in nonfatal CHF encountered by a much smaller group of treated subjects. However, this remains an important topic for further consideration. Regardless of the exact origin, the edema and/or CHF seen with TZDs are clinically significant and must be considered when patients are treated with these agents. The American Diabetes Association/American Heart Association joint consensus statement on TZD use in patients with diabetes recommends a thorough evaluation for occult heart failure, left ventricular dysfunction, or coronary artery disease to avoid precipitating a CHF exacerbation with TZD therapy.<sup>163</sup> Moreover, patients should report any weight gain, pedal edema, or new dyspnea or fatigue that might be indicators of serious cardiac disease after starting a treatment with TZDs. TZD therapy is not recommended for patients with class III or IV heart failure; fluid retention also can be a clinical issue for a subset of patients with class I or II CHF.

Another clinically significant side effect of TZDs is the increase in body weight induced by these agents. This change, which likely involves both increases in adiposity and fluid retention, is typically in the range of 2 to 5 kg.<sup>173</sup> Recent work suggests that TZDs may modulate mitochondrial biogenesis.<sup>174</sup> Some of the weight induced by TZDs may be beneficial, involving a shift from visceral to subcutaneous depots, and also track the increase in the antiinflammatory protein adiponectin induced by TZDs.<sup>175</sup> The change in fat distribution seen with TZDs also must include a change in energy balance and possible effects on other pathways and factors influencing body weight because a simple shift in fat location would not account for an overall net increase in body mass.<sup>176</sup> Regardless, the weight increase seen with PPAR $\gamma$  activation has undoubtedly contributed to TZDs remaining a relatively limited percentage of antidiabetic drug use. When combined with insulin, the weight gain and fluid retention seen with TZDs may be more substantial and serious. Selective PPAR modulators, PPAR $\delta$  agonists, and PPAR $\gamma$  antagonists are all being considered as possible approaches to limit the weight gain and/or edema seen with current TZDs.<sup>177</sup> The particularly large size of the PPAR LBD and

the fact that distinct biological responses derive from specific ligand-receptor physical interaction provide a scientific basis for such efforts. Certainly, clinical experience establishes that agonists for the same PPAR isotype can have unique effects. For example, both rosiglitazone and pioglitazone are free of the idiosyncratic liver failure seen with troglitazone.<sup>178–180</sup> Another strategy to limit TZD weight gain is a combination therapy with weight-reducing non-PPAR drugs that either are already approved (exenatide) or under development (rimonabant).

### PPAR $\beta/\delta$

The lack of a PPAR $\beta/\delta$  agonist in current clinical use may have contributed to less being known about this PPAR isotype. The expression of PPAR $\beta/\delta$  in essentially all cell types and tissues also suggests its potential fundamental role in cellular biology and possible widespread effects of PPAR $\beta/\delta$  agonists.<sup>39,40</sup> The highest levels of PPAR $\beta/\delta$  are found in small intestine and colon, heart, adipose tissue, and brain. Some early functional studies indicated PPAR $\beta/\delta$  involvement in epidermal differentiation, maturation, and skin wound healing.<sup>181</sup> PPAR $\beta/\delta$ -null mutant mice die in utero as a result of placental malformation.<sup>182,183</sup> Notably, the placental vessels develop normally (in contrast to PPAR $\gamma$ -deficient mice), but the connections between the placenta and the maternal deciduas are prone to disruption. The mice that survive are significantly smaller in size, weight, and adiposity. Selective overexpression of a constitutively active form of PPAR $\beta/\delta$  in mouse adipose tissue induces significant weight loss and protects against the obesity and dyslipidemia induced by a high-fat diet.<sup>184</sup> This PPAR $\beta/\delta$  effect correlated with activation of genes involved in fatty acid oxidation and adaptive thermogenesis. Importantly, PPAR $\beta/\delta$  did not have an effect on genes involved in lipid storage and thus appears to be involved primarily in energy consumption in adipose tissue. This enhancement of fatty acid oxidation also was found in genetically altered mice that overexpress PPAR $\beta/\delta$  in skeletal muscle.<sup>185</sup> In the presence of a PPAR $\beta/\delta$  agonist, the mouse skeletal muscle fibers reportedly switch from type II “glycolytic/fast twitch” to type I “oxidative/slow twitch.” This change may explain why these mice can run twice the distance of control mice.<sup>184</sup> The muscle fiber type switch also confers resistance to obesity. Together, these data implicate PPAR $\beta/\delta$  in fuel combustion and suggest that single, dual, or pan-PPAR agonists that include a component of PPAR $\beta/\delta$  activation might offset some of the weight gain issues seen with TZDs.<sup>186</sup> PPAR $\beta/\delta$  also increases HDL levels.<sup>187</sup> This effect has promoted additional interest in this receptor as a therapeutic target.

### PPAR $\beta/\delta$ in the Vasculature and Inflammation

In terms of the vasculature, PPAR $\beta/\delta$  has been studied most in monocytes/macrophages. PPAR $\beta/\delta$  expression is induced in vitro during monocyte differentiation when its activation promotes intracellular lipid accumulation.<sup>188</sup> This effect may occur through increased expression of scavenger receptor class A and CD36, which are proteins involved in lipid storage, and repression of genes involved in lipid efflux. Potentially consistent with this, LDL receptor-deficient mice treated with a PPAR $\beta/\delta$  agonist had no significant differences in aortic atherosclerosis.<sup>85</sup> Analyses of aortic valve sections

taken from LDL receptor/PPAR $\beta/\delta$ -null mice revealed 50% to 60% reduction in fatty streak size.<sup>85</sup> However, this study found no change in macrophage-dependent lipid transport, suggesting that PPAR $\beta/\delta$  does not directly regulate foam cell formation in this model. Macrophages from PPAR $\beta/\delta$ -null mice also express lower levels of monocyte chemotactic protein-1, MMP-9, and interleukin-1 $\beta$ , implicating this receptor in limiting inflammation.<sup>189</sup> PPAR $\beta/\delta$ -mediated regulation of inflammation in macrophages may differ in the presence versus absence of ligand and subsequent accessory molecule recruitment or release, which has been suggested to occur with BCL6.<sup>189</sup> Both the nature of this PPAR $\beta/\delta$  data and their complexity reveal the issues in oversimplifying activation of any PPAR as being either biologically good or bad. The role of PPAR $\beta/\delta$  in smooth muscle is largely unknown. PPAR $\beta/\delta$  activation by a prostaglandin derivative blocked apoptosis in cultured endothelial cells by upregulating the 14-3-3 protein, which binds and sequesters the proapoptotic factor Bad.<sup>190</sup> Recent work suggests that PPAR $\beta/\delta$  may induce endothelial proliferation and angiogenesis.<sup>191</sup> Further studies are necessary to explore the implications of PPAR $\beta/\delta$  activation related to inflammation and atherosclerosis in humans.

### PPAR Therapeutics: Where Do We Go From Here?

Since their initial characterization, the PPAR nuclear hormone receptor family has emerged in a relatively short period of time as key regulators of metabolism and established therapeutic targets. The troubling rise in the incidence of obesity, insulin resistance, prediabetic conditions, and diabetes mellitus, coupled with the realization that these metabolic disturbances can profoundly alter vascular function, frames the potential importance of PPARs. The molecular intersection between metabolic disorders and inflammation remains poorly understood but may reflect a crucial evolutionary need to link fuel utilization and fuel stores with wound healing and tissue repair. The metabolic disarray associated with and often induced by diabetes and/or dyslipidemia may lead these metabolic networks to become maladaptive. Environmental changes such as increased access to calories, alterations in the nature of those calories, and a decline in physical activity likely exacerbate these issues, with long-term consequences such as diabetes and atherosclerosis.

The torrent of rapidly emerging molecular and clinical data regarding PPARs has established these nuclear receptors as transcriptional regulators of key metabolic pathways, with roles that extend to vascular and inflammatory systems. The therapeutic targeting of PPARs stands as a separate issue. Clearly, PPAR activation improves dyslipidemia and insulin sensitivity. The extent of these benefits and whether they extend to atherosclerotic complications remain to be established. At the same time, the boundaries for such potential benefits continue to move, eg, with evidence that TZDs can delay or even conceivably prevent the development of diabetes among patients who often also are at increased cardiovascular risk. Regardless of the future of therapeutic targeting of PPARs, it is important to distinguish between the effects of the various synthetic PPAR-modulating molecules currently

available or in development and the biological role of PPARs themselves in vivo. All PPAR isoforms regulate central metabolic pathways in human physiology, making them inherently important for further study. Separate from this biological function is the question of how to best exploit PPARs for treating metabolic disorders like dyslipidemia, diabetes, and their vascular complications.

For the clinician, the use of currently available PPAR agonists involves understanding the interface between biology and clinical responses considered here. Despite our clinical experience with PPAR agonists, further progress in targeting PPARs safely and therapeutically and with a scientific rationale requires a deeper understanding of PPAR biology, the effects of PPAR modulation, and how such responses differ between structurally distinct molecules. The recent abandonment of novel dual PPAR $\alpha/\gamma$  agonists because of disappointing and/or worrisome clinical effects<sup>192,193</sup> and ongoing attempts to develop novel PPAR modulators only underscore the need for such insight.

In the present scientific era, the longstanding need for more biological data is now countered by a competing and perhaps equally important demand for understanding how such pathways converge. This is particularly true for the clinician and may be especially relevant to diabetes, atherosclerosis, and their complicated relationship. Although resolving such questions remains a challenge, PPARs, as nuclear receptors that can sense the extracellular environment and respond by orchestrating gene expression in multiple pathways, are well positioned to provide answers to how metabolism, inflammation, and vascular function are integrated.

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## Peroxisome Proliferator–Activated Receptors as Transcriptional Nodal Points and Therapeutic Targets

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