PPARs and lipid ligands in inflammation and metabolism

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) belong to a family of ligand-activated nuclear receptors that includes the estrogen, thyroid hormone, and glucocorticoid receptors[1]. The PPAR family consists of three subtypes encoded by three separate genes: PPAR-α (NR1C1), PPAR-β/δ (NR1C2), and PPAR-γ (NR1C3). Distinct PPAR subtype tissue distributions[2,3] and unique ligand-binding pockets drive separate but often complementary patterns of gene expression in response to PPAR ligands[4]. PPAR activation occurs upon cognate synthetic or endogenous ligand binding to the ligand-binding domain (LBD). Activated PPARs heterodimerize with retinoid X receptors (RXRs), another class of nuclear receptor, which subsequently bind to the hexameric direct repeat peroxisome proliferator response elements (PPRE)[56] and recruit co-activator protein complexes to positively regulate expression of target genes (Figure 1A). PPARs also mediate ligand-dependent repression of inflammatory gene expression through the association with co-repressor protein complexes[7].

Nearly all nuclear receptors share structural similarity consisting of a conserved DNA-binding domain (DBD) and LBD[1]. The PPAR subtype structural similarities contribute to the partial overlapping function of PPARs across different tissues. In hepatocytes, PPAR-α positively regulates fatty acid β-oxidation, ketogenesis, and gluconeogenesis, while suppressing amino acid catabolism and inflammatory responses[8]. PPAR-α plays anti-inflammatory roles in smooth muscle cells and vascular endothelial cells[9,10]. PPAR-β/δ (PPAR-δ) plays roles in lipid metabolism[11], fatty acid oxidation and energy dissipation[12], anti-inflammation[13], and colon cancer[14]. PPAR-γ is an essential modulator of fat cell differentiation[15-17] and lipid storage and plays important anti-inflammatory roles in macrophages[18,19] and other tissues such as the colon[20]. PPAR-γ also contributes to insulin sensitivity[21], in part through the regulation of adiponectin, an adipo(cyto)kine that enhances insulin sensitivity[22].

PPAR-γ is activated by synthetic ligand thiazolidinediones (TZDs)[7]. TZDs, including rosiglitazone and pioglitazone, are potent insulin sensitizers that have a myriad of potential benefits for patients with cardiovascular disease including improvements in endothelial function, lipid profiles and atherosclerosis[23-25]. TZDs, however, augment renal sodium reabsorption, leading to fluid retention that can exacerbate heart failure[26-28]. Recent meta-analyses have raised questions surrounding the safety of TZDs, linking the drugs to the occurrence of myocardial infarction and death[29,30]. While some studies suggest that the

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relative risks of rosiglitazone are higher than pioglitazone, the possibility that all TZDs may have adverse risk profiles has not been excluded. The unwanted side effects of TZDs have raised the prospects for the development of newer and safer PPAR ligands, such as the therapeutic usage of natural PPAR ligands. Recent studies have identified physiologically relevant endogenous PPAR ligands linked to the expression of their endogenous synthetic enzymes in specific tissues. Examples include 15-keto-prostaglandin E_2 produced by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in colonic epithelial cells and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 produced by prostaglandin D_2 synthase (PGD$_2$S) in macrophages (Figure 1B). Further study of the effectiveness of natural PPAR ligands or synthetic molecules that mimic the actions of natural ligands is needed to determine their potential as clinical therapeutics.

This review will characterize the structural and functional relationships of PPARs, define the regulatory mechanisms that control PPAR activities, and review the candidate natural ligands of PPARs to provide a framework for understanding the roles of PPARs as anti-inflammatory therapeutics.

2. PPAR Structure

PPARs share similar structural features with other nuclear receptors[5], including a poorly conserved amino-terminal domain, a highly conserved DBD, a connecting hinge region (also referred to as the C-terminal extension; CTE), and a discrete LBD[31]. The central DBD is highly conserved among PPAR isoforms. The LBD contains an interior binding pocket specific for the cognate ligand. The domain also carries the moderately conserved ligand-regulated transcriptional activation function-2 (AF-2)[32] that forms part of the ligand-binding pocket and is required for recruitment of co-activators such as NCoA-1/SRC-1[33]. The N-terminal region (the A/B domain) is variable in length between receptors and contains a poorly conserved transcriptional activation function domain (AF-1), the activity of which is controlled by the cognate ligand[34]. The AF-1 region of the PPAR family members plays a role in determining PPAR isotype-selective gene expression differences[35]. The activity of the A/B domain is regulated by post-translational modifications.

3. Ligand-binding affinity

Resolution of the crystal structure of ligand-free (apo) or ligand-bound (holo) nuclear receptor LBDs with the associated co-activator fragments[36-39] has provided the molecular details of ligand-induced transcriptional activation by nuclear receptors. Nuclear receptor LBDs are folded into three layers of α-helices that allow the formation of a ligand-binding pocket buried within the core of the α-helices. A globular domain consisting of 11-13 α-helices is arranged in anti-parallel helical sheets that combine to make what is described as an α-helical sandwich[5]. Three long helices (helices 3, 7, and 10) form the two outer layers of the sandwich, while the middle layer of helices (helices 4, 5, 8, and 9) is present in only half of the globular domain, creating a cavity in the structure for binding of the ligand in most of the receptors[40].

The first step of nuclear receptor activation is initiated by ligand binding. The specificity of the LBD-ligand complex is largely based on hydrophobic interactions, hydrogen-bonding networks, and the steric size and shape of the binding pocket[5]. The ligand-binding pocket varies greatly in size and shape between nuclear receptors as do the binding affinities toward their respective ligands. For example, estrogen receptor, retinoic acid receptor and vitamin D receptor have smaller ligand-binding pockets and bind their respective ligands, 17β-estradiol, retinoic acid, and vitamin D_3, with high affinities (subnanomolar)[41]. The PPAR-γ LBD is composed of 13 α-helices and a small four-stranded β-sheet that forms a large (approximately 1440 Å^3) hydrophobic ligand-binding pocket typical of the promiscuous

nuclear receptors, including the PPARs and the pregnane X receptor (PXR), that bind many different ligands with low affinities[42]. The LBD pockets of PPARs have a distinct three-arm T shape, allowing them to bind ligands with multiple branches such as phospholipids and synthetic fibrates in addition to singly-branched fatty acids[43]. Similarly, the LBD pocket of PXR is large (1200Å³) with an elliptical shape and structural plasticity allowing the recognition of a broad range of xenobiotic compounds and endogenous C21 steroids (pregnanes)[44]. The LBD of PPAR-δ is narrower than those of PPAR-γ and PPAR-α, prohibiting binding of many large ligands that activate the other receptors[45]. PPAR-α contains the most lipohilic ligand-binding pocket, explaining the affinity for a variety of saturated fatty acids[43]. As a result of the LBD pocket structures, PPARs possess a relatively low binding affinity for a broad range of putative ligands including fatty acids, phospholipids, eicosanoids and prostaglandin metabolites[7].

Activation by ligand-binding modulates transcriptional activity of PPARs. Ligand-triggered activation stabilizes the interaction between PPAR and RXR and enhances the formation of functional PPAR-RXR heterodimers[46]. Evaluation of the crystal structure of the PPAR-γ-RXR-α-DNA complex demonstrated that the LBD assists the DBDs of both heterodimeric partners in binding to DNA. In addition, the LBD forms the centerpiece of the complex around which the other domains from PPAR-γ and RXR-α are arranged[47]. The LBD-ligand interaction, therefore, plays a key role leading to complex formation that results in gene activation or repression.

4. Transcriptional Control by PPARs

4.1 Peroxisome Proliferator Response Elements

PPARs, like most nuclear receptors, function as ligand-gated transcription factors. In contrast to steroid hormone receptors, which bind to DNA as homodimers in a ligand-dependent manner, PPAR/RXR heterodimers can bind to DNA in the presence or absence of ligands for either heterodimeric partner. However, interaction of PPARs with lipid or synthetic ligands enhances heterodimerization with RXR and increases DNA binding[48]. Early studies of PPARs demonstrated that the nuclear receptor PPAR-α heterodimerizes with RXR in response to natural ligand polyunsaturated fatty acids and the fibrate Wy14,643[49]. The PPAR-RXR heterodimer subsequently binds to regulatory DNA elements called PPREs and initiates transcription.

The PPRE is a direct repeat type 1 (DR1) consisting of a hexameric nucleotide recognition motif (AGGTCA) spaced by one nucleotide. An additional AACT consensus motif is positioned 5′ to the DR1[50]. The PPAR-RXR-DNA crystal structure revealed that the PPAR-γ hinge region (CTE portion) makes an extensive DNA interaction, binding to the element 5′ upstream of the hexameric repeat[47]. Genome wide chromatin immunoprecipitation sequencing (ChIP-seq) studies to determine the DNA elements bound specifically by PPAR-γ confirmed the localization of the receptor to DR1-like element PPAR-γ consensus motifs under physiologic conditions in macrophages and adipocytes and demonstrated that PPRE-binding sites may be either shared between cell types or cell-type specific[51].

4.2 PPAR expression

Individual PPAR receptor expression levels underlie the tissue-specific activation profiles of PPAR ligands. PPAR-α may bind to a given PPRE in tissues where it predominates, such as the liver, while PPAR-γ may regulate the same response element in other cells types such as adipocytes or macrophages where PPAR-γ expression is higher. The lipoprotein lipase (*LPL*) gene, for example, contains a PPRE that is regulated by both PPAR-α and PPAR-γ depending upon the cell type. Treatment with PPAR-α agonists such as fenofibrate induces *LPL* exclusively in liver, while the PPAR-γ agonist BRL49653 induces expression in

adipose tissue without affecting transcription in liver[52]. Similarly, a shared bi-directional PPRE located in the region between the murine PEX11α and Perilipin genes is regulated by both PPAR-α and PPAR-γ, however, the direction of activation determining which gene is activated is cell type specific. In the liver, PPAR-α binds to this PPRE and selectively activates the PEX11α gene, leading to peroxisome proliferation. In adipose tissue, PPAR-γ binds the same PPRE and selectively activates the Perilipin gene which promotes triglyceride accumulation by shielding lipid droplets[53]. Despite shared PPRE utilization in some instances, PPARs maintain a high degree of subtype specificity, and one PPAR isotype does not necessarily complement the function of another when expressed in a given cell type. Adenoviral expression of PPAR-γ1 in mouse liver, for example, leads to induction of genes involved in lipid accumulation and adipogenesis that are not readily activated by PPAR-α present in the liver[54]. The data suggests that PPAR subtype expression levels contribute but do not fully account for different PPAR responses across cell types.

Differences in PPAR-γ isoform expression also contribute to tissue specific differences in PPAR-mediated gene expression. Alternative splicing and differential promoter usage result in expression of two PPAR-γ isoforms, PPAR-γ1 and PPAR-γ2. PPAR-γ2 carries an additional 30 amino acids at the N-terminus and is largely expressed in adipocytes where it drives a higher ligand-independent basal activity compared to PPAR-γ1. The N-terminal ligand-independent activation function 1 (AF-1) domain is responsible for the difference in basal transcription between the two isoforms. The activation function of the N-terminal domain of PPAR-γ2 is 5-6 fold greater than that for PPAR-γ1[55]. Deleting PPAR-γ2 confirmed a role of the receptor in the adipose proliferative response[56]. Using chimeric PPAR-γ/PPAR-δ proteins, the AF-1 region of PPAR-γ was shown to be essential for adipogenesis[35,57] while comparison of the PPAR-γ1 and PPAR-γ2 isoforms confirmed that PPAR-γ2 was more effective at inducing adipogenesis[58]. These data imply that the relative cell specific distribution of PPAR subtypes and isoforms contributes to differences in PPAR-mediated target gene expression and ligand responsiveness among cell types.

4.3 Post-translation Modification of PPARs

PPAR activity can be modulated by post-translational modification. In the case of PPAR-α and -γ, phosphorylation of the A/B segment has been found to play an important role in modulating receptor activation. Mitogen-activated protein (MAP) kinase mediated phosphorylation of serine 82 in the PPAR-γ1 A/B-domain (or the corresponding serine 112 in PPAR-γ2) has been shown to inhibit both ligand-dependent and independent PPAR transactivation[59]. PPAR-γ activity is reduced upon serine 82 phosphorylation following MAPK activation by growth factors (epidermal growth factor, platelet-derived growth factor, transforming growth factor-β, or insulin) as well as c-Jun N-terminal kinase 1/2 or p38 activation[59-63]. Conversely, modification of the same residue by cyclin-dependent kinases, cdk7 and cdk9, was found to increase PPAR-γ activity[64,65]. Phosphorylation of the PPAR-γ A/B-domain, therefore, may either inhibit or stimulate transcriptional activity, depending upon the cellular context and/or kinases involved. Additionally, recent work has demonstrated that reductions in insulin-sensitizing genes dysregulated during obesity, such as adiponectin and adipsin, are linked to the phosphorylation of serine 273 in the PPAR-γ2 induced by cyclin-dependent kinase 5[66]. Synthetic ligands blocked cdk5-mediated phosphorylation at serine 273 in concert with improvements of insulin sensitivity. The results provide a mechanistic framework for the development of novel PPAR-γ ligands that have insulin-sensitizing but fewer adverse side effects.

Sumoylation of PPARs involving the covalent attachment of small ubiquitin-like modifier (SUMO) peptides also modifies PPAR activity. SUMO is a 100-amino acid polypeptide that is covalently attached to proteins through a process typically coordinated by E3 ligases. Four SUMO proteins have been described. The modification occurs at the lysine residue of the

consensus motif φKXE/D (φ: large hydrophobic residue, X: any residue) in the substrate proteins. Sumoylation of transcription factors typically leads to repression of transcription. Androgen receptor[67], glucocorticoid receptor[68], liver X receptor[69], PPARs and other nuclear receptors have been shown to be SUMO modified. Two functional sumoylation sites have been identified for PPAR-γ, lysine 107 in the AF-1 domain and lysine 395 in the AF-2 domain (lysine 77 and lysine 365 in PPAR-γ1, respectively). Conjugation of SUMO-1 or -2 to lysine 107 by PIAS1 or PIASxβ inhibits PPAR-γ activity. The sumoylation defective K107R mutant of PPAR-γ2 exhibited stronger transactivation than the wild type protein[53,70,71]. In contrast to K107 SUMO conjugation, SUMOylation of K395 contributes to the anti-inflammatory function PPAR-γ on inflammatory genes such as inducible nitric oxide synthase (*Nos2*) in macrophages[72]. Treatment with ligand resulted in sumoylation of K395, targeting PPAR-γ to the NCoR-containing corepressor complexes bound on NFκB target genes, sustaining inflammatory gene repression.

PPAR-γ activity can in part be regulated by ubiquitination. Ubiquitination is the covalent attachment of ubiquitin, a 76-amino-acid peptide to lysine residues in the substrate protein. The PPAR-γ protein is polyubiquitinated and targeted for degradation by the proteasome. Ligand-dependent activation of PPAR-γ is accompanied by ubiquitination and subsequent proteasomal degradation of the receptor, resulting in selective down-regulation of PPAR-γ expression after activation[73]. Treatment of adipocytes with interferon-γ also increased ubiquitination and led to degradation of PPAR-γ[74,75].

PPAR-α activity is regulated by post-translational modifications including phosphorylation[76], ubiquitination[77] and SUMOylation. Murine PPAR-α is SUMOylated at K358, promoting interaction with GA-binding protein alpha bound to the Cyp7b1 promoter and down-regulating this gene[78]. SUMO-1 conjugation of human PPAR-α at K185 of the hinge region of the receptor down-regulates its transcriptional activity by recruiting the corepressor NCoR. Mutation of this lysine K185R increased transcriptional activity while ligand activation inhibited SUMOylation and preventing NCoR binding[79].

4.4 Coactivators and Corepressors

Nuclear receptors can function as molecular switches, alternating between states of transcriptional repression and activation, depending on the presence or absence of ligand and association with coactivators or corepressors[80]. Coregulator proteins are often components of large multiprotein complexes that act sequentially and/or in combinatorial fashion to remodel chromatin, mobilize nucleosomes and recruit the transcription machinery[81]. Many of the common cofactors for the nuclear receptor family of proteins regulate PPAR activity, including the p160 co-activator members SRC-1/NCoA1[33,82], TIF2/GRIP1/NCoA2/SRC-2[83,84], and pCIP/ACTR/AIB1/SRC-3[85]. The nuclear receptor interaction domain of these coactivators is highly conserved and contains one or more copies of the consensus motif LXXLL which can mediate ligand-dependent interaction with nuclear receptors[86-88]. In contrast, the nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) corepressor molecules contain an extended LXXXIXXXL motif that binds to the coactivator binding pocket in the absence of agonists or the presence of antagonists. Binding of agonists to the LBD induces a conformational change that displaces the LXXXIXXXL motif and generates a 'charge clamp' for LXXLL motifs[42, 89].. The p160 proteins serve as foundations upon which coactivator complexes are assembled. The p160 proteins recruit factors such as cAMP responsive element binding protein (CREB) binding protein (CBP)/p300 which possesses the histone acetyltransferase (HAT) activity for remodeling chromatin and allowing transcriptional activation[90]. PPAR activity, therefore, depends on the presence of the associated coactivator complex proteins.

PPAR subtype specificity is partly imposed by differential affinity of the receptors towards their individual cofactors. The A/B domain (containing AF-1) plays an important role in defining the PPAR isotype-specific transcriptional responses. Three A/B-interacting proteins have been identified that display isotype-specific interactions: PPAR-γ co-activator 2/ SCAN domain protein 1 (PGC-2/SDP1), HIV-1 Tat-interacting protein 60 (Tip60), and the corepressor *tribbles* homolog 3 (TRB3). PGC-2 and Tip60 are co-activators of PPAR-γ that promote while TRB3 is a corepressor of PPAR-γ that suppresses adipogenesis[57,91,92]. Additionally, a portion of the transcriptional activation of PPAR-γ depends upon the presence of an intact A/B domain. The A/B domain dependent genes are the highly selective PPAR-γ target genes, many of which are involved in lipid storage. Deletion of the PPAR-γ2 A/B-domain reduced the recruitment of CBP and p300 to the PPREs of genes that required this domain for full activation[93]. The results confirm that PPAR subtype specific domain-interacting cofactors, such as those interacting with the A/B-domain, help define PPAR-subtype specific functions.

The ligand-independent silencing activity of nuclear receptors is due to an ability to recruit NCoR/SMRT corepressors and establish a corepressor complex on target gene promoters that can affect chromatin structure through the associated histone deacetylase (HDAC) activity of the complex proteins[94]. PPAR-γ is capable of recruiting the corepressors SMRT and NCoR in 3T3-L1 cells, repressing basal PPAR-mediated transcriptional activity in the absence of ligand[95]. PPAR agonists therefore convert PPAR/RXR heterodimers from repressors of transcription to transcriptional activators at these sites by inducing a conformational change in the ligand binding domain that results in exchange of NCoR/ SMRT corepressor complexes for coactivator complexes that contain components with LXXLL interaction motifs (Figure 2A) .

4.5 Permissive Chromatin

In addition to the role of PPAR isotype expression in defining tissue-specific activation by PPARs, the specificity and potency of activation by PPARs are highly dependent upon cell type[96]. Cofactor differences between cell types contribute to PPRE activity by altering the available binding sites in addition to roles they play in PPAR-containing activation or repression complexes. Genome-wide studies of adipocytes demonstrated that PPAR-γ localizes preferentially to lipid and carbohydrate metabolism genes, many of which are downregulated after the knockdown of PPAR-γ expression. Chromatin immunoprecipitation (ChIP) of PPAR-γ in adipocytes confirmed enrichment of PPRE binding sites (DR1-like elements) in the majority of the binding regions identified by sequencing PPAR-γ bound DNA regions (ChIP-seq)[97,98]. C/EBP-α and C/EBP-β binding motifs were enriched in the proximity of PPAR-γ binding regions, suggesting a cooperative role for the CCAAT/ enhancer binding protein (C/EBP) family in the PPAR-γ-dependent target gene expression of adipocytes that was confirmed after C/EBP knockdown.

PPAR binding site specificity, however, is cell-type specific. The cell-specificity of binding is determined in part by the presence of heterochromatin in a non-permissive, transcriptionally silent or active (euchromatin) state. Dimethy lysine 9 of histone 3 (H3K9Me2) and trimethyl lysine 27 of histone 3 (H3K27Me3) markers are present on transcriptionally silent heterochromatin[99,100]. When comparing macrophages and adipocytes, the macrophage-unique PPAR-γ-binding sites were marked by these repressive histone methylation marks in adipocytes[51] consistent with inactive chromatin in the adipocyte that prevented PPAR-γ from binding. The data suggest a model where cell specificity of PPAR binding is restricted by the presence of heterochromatin in permissive or nonpermissive states for a given PPAR binding site. Whereas C/EBP was an important factor defining functional binding sites in adipocytes, the hematopoietic transcription factor PU.1 co-localized with PPAR-γ in areas of open chromatin in the subset of immune genes

unique to the macrophage[51]. The data supports the hypothesis that cell specific factors may establish open chromatin configurations that permit PPAR binding site accessibility. Co-factors such as PU.1, therefore, help to define the macrophage specialized functions of PPAR-γ, such as maintenance of the alternative macrophage phenotype[101,102], cholesterol uptake and efflux in atherosclerotic plaques[103,104], antigen cross-presentation to T lymphocytes[105], and dendritic cell immunogenicity[106] that are unique from the metabolic functions of the receptor presumably by allowing additional PPAR binding sites that are unique to the macrophage.

4.6 Endogenous Ligand Regulation

PPARs are activated by fatty acids and naturally occurring fatty acid-derived molecules including eicosanoid and prostaglandin derivatives. The relative tissue quantity of endogenous ligands for PPARs is expected to play a role in defining the activation state of a given PPAR subtype in a given cell. Co-repressor displacement[107] or co-activator recruitment[108,109] along with histone acetylation[110,111] in the setting of ligand-dependent PPAR-γ activation (either endogenous lipid species or exogenous ligand such as fibrates or thiazolidinediones) guides temporal or disease-state-specific PPAR-γ enhancer activation. A representative example is the loss of TRAP220 mediator complex component at PPAR-γ-specific binding regions in the cystic fibrosis transmembrane regulator (*Cftr*)-null mouse colonic epithelial cells associated with a concomitant reduction of target gene expression and the endogenous PPAR-γ ligand 15-keto-prostaglandin E_2(15-keto-PGE_2)[112]. Measurable differences in endogenous PPAR-γ ligand quantities defined the disease-state difference in PPAR-γ activation between the *Cftr*-null and wild type colonic cells. The relevant endogenous PPAR ligands in a given cell type, therefore, can control the cell-type and PPAR-isotype specific transcription activity under physiologic conditions.

Specific PPAR ligand species are present in different tissue types, accounting for a component of cell-specific PPAR activation. Prostaglandin D_2 synthase (PGD_2S), the enzyme responsible for producing PGD_2, the precursor of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), is expressed predominantly in macrophages and specialized antigen-presenting cells and is absent in epithelial cells[113]. As a result, 15d-PGJ_2 can be found in monocytes after IL-13 treatment[114] and is present in the cytoplasm of foamy or spindle macrophages of human atherosclerotic plaque[115] but was not detected by liquid chromatography/tandem mass spectrometry from colonic epithelial cells (author's unpublished observation). 15d-PGJ_2 production increases during the inflammatory process coordinate with increased expression of PGD_2S. Similarly, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the enzyme responsible for producing 15-keto-PGE_2, is highly expressed in pulmonary and colonic epithelial cells but is not expressed in macrophages. As a result,15-keto-prostaglandin E_2 was found to be an important physiologically relevant PPAR-γ ligand in colonic epithelial cells[112]. The regulation of cell-specific or tissue-specific ligand production, therefore, contributes to cell-specific PPAR isoform activation states (Figure 1A).

5. PPARs and Transrepression

5.1 PPARs and inflammatory responses

NF-κB is a critical activator of genes involved in the inflammatory process[116]. Pro-inflammatory cytokines activate the IκB kinase (IKK) complex that phosphorylates the NF-κB inhibitors, triggering their degradation and freeing NF-κB to translocate to the nucleus where it induces target genes such as the inducible cyclooxygenase (COX2) gene[117]. Additionally, AP-1, STAT and IRF transcription factors contribute to inflammatory

responses. AP-1 is composed of homo- or heterodimers among members within the Jun and Fos families. c-Jun in association with c-Fos is the major AP-1 heterodimer[118,119].

Several mechanisms have been described for the negative regulation of inflammatory gene expression by nuclear receptors. In addition to the roles of PPARs in lipid and glucose metabolism, the three PPAR isoforms have emerged as key regulators of inflammatory and immune responses. PPARs are expressed in vascular and immunological cell types such as monocytes/macrophages, endothelial cells, lymphocytes and dendritic cells. PPARs can inhibit inflammatory gene expression by several mechanisms, including direct interaction with NF-κB or AP-1 subunits, modulation of kinase activities, competitive binding for limiting pools of coactivators, and interaction with co-repressors. PPAR-γ has been demonstrated to mediate anti-inflammatory signaling in a number of inflammatory model systems, antagonizing the production of cytokines induced by gamma interferon (IFN-γ) and lipopolysaccharides (LPS) through inhibition of STAT1, NF-kB, and AP-1[18,19]. Additional research has suggested that PPAR ligands display anti-inflammatory effects in several disease models including atherosclerosis, obesity-induced insulin resistance, autoimmune encephalomyelitis, psoriasis, inflammatory bowel disease, and arthritis[7].

Some of these models have been described using the ligand 15d-PGJ$_2$ which has been demonstrated to show PPAR-γ independent anti-inflammatory effects. It is probable that some of these mechanisms only operate in the case of specific cell types or PPAR isoforms. Consistent with this, PPAR-γ agonists do not interfere with NF-κB nuclear entry in macrophages and specifically regulate a subset of LPS-inducible genes[120-122], indicating promoter-specific mechanisms underlying transrepression in the macrophage rather than a global effect of antagonizing inflammatory signaling.

5.2 Direct Interactions Between PPARs and Inflammatory Transcription Factors

Many nuclear receptors can exhibit inhibitory effects that do not involve direct sequence-specific DNA binding by the DNA binding domain. One established mechanism is the tethering of nuclear receptors to negatively regulated transcription factors, resulting in inhibition of DNA-binding and/or transactivating functions. Studies of the anti-inflammatory effects of glucocorticoids initially described this model. Following glucocorticoid treatment, the glucocorticoid receptor (GR) interacts directly with AP-1[123-125] and NF-κB[126,127] subunits to mediate an anti-inflammatory effect. Similar inhibition of AP-1 has been seen with the RXR-α ligand 9-cis-retinoic acid[128].

Several reports have indicated that PPARs may exert a component of their anti-inflammatory effects in certain cell types through mechanisms involving direct interactions with inflammatory transcription factors including NF-κB, AP-1, or STAT, resulting in inhibition of coactivator recruitment (Figure 2B) or recruitment of corepressors. Ligand-activated PPAR-α has been shown to interact with p65 and c-Jun and prevent inflammatory activation by IL-1α[129]. PPAR-α ligands have also been shown to induce expression of the inhibitory protein inhibitor of kappa B (IκBα) in liver and smooth muscle cells, which sequesters NF-κB in the cytoplasm and reduces activity[130-132]. PPAR-γ has similarly been shown to interfere with c-Jun promoter binding in vascular endothelial cells[10] and to interfere with NF-κB nuclear localization in macrophages[133] and colonic epithelial cells[134] under specific experimental conditions. PPAR-γ agonists including ciglitazone and 15d-PGJ$_2$ also stabilized IκBα levels in lungs, resulting in reduced activation of NF-κB[135]. The overall contribution of these mechanisms to the anti-inflammatory role of PPARs is unclear since PPAR ligands appear to have promoter-specific anti-inflammatory functions, particularly in the low micromolar range of concentration that is receptor dependent, rather than general anti-inflammatory effects on all promoters.

5.3 Regulation of Kinase Activity

A second mechanism for transrepression involves inhibition of signal transduction pathways necessary for transcriptional activation. c-Jun transcriptional activity is enhanced by amino-terminal phosphorylation on Ser63 or Ser73 by members of the Jun amino-terminal kinase (JNK) and mitogen-activated protein-serine (MAP) families[136-138]. Activation of c-Jun, for example, is greatly increased by phosphorylation of Ser63 or Ser73 by members of the Jun amino-terminal kinase (JNK) superfamily. Glucocorticoids, retinoic acid, and T3 have been shown to inhibit JNK, blocking c-Jun phosphorylation and inhibiting AP-1 dependent activation mediated by inflammatory stimuli such as TNF-α[139,140]. Inhibiting JNK and other signaling mediated phosphorylation of c-Jun, reduces the induction of AP-1-related inflammatory target genes.

A role for the anti-inflammatory effects of PPAR-γ ligands in regulating kinase activity has been demonstrated in the colon, where ligand activation of PPAR-γ and RXR-α reduced JNK and p38 MAPK activation induced by the chemical colitis agent 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Figure 2C). The study confirmed an anti-inflammatory effect of PPAR-γ ligands, although an impact on c-Jun phosphorylation or AP-1 activation was not confirmed[141]. PPAR-α agonists have been shown to antagonize the phosphorylation of c-Jun in microglial BV-2 cells in response to whole body radiation[142]. Other studies failed to show that PPAR-α and/or -γ agonists inhibit c-Jun or p38 phosphorylation and that they primarily act on transcription events in the nucleus[143,144].

5.4 Competitive Interference with Coactivators

One studied model of repression by nuclear receptors describes that inhibition of inflammatory gene activation is mediated through competitive binding of limiting quantities of the general coactivators CREB-binding protein (CBP) and p300. A recruitment model has been proposed for proteins that interact with CBP/p300, such as the c-Jun and p65, demonstrating that interference or squelching of one another can occur when the intracellular levels of CBP are limiting. CBP/p300 is recruited to the promoters of activated genes by association with the stress-inducible factors NF-κB, IRF, and AP-1 where it contributes to transcriptional activation[145-147]. Consistent with this, recent work has confirmed LPS-inducible p300 chromatin association with transcriptional enhancers in LPS-inducible transcripts on a genome-wide scale[148]. Activation of alternative parallel pathways that utilize the same co-activators (CBP/p300) might diminish the inflammatory stimulus response if the co-activator concentration is limiting. Competition for CBP/p300 has been proposed to contribute to the anti-inflammatory effects of glucocorticoids[147], although this mechanism did not explain glucocorticoid repression of the IL-6 promoter in human embryonic kidney cells[149,150].

Some evidence suggests that a portion of the anti-inflammatory effects of PPAR ligands may be mediated through coactivator competition (Figure 2D). Upon stimulation of PPAR-γ with ligand, the interaction between PPAR-γ and CBP is increased[151]. In the case of the inducible nitric oxide synthase (*Nos2*) promoter, PPAR-γ mediated repression occurred only in the presence of wild type receptor that was capable of recruiting CBP[152], while transrepression did not occur with the 'charge clamp' containing mutant PPAR-γ that fails to interact with coactivators. Similar studies in epithelial cells found that overexpression of CBP/p300 could partially block the anti-inflammatory effects of PPAR-γ agonists on COX2 expression[153]. The data supports a model where PPAR-mediated transrepression is correlated with transactivation. Other studies of PPAR-α have shown that PPAR-dependent repression was independent of the CBP co-expression in the cell[129]. The basis for the differences between these experimental conclusions is unclear, although many of the experiments performed have been completed using overexpression systems, and it is

suspected that the earlier work may not fully reflect the native levels of co-regulators in the cell.

5.5 Co-repressor Model of Transrepression

Two distinct models for corepressor-dependent transrepression have been proposed by PPARs. In the case of PPAR-δ, an association with the transcriptional repressor BCL-6 occurred in the absence of PPAR-δ ligands, antagonizing the BCL-6 repressor function. In the presence of ligand, PPAR-δ released BCL-6, which exerted anti-inflammatory effects by repressing transcription from NF-κB-dependent promoters[13]. In the second model, recent studies have highlighted the role of PPAR-γ in stabilizing corepressor nuclear receptor corepressor (NCoR)-histone deacetylase-3 (HDAC3) containing complexes at target promoters[72]. In addition to mediating repressive functions of unliganded nuclear receptors, NCoR corepressor complexes also confer repression functions to other classes of transcription factors. Recent studies indicate that NCoR complexes occupy subsets of inflammatory response genes in macrophages and other cell types and play roles in maintaining these genes in a repressed state under basal conditions. In the presence of an inflammatory stimulus such as LPS, the NCoR-containing repressor complex is rapidly cleared from the promoter, resulting in de-repression as prerequisite for subsequent binding of activating factors such as NF-κB and subsequent transcriptional activation. Treatment with PPAR-γ ligands reduced NCoR clearance from the promoter, resulting in maintenance of repression (Figure 2E). This mechanism was linked to SUMOylation of lysine 365 of PPAR-γ. Mutation of the K365 residue, prevented SUMOylation of the receptor, eliminated PPAR-γ recruitment to the inflammatory promoter, and allowed signal-dependent NCoR clearance. It is important to note at this point that all mechanistic studies of anti-inflammatory actions of PPARs have been carried out in cell culture systems and the biological roles of any specific mechanism remains to be determined in an in vivo context.

6. Synthetic and Endogenous PPAR Ligands

Because of the broad roles of PPARs in regulating metabolism, inflammation, differentiation, and cellular growth, a large number of specific and potent synthetic ligands have been generated (Figures 3-5). While PPAR-α is activated by the fibrate drugs such as clofibrate and Wy14,643, PPAR-γ is the receptor for the thiazolidinedione (TZD) class of antidiabetic drugs. TZDs stimulate pre-adipocyte differentiation[154] and induce insulin sensitization[155], leading to their usefulness in treating diabetes. TZD drugs were recognized to activate the *aP2* (*FABP4*) gene transcription[156] through an enhancer element identified to bind the PPAR-γ/RXR heterodimer[157]. Subsequent studies proved direct binding of the TZD BRL49653 (rosiglitazone) to the PPAR-γ protein[158] and demonstrated that the adipogenic program of TZDs were mediated though ligand-dependent PPAR-γ activation. The affinity of common PPAR-γ agonists measured by competition binding is 40 nM for rosiglitazone and 4.8 μM for pioglitazone[159]. GW2433 is a synthetic agonist for PPAR-δ. Synthetic agonists and antagonists for PPARs have served as the basis for countless studies of the effects of PPARs.

Natural ligands for PPARs consist of fatty acids and cyclooxygenase-derived eicosanoids and prostaglandins that bind to PPARs with relatively low affinity in many cases (i.e. binding constants in the micromolar range) (Tables 1-3). Identification of the potential endogenous ligands for PPARs has often rested on screening of candidate molecules rather than mass spectroscopy. This approach combined with the promiscuous nature of the LBD structure of PPARs has led to claims that numerous natural lipid species are PPAR agonists. The importance of many of these compounds have not been fully established *in vivo*, although several recent studies have characterized specific lipid species that appear to be

relevant and at the appropriate ambient tissue concentrations to act as ligands in specific cell types.

Many of the endogenous ligands are derived from arachidonic acid and linoleic acid as products of the lipoxygenase and cyclooxygenase pathways (Figure 6). Prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, is the key rate-limiting enzyme in prostaglandin biosynthesis. Two isoforms of the enzyme exist. COX1 (*PTGS1*) is constitutively expressed in most tissues while COX2 (*PTGS2*) is inducible and is considered a pro-inflammatory enzyme. Phospholipase A2-derived arachidonic acid is first converted to an unstable endoperoxide intermediate by cyclooxygenases and subsequently to one of several related prostanoids including PGD_2, PGE_2, PGF_{2a}, prostacyclin (PGI_2) or thromboxane A_2[117]. While synthesis of prostaglandins and release of arachidonic acid is regulated by activation of phospholipases, prostanoid production is dependent on the expression levels of the metabolizing synthases that vary in a cell-specific and stimulus-specific manner. Prostaglandins, for example, are generally present physiologically in body fluids at picomolar to low nanomolar concentrations[160], however, local prostaglandin concentrations in the micromolar range have been detected at sites of inflammation[161].

The endogenous ligands for PPAR-γ in various tissues have not been firmly established. Physiological agonists of PPAR-γ include the lipoxygenase products 13-HODE and 15-HETE, the prostaglandins 15d-PGJ_2 (15-deoxy-$\Delta^{12\text{-}14}$-prostaglandin J_2) and 15-keto-prostaglandin E_2, as well as nitroalkene fatty acids. 16:0/18:1-GPC (1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine) and possibly leukotriene B4 (LTB4) serve as endogenous ligands for PPAR-α. Eicosapentaenoic acid and possibly 15-HETE are ligands for PPAR-δ. Species of lysophosphatidic acid (1-acyl-2-hydroxy-sn-glycero-3-phosphate, LPA) have been proposed to act via PPAR-γ[162,163]. LPA has been shown to bind PPAR-γ, activate transient reporter assays, and stimulate lipid accumulation in human monocytes. LPA, however, generally mediates cell signaling through the activation of surface LPA receptors and pro-inflammatory functions through the activation of NF-κB, AP-1 and C/EBPβ[164], and its role as an endogenous PPAR-γ ligand has not been fully clarified. Dietary conjugated linoleic isomers have also been proposed to act through PPARs.

7. PPAR-γ endogenous ligands

7.1. PPAR-γ agonists

7.1.1 Cyclopentenone prostaglandin 15d-PGJ_2—The J-series cyclopentenone prostaglandins are metabolites of prostaglandin D_2. Prostaglandin D_2 synthase (PGD_2S) metabolizes cyclooxygenase (COX)-derived PGH_2 to the PGD_2 that is spontaneously converted to 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) by non-enzymatic dehydration (Figures 3 and 6). The J_2 series of prostaglandins differ from other classes of prostanoids by the presence of an electrophilic α,β-unsaturated carbonyl group in the cyclopentenone ring, a structure proposed to account for some of their receptor-independent biological actions. 15d-PGJ_2 was recognized in 1983 as a degradation product of PGD_2, formed in the presence of albumin[165]. Subsequently, 15d-PGJ_2 was identified as the first natural ligand agonist for PPAR-γ, inducing the transcription of PPAR target genes[166,167] (Figure 3). 15d-PGJ_2 induced the interaction between PPAR-γ and all coactivators tested (SRC-1, TIF2, AIB-1, p300, TRAP220/DRIP205)[151] consistent with its function as an activating ligand. 15d-PGJ_2 was found to inhibit synthetic ligand binding for PPAR-γ, with IC_{50} of approximately ~1 μM for [^{3}H]-BRL49653[167]. It was subsequently demonstrated to covalently bind to the sulfhydryl group of cysteine 285 in the PPAR-γ ligand binding pocket through a Michael addition reaction by the α,β-unsaturated carbonyl[168] that readily reacts with substances containing nucleophilic groups such as cysteinyl thiols. Mutation of the cysteine residue in the PPAR-γ LBD resulted in the loss of activation by 15d-PGJ_2, while the mutant was still

activated by synthetic ligands. Based on the model of the α,β-unsaturated carbonyl of 15d-PGJ$_2$ covalently bound to the cysteine residue of the PPAR-γ LBD through a Michael addition reaction, a number of potential activating endogenous ligands including several oxidized eicosanoid and keto-prostaglandins have been proposed[168].

A major question in subsequent studies has been whether 15d-PGJ$_2$ is present in sufficient quantities at physiological levels to act as an activating ligand *in vivo*. The fact that there is no enzymatic formation of cyclopentenone prostaglandins initially raised this concern. PGD$_2$ undergoes chemical dehydration to form the cyclopentenone prostaglandin PGJ$_2$, which is further dehydrated by loss of the 15-hydroxyl group and migration of the 13,14-double bond of PGJ$_2$ to form 15d-PGJ$_2$. This reaction proceeds at a slow rate compared to the formation of other prostaglandins.

In addition to a role in PPAR-γ activation, 15d-PGJ$_2$ also functions to block inflammatory stimuli mediated through various transcription factors, including NF-κB, AP-1, and signal transducer and activator of transription-1 (STAT-1). Treatment of LPS-stimulated macrophages demonstrated that the compound was more potent than synthetic PPAR ligands at inhibiting the induction of inflammatory genes, including inducible nitric oxide synthase (*Nos2*), gelatinase B (*Mmp9*), and scavenger receptor (*Msr1*)[18,19]. This result led to the discovery that 15d-PGJ$_2$ has anti-inflammatory effects that were independent of PPAR-γ[169], a result that was confirmed in PPAR-γ null macrophages treated with 15d-PGJ$_2$ in the micromolar (6-10 μM) concentration range[170,171]. These anti-inflammatory effects were mediated at least in part through the prevention of phosphorylation and degradation of the NF-κB inhibitor IκBα by IKK inhibition[172,173]. 15d-PGJ$_2$ covalently binds to and inhibits IκB kinase, a key activator of NF-κB[174]. This covalent binding is mediated through a Michael addition reaction to the reactive cylopentenone ring.

The levels of cyclopentenone prostaglandins, PGD$_2$ and 15-deoxy-$\Delta^{12,14}$-PGJ$_2$, have been shown to increase *in vivo* in response to inflammatory stimulus, corresponding to the inflammatory resolution phase. In the rat carrageenin-induced pleurisy model, COX2 levels and PGE$_2$ levels increase 2 hours after experiment onset, associated with a polymorphonuclear leukocyte (PMN) infiltrate. The PMNs are replaced by migrating mononuclear cells, which differentiate into macrophages. Later in the process, PGE$_2$ levels fall and the cyclopentenone prostaglandin levels including 15d-PGJ$_2$ peak, associated with the activation mechanisms that result in inflammatory resolution[175]. In other studies of a murine polymicrobial sepsis model, 15d-PGJ$_2$ was shown to be an effective anti-inflammatory agent, where injecting 15d-PGJ$_2$ following LPS treatment reduced lung injury and neutrophil trafficking to lung and small intestines, blocked the expression of adhesion molecules, and improved survival[176]. It was also found to be an endogenously produced anti-inflammatory mediator in the murine zymosan peritonitis model. The authors demonstrated production of both PGD$_2$ and subsequently 15d-PGJ$_2$ *in vivo*. 15d-PGJ$_2$ contributed to the resolution phase of inflammation by dampening pro-inflammatory cytokines and augmenting the production of interleukin-10[177]. PGD$_2$ synthase-null mice did not produce 15d-PGJ$_2$ during zymosan peritonitis and developed prolonged macrophage and lymphocyte accumulation associated with increased TNF-α and reduced IL-10 levels. These experiments mechanistically link endogenous PGD$_2$ production with 15d-PGJ$_2$ and the resolution of acute inflammation.

Thus 15d-PGJ$_2$, in addition to its role as an activating ligand for PPAR-γ, plays anti-inflammatory roles that contribute to the resolution and dampening of inflammation. While it clearly acts as a PPAR-γ ligand at micromolar concentrations, it is unclear to what extent 15d-PGJ$_2$ plays anti-inflammatory or PPAR-regulating roles *in vivo*. Studies of adipocyte differentiating 3T3L1 cells by liquid chromatography/tandem mass spectrometry (LC-MS/

MS) demonstrated that the cell-associated 15d-PGJ$_2$ concentrations were three orders of magnitude lower (~1 nM) than concentrations required for PPARγ-dependent effects[178]. LC-MS/MS studies from zymosan-stimulated peritoneal macrophages demonstrated concentrations an order of magnitude higher (~ 5-15 nM), however this was still far below the levels previously used for PPAR-activation and NF-κB inhibition studies[177]. Furthermore, 15d-PGJ$_2$ was not increased in the joint fluid of patients with arthritis nor in the urine of patients with diabetes or obesity[178]. These data provide evidence that while 15d-PGJ$_2$ can be produced *in vivo* under specific experimental conditions where macrophages are involved, its levels may be too low to be compatible with roles as an endogenous activator of PPAR-γ. Regional differences in lipid concentrations occurring as a result of active transport or nuclear accumulation of 15d-PGJ$_2$, however, might account for higher local concentrations than have been measured in these experiments. Cyclopentenone prostaglandins have been shown to be actively transported into cells and transferred to the nuclei through a process involving binding to the liver fatty acid binding proteins (L-FABP)[179]. Similarly, PGE$_2$ has been shown to undergo active uptake, leading to cytoplasmic compartment concentrations 25-fold higher than that of the extracellular medium[180]. It is possible that under specific physiologic conditions, the nuclear concentrations may exceed that needed for mediating the anti-inflammatory or PPAR-activation effects. Since the interaction with the PPAR-γ LBD is covalent, regional and temporal concentration changes coupled to the receptor may be more capable of modulating PPAR receptor function than if the ligand binding was reversible. Future studies aimed at determining the physiological relevance of 15d-PGJ$_2$ particularly in monocytes and macrophages where the production is inducible would be helpful to clarify the *in vivo* affects of the lipid while utilizing more specific measures of receptor-dependent gene activation such as chromatin immunoprecipitation to measure coactivator promoter recruitment.

7.1.2 15-Lipoxygenase metabolites—Lipid peroxides are formed by the action of lipoxygenases, a group of enzymes implicated in the pathogenesis of a number of inflammatory disorders including asthma[181], diabetic vascular disease[182], and atherosclerosis. Lipoxygenases catalyze the region- and stereospecific oxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives[183]. These fatty acid hydroperoxides are further transformed to bioactive compounds such as the leukotrienes and lipoxins, which play pro-inflammatory or anti-inflammatory roles[184]. Three major classes of mammalian lipoxygenases (5-, 12-, and 15-LOX) exist which catalyze the oxygenation of their substrate. Some lipoxygenase enzymes may exhibit multiple positional specificities[185]. The role of oxidized lipids in the development of atherosclerosis has been studied since the presence of lipid peroxides was discovered in human atherosclerotic aorta in 1952[186]. The lipid peroxides 13-hydroxy-9Z,11E-octadecadienoic acid (13-HODE) and 9-hydroxy-10E,12Z-octadecadienoic acid (9-HODE) were found within atherosclerotic plaques[187,188], and a role of peroxidized linoleic acid was postulated.

The human 15-lipoxygenase is expressed in the macrophages of atherosclerotic lesions and has been implicated in the pathogenesis of atherosclerosis based on the associated lipid peroxides[189-191]. Oxidative modification of cholesterol esters in low density lipoprotein (LDL) particles leads to the formation of oxLDL which is rapidly taken up by macrophages or vascular smooth muscle cells via scavenger receptors like SR-A or CD36, leading to foam cell formation. Analysis of the oxygenated lipids from aortas of rabbits fed an atherogenic diet and human atherosclerotic lesions confirmed the presence of 13-HODE and 9-HODE, the same lipid peroxides that are generated by lipoxygenase-treatment of LDL[192,193]. The S-isomer of 13-HODE was the predominant oxygenated fatty acid isolated. In 1998, 13-HODE and 9-HODE of oxidized low-density lipoproteins (oxLDL) were identified as endogenous PPAR ligands, mediating PPAR-γ-dependent transcription in macrophages[194] (Figure 3).

PPAR-γ was demonstrated to be expressed in foam cells of human atherosclerotic lesions, with the expression correlating with the oxidized lipid component[195], and the receptor was found to promote the uptake of oxLDL by inducing scavenger receptor CD36[196]. IL-4 stimulated the production of endogenous PPAR ligands by up-regulating the murine 15-LOX homolog, 12/15-lipoxygenase[197].

After the early studies suggested a pro-atherogenic role of 15-lipoxygenase, a possible anti-atherogenic role was demonstrated in a 15-lipoxygenase transgenic rabbit model. Transgenic expression of the human 15-LOX in rabbits fed a high fat, high cholesterol diet or regular diet on the Watanabe heritable hyperlipidemic (WHHL) genetic background, were less prone to atherosclerotic disease[198]. Following this work, however, several subsequent studies demonstrated a pro-atherogenic function of the 15-LOX enzyme. Pharmacological inhibition of 15-LOX in hypercholesterolemic rabbits attenuated atherosclerosis[199] while genetic disruption of the 12/15-LOX in the background of two high risk mouse models of atherosclerosis (apoE and LDLR null) or in the hematopoietic cells of apoE null mice by transplantation of 12/15-LOX null bone marrow retarded the initiation and progression of atherosclerosis[200-202]. The latter model is interesting because it implicates macrophages rather than the vascular endothelial cells as the source of genetic protection of 12/15-LOX deletion in the mouse model.

15-Lipoxygenase dioxygenates arachidonic acid mainly to 15S-hydroperoxy-5Z,8Z,11Z, 13E-eicosatetraenoic acid (15-HpETE). A minor reaction product 12S-hydroperoxy-5Z,8Z, 10E,14Z-eicosatetraenoic acid (12-HpETE) is always detectable. The major product of metabolism of linoleic acid is 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13-HpODE). A high degree of stereo-selectivity for the S enantiomers differentiates the enzymatic 15-lipoxygenase reaction from the non-enzymatic lipid peroxidation reaction.[203] In mammalian cells, the hydroperoxy products 15-HpETE, 12-HpETE, and 13-HpODE, are rapidly reduced to their corresponding hydroxy-lipids (15-HETE, 12-HETE, and 13-HODE) by selenium-containing glutathione peroxidases (GPXs). In addition to 13-HODE and 9-HODE, 15-HETE is an endogenous ligand for PPAR-γ, mediating PPAR-dependent transcription[194]. Two human 15-LO isotypes have been demonstrated which display slightly different specificities. Human 15-lipoxygenase-1 (15-LOX-1) is expressed in reticulocytes, lung and colonic tissue and primarily converts linoleic acid to 13-HODE. The 15-lipoxygenase-2 (15-LOX-2) is expressed in epidermis, prostate, lung, and colonic tissue and primarily generates 15(S)-HETE from arichidonic acid[204,205]. 15(S)-HETE is also generated by 15-LOX-1. The oxo metabolite 13-oxoODE has also been shown to act as a ligand for PPAR-γ, showing greater affinity for the receptor than 13-HODE[194].

In addition to the pro-atherogenic role of 12/15-LO in mouse models, evidence suggests that 15-LOX metabolites play mostly athero-protective and anti-inflammatory roles. 15-HETE was demonstrated to be increased in the atherosclerotic aortas of cholesterol-fed and WHHL rabbits and was the predominant eicosanoid present[206,207]. 15-HETE inhibits superoxide production and degranulation of PMNs when stimulated with agonists such as the granulocyte activator N-formylmethionylleucylphenylalanine (fMLP), leukotriene B4 (LTB4), or platelet activating factor[208]. 15-HETE production also inhibits PMN migration across cytokine-activated endothelium in cell culture[209] by reducing the presence of cell-surface LTB4 receptors[210]. Additionally, 15-LOX activity is linked to the anti-inflammatory lipoxins. 15-HpETE can be further metabolized by 5-lipoxygenase leading to the anti-inflammatory products 5S,6R,15S-trihydroxy-eicosatetraenoic acid (lipoxin A4) and 5S, 14R,15S-trihydroxy-eicosatetraenoic acid (lipoxin B4). Lipoxins counteract the actions of pro-inflammatory factors like leukotrienes and inhibit neutrophil chemotaxis and transmigration of PMNs through epithelial cells[211]. Down-regulation of anti-inflammatory lipoxin A4 has recently been associated with several inflammatory diseases in human

patients including asthma[212], cystic fibrosis[213], and ulcerative colitis[205]. Reduced expression of 15-LOX-2, the enzyme primarily responsible for production of the lipoxin precursor 15-HpETE, was confirmed in the case of patients with asthma and colitis. 15-HpETE and metabolites, therefore, play significant anti-inflammatory roles.

The 15-lipoxygenase lipid peroxide metabolites 9-HODE and 13-HODE as well as the arachidonic acid metabolite 15-HETE have been demonstrated to have anti-inflammatory effects through PPAR-γ. 13-HODE and 15-HETE (34 μM) down-regulated inducible nitric oxide synthase (*Nos2*) promoter activity in macrophages[197]. Both 9-HODE and 13-HODE down-regulated C-C chemokine receptor 2 (CCR2)[214], a receptor for monocyte chemoattract protein-1 (MCP-1) in human monocyte Thp1 cells. MCP-1 is an important cytokine responsible for monocyte accumulation in areas of inflammation such as atherosclerotic plaques[215]. PPAR-γ agonists have been shown to antagonize gelatinase B (MMP-9), potentially contributing to atherosclerotic plaque stability[216]. Additionally, concomitant with the induction of the 15-lipoxygenase, monocytes treated with IL-4 become resistant to inflammatory stimulus by LPS. IL-4, which induces PPAR-γ and 15-LO expression, was found to exert an anti-inflammatory effect on monocytes, suppressing the secretion of proinflammatory cytokines including IL-1β, TNF-α, and IL-6 upon stimulation by LPS or IFN-γ[217].

15-LOX metabolites have been demonstrated to show cell cycle effects in several cell types. While macrophage treatment with IL-4 was shown to be anti-inflammatory, IL-4 treatment of A549 lung adenocarcinoma cells increased 15-LOX activity, 15(S)-HETE production, and apoptosis induction[218]. 15-LOX-2 mediated 15(S)-HETE was found to mediate PPAR-γ-dependent transcription in PC-3 prostate cancer cells, while reducing proliferation and clonigenic capacity of the cells[219]. Additionally, 15-LOX-2 expression and 15(S)-HETE formation have been shown to be reduced in prostate carcinoma and absent in colon cancer[220]. Finally, the 15-LOX metabolite, 13(S)-HODE, was found to bind to and suppress activation and expression of PPAR-δ, leading to apoptosis in DLD colorectal cancer cell lines[221].

Recent studies of the role of 12/15-lipoxygenase have raised questions about the anti-inflammatory role of 12/15-LOX metabolites. 12/15-LOX null mice, for example, were resistant to the streptozotocin inflammatory model of type 1 diabetes[222] as well as the inflammatory effects of diet-induced obesity[223]. Macrophages from 12/15-LOX null mice were defective in interleukin-12 production but not TNF-α or nitric oxide release in response to LPS[224], suggesting a gene-specific anti-inflammatory effect of the knockout. Select studies of the human 15-LOX-1 have also shown a pro-inflammatory phenotype. Over-expressing the human 15-LOX-1 in bronchial epithelial A549 cells, for example, increased inflammatory chemokine expression including the macrophage inflammatory protein 1-α (MIP-1α), RANTES, and IP-10 and increased the chemotaxis of monocyte-derived immature dendritic cells. Exogenous treatment of 15-HETE (50 μM) to control cells, however, did not increase MIP-1α expression, and PPAR-dependent transcriptional events were not assessed[225]. The mechanisms of the pro-inflammatory effects in these studies have not been resolved.

Study of the differences between the murine 12/15-LOX and human 15-LOX isotypes revealed that the murine 12/15-LO produces more 12-HETE than 15-HETE (3:1) while the human reticulocyte 15-LO produces less 12-HETE and more 15-HETE (1:12)[185]. The ratio of HETEs and the respective lipoxygenase activities may also vary depending upon the physiologic stimulus of the experimental condition and the specific 15-LOX isozyme expressed in a given cell type. 15-HETE was the predominant eicosanoid produced upon arachidonic acid incubation of aortas from rabbits receiving a high cholesterol diet while 12-

HETE was produced in rabbits feeding on normal chow[207], suggesting that the hypercholesterolemic state selectively induced 15-lipoxygenase activity in aortic tissue of rabbits. Furthermore, 12-HETE increases monocyte adhesion to endothelial cells[226], an important early step in the formation of the early atherogenic lesion. The loss of 12-HETE in the 12/15-LOX null mouse may over-ride a possible role of the anti-inflammatory 15-lipoxygenase peroxides in murine atherogenic models due to a predominance of 12-lipoxygenase activity. Discrepancies between experimental data surrounding the murine 12/15-LOX and human 15-LOX genes, therefore, may potentially be explained by the differences in the lipoxygenase activities of the respective enzymes. Careful quantitative study of the lipoxygenase enzymes and lipid species by mass spectrometry in future studies to clarify the roles of lipid peroxides would help resolve conflicting data surrounding the 12/15-lipoxygenases and solidify their role in mediating protection against biological inflammatory diseases.

7.1.3 Nitroalkene fatty acids—Nitro fatty acids are also candidate natural ligands for PPAR-γ. The nitroalkene derivatives of linoleic acid (nitrolinoleic acid, LNO$_2$) and oleic acid (nitro-oleic acid, OA-NO$_2$) are formed via nitric oxide-dependent oxidative inflammatory reactions. LNO$_2$ and OA-NO$_2$ are endogenous ligands for PPAR-γ, robustly activating PPAR-γ-dependent reporter assays, endogenous target gene expression, and cell functions including adipocyte differentiation and glucose uptake[227,228]. Despite these functions in cell models, nitroalkene fatty acids would be expected to be short-lived inside the cell due to their capacity to undergo non-enzymatic Michael addition reactions. Both the nitroalkene derivatives of oleic acid (OA-NO$_2$) and linoleic acid (LNO$_2$) have been demonstrated to react readily with glutathione (GSH) and protein cysteine residues via Michael addition reactions[229]. Covalent modification of the PPAR-γ LBD at cysteine 285 has been demonstrated at micromolar concentrations[230]. Because glutathione is present at high concentrations in vivo, it is unclear whether nitro fatty acids are sufficiently stable to undergo this reaction at physiologic levels in order to activate PPAR-dependent transcription.

Nitro fatty acids have been demonstrated to show anti-inflammatory effects in several cell models. LNO$_2$ and OA-NO$_2$ prevent TNF-α-stimulated inflammatory and atherogenic responses in human umbilical vein endothelial cells (HUVECs) by blocked cytokine release and NF-κB activation[231]. In murine macrophage RAW264.7 cells, LNO$_2$ and OA-NO$_2$ exhibited anti-inflammatory effects by inhibiting LPS-induced STAT1 activation and suppressing *Nos2* and *Mcp-1* expression. This effect was not blocked by the addition of glutathione or GW9662, a PPAR-γ synthetic antagonist, suggesting a mechanism independent of thio-nitroalkylation or PPAR-γ[232].

Whereas the first studies of LNO$_2$ and OA-NO$_2$ in the plasma of healthy subjects demonstrated concentrations of ~500 nM for free/nonesterified fatty acids as measured by LC-MS/MS[228], other investigators demonstrated substantially lower concentrates by gas chromatography tandem mass spectrometry (GC-MS/MS). The two OA-NO$_2$ isomers, 9-NO$_2$-OA and 10-NO$_2$-OA, were found at mean plasma concentrations of 880 and 940 pM, respectively[233]. A second study by the same group eliminating the high-performance liquid chromatography (HPLC) step to resolve the discrepancy between data sets by using a modified protocol demonstrated plasma OA-NO$_2$ concentrations that were even lower but still in the pM range[234]. Furthermore, LNO$_2$ occurred at lower concentrations than OA-NO$_2$ and was found to be in the lower end of the pM range. While OA-NO$_2$ is the most abundant nitrated unsaturated fatty acid in human plasma, the concentrations of both isomers in the pM range are far below those used in studies demonstrating anti-inflammatory and PPAR-γ-dependent effects. These findings raise questions about the endogenous roles of nitroalkene fatty acids. While nitroalkene fatty acids appear to act as potent agonists of PPAR-γ and

have anti-inflammatory effects at high concentrations, their physiologic relevance remains to be established. Future studies in PPAR-γ and NO synthase deficient macrophages or other cell types would be helpful to establish the *in vivo* relevance and receptor-dependent effects of LNO_2 and $OA-NO_2$.

7.1.4 15-keto-prostaglandin E₂—PGE_2 is a short-lived mediator that is produced through the action of constitutive and inducible cyclooxygenase enzymes. The primary catabolic pathway of prostaglandins such as PGE_2 is initiated by the oxidation of the 15(S)-hydroxyl group catalyzed by NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), generating 15-keto-prostaglandin E_2 (15-keto-PGE_2). Subsequent enzymatic metabolism of 15-keto-PGE_2 takes place through the reduction of the Δ^{13} double bond catalyzed by the NADPH/NADH-dependent Δ^{13}-15-ketoprostaglandin reductase (13-PGR)[235]. This later enzyme also exhibits $NADP^+$-dependent leukotriene B_4 12-hydroxydehydrogenase (12-LTB_4DH) activity.

15-PGDH expression has been demonstrated in the prostate, uterus, liver, lung, adipocyte and gastrointestinal epithelium. 15-PGDH acts as a tumor suppressor and its expression is down-regulated in lung[236], gastric[237], colon[238], and bladder cancers[239]. Over-expressing 15-PGDH induced apoptosis of A549 lung cancer cells[236] and slowed the growth of SGC7901 gastric cancer cells[240]. Furthermore, mice with a null deletion of 15-PGDH were more susceptible to models of colon carcinogenesis[241]. It has not been determined which physiological effects of 15-PGDH deletion result from up-regulation of prostaglandins or concomitant down-regulation of oxidized prostaglandins.

While 15-keto-prostaglandins were generally thought to contain significantly reduced biological activity compared to their non-oxidized prostaglandins[242], the dehydrogenase reaction produces an α,β-unsaturated ketone similar to that seen in $15d-PGJ_2$ that can undergo Michael addition with thiol groups. The oxidized prostaglandins 15-keto-PGE_1, 15-keto-PGE_2, 15-keto-PGF_1, and 15-keto-PGF_2 were all demonstrated to act as ligands for PPAR-γ in transient transfection assays[168]. The oxidized prostaglandins were more capable of activating the PPAR reporter when compared to their prostaglandin counterparts. 15-keto-PGE_2 but not PGE_2, 13,14-dihydro-15-keto-PGE_2, nor 15-keto-PGF_{2a} was capable of displacing rosiglitazone from binding the PPAR-γ LBD, suggesting that the former is the more effective endogenous ligand[243] (Figure 3). 15-keto-PGE_2 in the micromolar range (10 μM) was also demonstrated to induce adipogenesis of 3T3L1 cells in a similar manner as the synthetic PPAR-γ ligand rosiglitazone.

Recent work from our group confirms a role of 15-keto-PGE_2 as a bona-fide endogenous ligand for PPAR-γ in the colon[112]. We found a defect in PPAR-γ mediated gene expression in the colonic epithelial cells and lungs of mice with a deletion in the cystic fibrosis transmembrane regulator (*Cftr*). While PPAR-γ protein levels were normal, recruitment of the co-activator TRAP220 to PPRE sites was reduced in *Cftr*-null colonic epithelial cells. We speculated that a defect in endogenous ligand levels might account for the reduced coactivator recruitment. We quantified 94 lipid analytes by LC-MS/MS and found that 15-HETE and 15-keto-PGE_2 were the two most abundant species in the wild-type colonic epithelial cells that were previously implicated to act as PPAR-γ ligands in the low micromolar range. While 15-HETE abundance was unchanged between the wild type and *Cftr*-null cells, 15-keto-PGE_2 abundance was reduced by 65% in *Cftr*-null cells. In concert with these results, we demonstrated 70% reduced expression of 15-PGDH in the *Cftr*-null cells.

We found levels of 15-keto-PGE_2 in colonic tissue of 90 pg/mg tissue (~250 nM). While this is slightly below the micromolar range tested to be effective in transient assays, we

demonstrated that the reduction of 15-keto-PGE$_2$ correlated with reduced PPAR-γ target gene expression and co-activator TRAP220 recruitment at PPRE sites in *Cftr*-null cells. The results are consistent with a role of 15-keto-PGE$_2$ as a physiologically relevant PPAR-γ ligand in the colon. Because 15-keto-PGE$_2$ presumably interacts with PPAR-γ through the LBD cysteine via a Michael addition reaction (based on the presence of the α,β-unsaturated ketone), covalent LBD modification may account for the effectiveness of 15-keto-PGE$_2$ in the sub-micromolar range. Alternatively, 15-keto-PGE$_2$ may be concentrated in the nuclear compartment by binding to fatty acid binding proteins (e.g. L-FABP) where it activates PPAR-γ as has been demonstrated for cyclopentenone prostaglandins. Future research should establish the mechanism of PPAR-activation by 15-keto-PGE$_2$ in the colon and other tissues where 15-PGDH is expressed.

7.1.5 Serotonin metabolites—Serotonin metabolites also act as endogenous agonists for PPAR-γ. The serotonin metabolite 5-methoxy-indole acetate (100 μM) was found to activate PPAR-γ mediated expression constructs in THP-1 macrophages and stimulate 3T3-L1 adipogenesis. [244] This study demonstrated that the 5-methoxy-indole acetate is bound to the AF-2 subpocket within the PPAR-γ LBD, while the other subpocket is simultaneously bound by a fatty acid metabolite. This suggests that PPAR-γ may function as a sensor for both the serotonin and fatty acid metabolic pathways. Serotonin levels are present in micromolar concentrations within the gastrointestinal tract and central nervous system, suggesting that the ligand may be present in these tissues. Future studies are needed to confirm the presence and functional activity of endogenous 5-methoxy-indole acetate in native tissues.

7.2 PPAR-γ antagonists

7.2.1 Prostaglandin F2α—PGF2α is synthesized by preadipocytes. It does not activative PPARs[4,245] but potently inhibits adipocyte differentiation[246,247]. Endogenous production of PGF2α is lower in differentiating compared to uninduced preadipocytes. PGF2α utilizes the G-protein-coupled prostaglandin F receptors at the cell surface, initiating signal transduction through inositol triphosphate[248,249]. PGF2α was found to stimulate MAP kinase activation leading to phosphorylation of the PPAR-γ1 and PPAR-γ2 at serine 112 while indirectly antagonizing PPAR-γ induction and limiting adipocyte differentiation[250]. The data implicates PGF2α as a cellular control mechanism regulating the phosphorylation state of PPAR-γ. While PPAR-γ-S112A knock-in mice did not show increased weight or adipose mass compared to wild type mice, they maintained insulin sensitivity in the setting of diet-induced obesity[251]. The result suggests that compounds inhibiting PPAR-γ phosphorylation or regulating PGF2α may enhance insulin sensitivity without increasing body weight or adiposity, although additional investigations are needed to characterize the role of PGF2α *in vivo*.

7.2.2 Cyclic Phosphatidic Acid—Recent work established the first evidence of a direct endogenously produced PPAR-γ antagonist, cyclic phosphatidic acid (1-acyl-2,3-cyclic-glycerophosphate)[252] (Figure 3). Cyclic phosphatidic acid (CPA) is generated in mammalian cells in a stimulus-coupled manner by phospholipase D2 (PLD$_2$). CPA binds to a site within the PPAR-γ LBD with Kd in the hundred nanomolar range and prevents activation by PPAR-γ agonists such as TZDs. Upon binding CPA, the interaction with the corepressor SMRT is stabilized, reducing PPAR-γ activity, while the homologous PPAR-α and PPAR-δ receptors were neither inhibited nor activated by CPA. Although it is nearly undetectable in resting cells, CPA production was stimulated in human monocytes by insulin, phorbol myristate acetate (PMA), LPS or H$_2$O$_2$ through phospholipase D$_2$. CPA was also shown to block TZD-stimulated adipogenesis and lipid accumulation in RAW264.7 and primary macrophages. CPA has been detected at concentrations of ~10 nM in human serum[253]. It is

unknown how a given cell coordinates PPAR-γ activity through simultaneous production of endogenous ligands such as 15-keto-PGE$_2$ in addition to ligand-binding antagonists such as CPA.

8. PPAR-α endogenous agonists

8.1 Conjugated linoleic acid

Conjugated linoleic acid (CLA) refers to a group of geometric and positional dienoic isomers of linoleic acid [18:2(n-6)] that is a natural fatty acid found in meats, cheeses and dairy products. The two predominant isomers of CLA found commonly in food and commercial preparations are 9Z,11E and 10E,12Z CLA. CLA has been shown to have potential beneficial effects in models of carcinogenesis, diabetes, colitis and atherosclerosis[254]. The 9Z,11E isomer constitutes up to 90% of total CLA and is thought to be responsible for the positive health benefits associated with CLA. These benefits include inhibition of allergic airway inflammation[255], suppression of NF-κB activation in dendritic cells[256], macrophages[257], and colonic epithelial cells[258], and suppression of inflammation in models of colitis[259,260]. These effects are at least in part mediated through enhanced production of IL-10[256].

CLA isomers have also been proposed to act as ligands for PPARs. In hepatoma cells, for example, conjugated linoleic acid isomers were found to be more effective at inducing PPAR target genes and PPRE-reporter constructs than linoleate. Using a scintillation proximity assay to assess PPAR-α binding, a rank order of potency of (9Z,11E)>(10E, 12Z)>(9E,11E)>furan-CLA was demonstrated, with IC$_{50}$ ranging from 140 nM to 400 nM. While CLA isomers appear to be among the most avid fatty acid ligands for PPAR-α (IC$_{50}$ = 140nM for the 9Z,11E isomer), the EC$_{50}$ for activation of the PPAR-α target gene Cyp4a1 was >2 orders of magnitude higher (~ 50 μM)[261], indicating that CLA may not be biologically available to act as a ligand due to metabolism effects such as excessive incorporation in phospholipid membranes or that it may be inefficient in recruiting co-activators required for gene activation despite binding to the receptor.

Conflicting results with respect to the CLA isomers and PPAR-activation have been demonstrated. While initial work suggested that CLA might activate PPAR-γ, as demonstrated by the effect of CLA on a PPAR-dependent reporter in rat CV-1 cells over-expressing PPAR-γ[262], subsequent work demonstrated CLA isomer specific effects and antagonism of PPAR-γ. The 10E,12Z CLA isomer antagonized ligand-dependent activation of PPAR-γ-dependent target genes *aP2* and *Perilipin* and reduced PPAR-γ expression in differentiating 3T3L1 adipocytes while the 9Z,11E isomer had no effect[263] (possibly resulting from the low expression of PPAR-α in adipocytes). The authors went on to demonstrate that 10E,12Z CLA promoted NF-κB activation and IL-6 induction in adipocytes which was in part responsible for the down-regulation of PPAR-γ activity[264]. In contrast to this, CLA demonstrated anti-inflammatory effects in models of colitis. A mixture of CLA isomers reduced disease activity index scores, NF-κB activation and colonic inflammatory cytokine TNF-α production[260]. While the authors suggested that the effects were dependent upon PPARs, mechanistic data linking CLA isomers to PPAR-dependent transcription events was not clarified in epithelial or immune cells. Furthermore, recent epidemiological data has suggested that diets rich in linoleic acid were not protective and actually increased the risk of colitis in human patients[265]. However, ingested linoleic acid may largely be metabolized to arachidonic acid, forming substrates for cyclooxygenase (COX) and lipoxygenase (LOX) enzyme systems, rather than undergoing conversion to conjugated linoleic acid. Future mechanistic studies of the effects of specific CLA isomers on PPAR-dependent transcription and receptor binding in epithelial and immune cells would

be helpful to unravel the roles of PPARs in mediating the effects of CLA on inflammation and gene activation.

8.2 16:0/18:1-GPC

PPAR-α is highly expressed in liver, where it regulates lipid transport, gluconeogenesis and fatty acid oxidation. PPAR-α has been demonstrated to respond to a variety of unsaturated and saturated fatty acids in reporter assays[4]. Because of its function in metabolism and data demonstrating binding to several fatty acids, PPAR-α was hypothesized to act as a sensor of nutritional status. Recently, a novel high affinity endogenous ligand for PPAR-α was identified in liver that links PPAR-α to the dietary carbohydrate responsive fatty acid synthase (FAS) responsible for palmitate (16:0) biosynthesis. A significant step towards this discovery was uncovered when it was demonstrated that liver-specific inactivation of fatty acid synthase (FAS) resulted in mice with decreased PPAR-α-dependent gene expression and a metabolic phenotype (hypoglycemia and steatohepatitis) resembling PPAR-α deficiency[266]. Subsequently, the authors identified the phospholipid 16:0/18:1-GPC (1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine) as the relevant PPAR-α-bound ligand generated by FAS using electrospray ionization mass spectrometry (Figure 4). They confirmed binding and co-activator recruitment to PPAR-α in vitro as well as acyl CoA oxidase (*Acox1*) and carnitine palmitoyl transferase (*Cpt1a*) induction in wild type but not PPAR-α-null mouse liver upon portal vein infusion of 16:0/18:1-GPC[267]. Binding affinity determined by scintillation proximity assay using ^{3}H-Wy14,643 demonstrated $IC_{50} = 33.25$ μM. 16:0/18:1-GPC accounted for $4.4\pm1.2\%$ of all nuclear phospholipids in liver.

In addition to CLA isomers and 16:0/18:1-GPC, the eicosanoid derivatives 8(S)-hydroxyeicosatetraenoic acid (HETE) and leukotriene B4 may act as PPAR-α ligands. 8(S)-HETE is the murine 8-lipoxygenase (8-LOX) product of arachidonic acid. The human 15-LOX-2 is regarded as the human ortholog of the mouse 8-LOX, sharing 78% identity in amino acid sequence. There is no overlap in the positional specificities of the two enzymes. The human 15-LOX-2 enzyme largely produces 15(S)-HETE rather than 8(S)-HETE[268].

8.3 LTB4

Leukotriene B_4 (LTB_4) acts as a chemoattractant and secretagogue for PMNs, eosinophils and T lymphocytes[269]. Several reports have suggested that LTB_4 might act as an endogenous PPAR-α agonist with affinities of $Kd = 60\text{-}90$ nM[270,271] under specific conditions. One study suggested that LTB_4 binds Xenopus PPAR-α with an affinity of 100 nM, although the IC_{50} for displacement was 10-50 mM of unlabeled LTB_4[270]. A second group demonstrated that calcium-ionophore-treatment of Jurkat T cells activated a PPAR reporter construct in a process dependent upon 5-lipoxygenase, the enzyme responsible for LTB4 biosynthesis. Activation of the PPRE reporter by 10 μM LTB4 occurred in CV-1 cells as well, although PPAR target gene activation was not assessed[272]. In contrast to these findings, others have failed to show activation or binding to PPAR-α[4]. The differences in these studies have not been resolved such that LTB_4 may play a role in PPAR activation under specific physiological conditions.

9. PPAR-δ endogenous agonists

Several synthetic agonists with high subtype selectivity have been described for PPAR-δ (Figure 5). The regulation of PPAR-δ by endogenous ligands, however, remains uncertain, largely due to the propensity of the receptor to bind a large number of lipid species with low affinity. Prostacyclin (PGI$_2$) [273,274] and all-*trans* retinoic acid[275] have been proposed to act as PPAR-δ ligands, however, conflicting results were found by other investigators[4,276-278]. Several fatty acids have been reported to induce PPAR-δ activity, although receptor

interaction and specificity has not been established. The crystal structure of PPAR-δ has been solved recently in interaction with the hydrophobic lipids 5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid (EPA) and the synthetic agonist GW2433. The structure demonstrated that the ligand-binding pocket of PPAR-δ is sufficiently large to allow the hydrophobic tail of EPA to bind in multiple configurations. The carboxylic acid of EPA interacts with the AF-2 helix in a similar fashion of rosiglitzone interacting to PPAR-γ[43]. A second study demonstrated PPAR-δ-dependent effects of 15(S)-HETE treatment in fibroblasts. 15(S)-HETE induced the PPAR target gene and coactivator recruitment to the *Angptl4* PPRE in fibroblasts in a PPAR-δ-dependent manner[279]. It is unclear if this effect is specific to fibroblasts. Whether anti-inflammatory effects of these putative PPAR-δ ligands are PPAR-dependent also remains to be established.

10. Conclusion

A number of recent studies have highlighted the increasing complexity of PPAR-mediated signaling. PPARs mediate transcriptional activation and repression of gene expression. Multiple molecular mechanisms contribute to the modulation of PPAR activity in a given cell type that regulate PPAR function. Lipid species have emerged as key regulators of PPAR activity. Comparisons between receptor-ligand affinities and the physiological concentrations of candidate ligands as measured by liquid chromatography/tandem mass spectrometry may serve as a guide for determining the relevance of putative ligands for a given PPAR receptor in a particular tissue. Additional studies of the potential therapeutic applications of natural PPAR-γ ligands, such as 15-keto-prostaglandin E_2 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, are needed because of the side effect profile of synthetic TZD agonists. Modulation of the activity of the synthetic pathways of these endogenous ligands may also prove to be of therapeutic interest as anti-inflammatory modulators.

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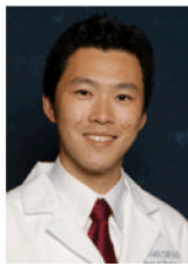
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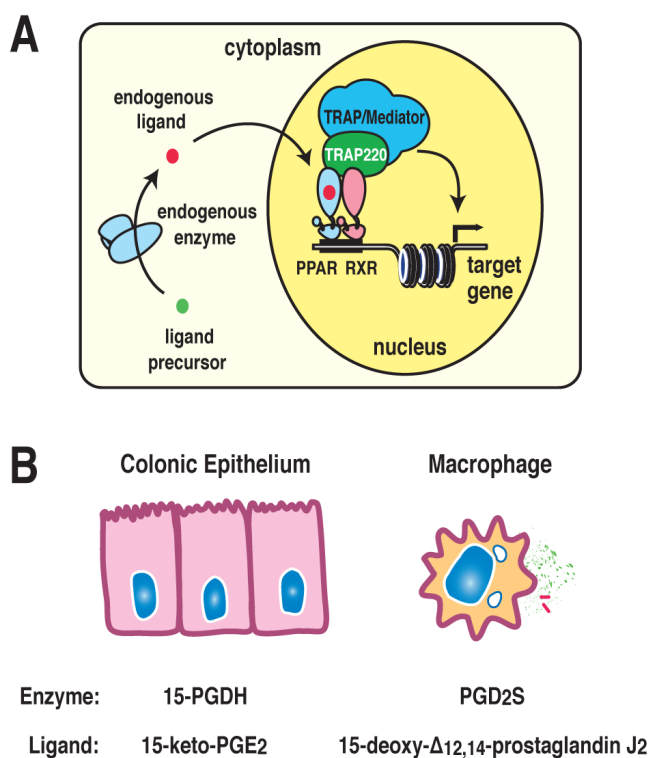
Figure 1.
PPAR transcriptional regulation and the production of endogenous ligands. A) Endogenous lipid ligand precursors undergo enzymatic conversion to active lipids, leading to their binding to PPAR/RXR heterodimers on target genes and recruitment of co-activator complexes that activate transcription. B) Cell-specific PPAR activation is regulated by the expression of metabolizing enzymes 15-hydroxyprostaglandin dehydrogenase (15-PGDH) expressed in colonic epithelial cells and prostaglandin D_2 synthase (PGD_2S) in macrophages, leading to the production of endogenous ligands 15-keto-prostaglandin E_2 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 respectively.
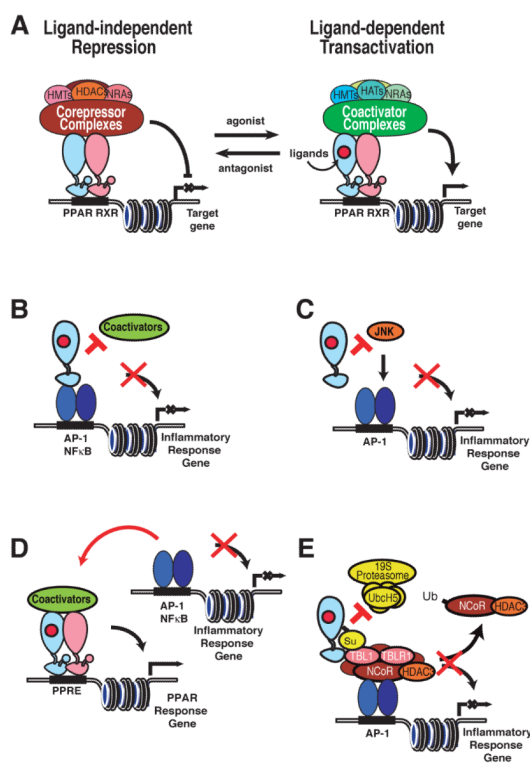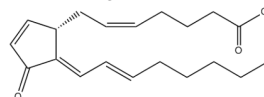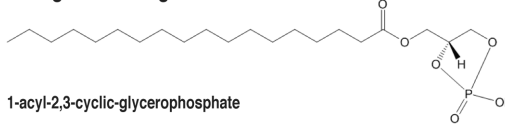
Figure 2.
Transcriptional activation and repression by PPARs. A) In the absence of agonist ligand, co-repressor complexes bind to PPAR/RXR heterodimers on response elements in target gene enhancers and promoters, inhibiting gene expression. Upon agonist binding, corepressor complexes dissociate and co-activator complexes are recruited, resulting in transcriptional activation of target genes. B) Transrepression of NFκB and AP-1 target genes by direct interactions of PPARs with NFκB and/or AP-1 proteins. Such interactions may prevent coactivator recruitement required for transcriptional activation. C) PPAR inhibition of AP-1-dependent gene expression by inhibition of c-Jun N-terminal kinase (JNK) activity required for activation of AP-1 target genes. D) PPAR inhibition of inflammatory response genes by competition for commonly used, rate-limiting coactivators. E) PPARγ repression of inflammatory response genes by inhibition of NCoR clearance. Ligand induced SUMOylation of PPAR-γ results in its docking to NCoR-containing co-repressor complexes bound to AP-1 elements via non-phosphorylated cJun. This interaction inhibits signal-dependent turnover of NCoR complexes required for transcriptional activation. Repression mechanisms may operate in a cell-specific and in some cases gene specific manner. The relative importance of specific mechanisms has not been established in vivo.

PPAR-γ
Synthetic agonist
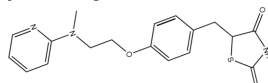
BRL-49653 (Rosiglitazone)

Endogenous agonists
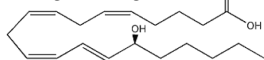
15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
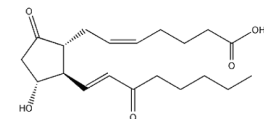
15-keto-Prostaglandin E2

15-deoxy-Δ12,14-Prostaglandin J2

Endogenous antagonist

1-acyl-2,3-cyclic-glycerophosphate

Figure 3.
Natural and synthetic PPAR-γ ligands. PPAR-γ ligands include the synthetic agonist BRL49653, the endogenous agonists 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (15d-PGJ$_2$), 15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE), and 15-keto-prostaglandin E$_2$, and the endogenous antagonist 1-acyl-2,3-cyclic-glycerophosphate.
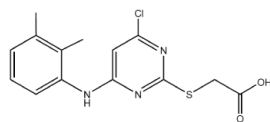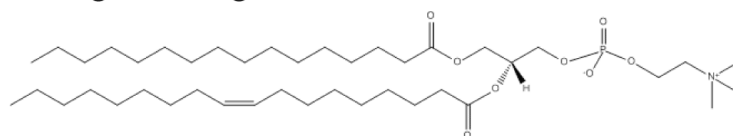
PPAR-α

Synthetic agonist

Wy14,643

Endogenous agonist

1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine

Figure 4.
Natural and synthetic PPAR-α ligands. PPAR-α ligands include the synthetic agonist Wy14,643 and the endogenous agonist 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine.
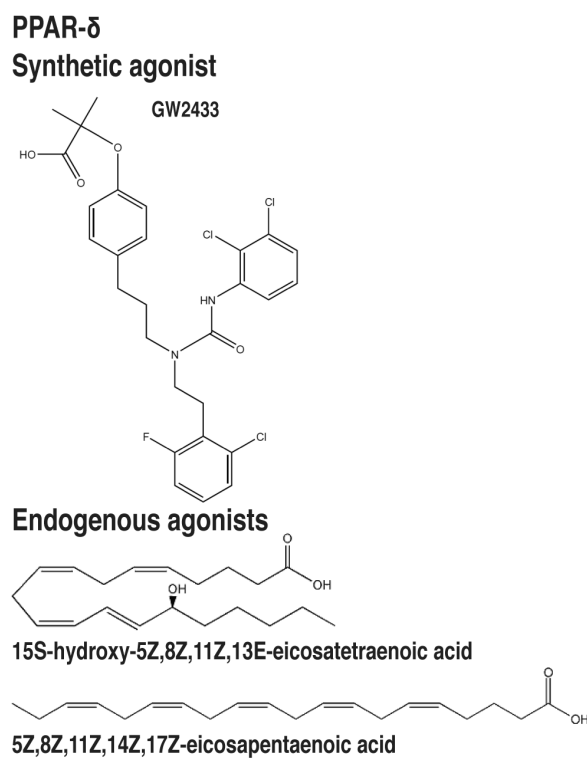
Figure 5.
Natural and synthetic PPAR-δ ligands. PPAR-δ ligands include the synthetic agonist
GW2433 and the endogenous agonists 15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
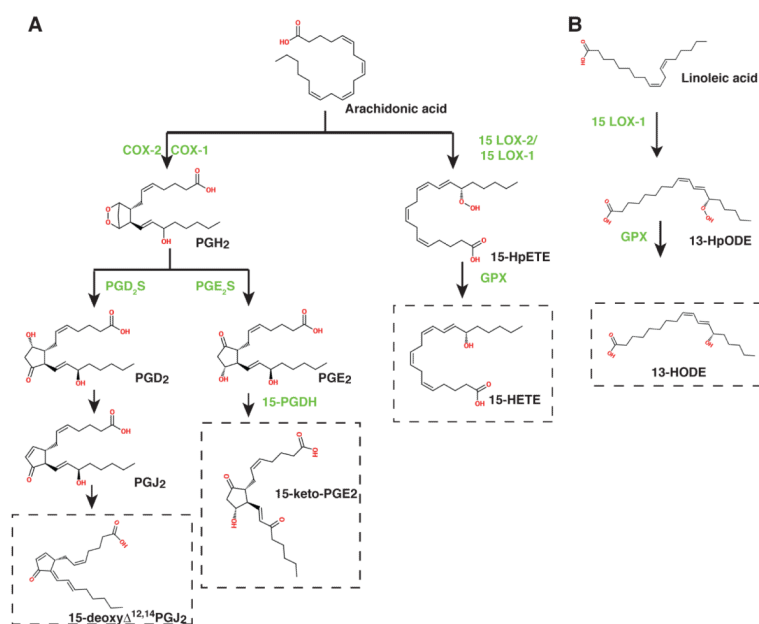(15-HETE) and 5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid (EPA).

Figure 6.
Biosynthetic pathways leading to generation of some of the naturally occurring PPAR ligands. A. Ligands generated from arachidonic acid. B. Ligands generated from linoleic acid.

Table 1

PPAR-γ ligands

Ligand	Activity	Biological Activity	Ref
15d-PGJ2	agonist	In murine sepsis model, administration of ligand reduced lung injury and PMN trafficking to lung and intestine, blocked expression of adhesion molecules, and improved survival	176
		Blocks inflammatory signaling through NF-κB, AP-1, and STAT-1, thus reducing expression of *Nos2*, *Mmp9*, and *Msr1*	18, 19
		Covalently interacts with PPARγ LBD at Cys285	168
		Inhibits NF-κB activation by preventing degradation of IkBα in a PPAR-γ independent fashion	174
		Inhibits MMP9 activity in human monocytes	216
13-HODE	agonist	Decreases *Nos2* expression in macrophages	197
		Decreases *CCR2* expression in THP1 human monocytes	214
		Found in atherosclerotic plaques	187
15-HETE	agonist	Decreases *Nos2* expression in macrophages	197
		Decreases superoxide production and degranulation of PMN stimulated by fMLP, LTB4, and PAF	208
		Inhibits PMN migration across cytokine-activated endothelium in cell culture by reducing LTB4 receptors	209, 210
9-HODE	agonist	Found in atherosclerotic plaques	188
		Decreases *CCR2* expression in THP1 human monocytes	214
Nitroalkene fatty acids		Induce adipocyte differentiation and glucose uptake	227, 228
		Block cytokine release and NF-κB activation in HUVECS stimulated by TNFα or atherogenic response	231
		Decrease *Nos2* and *Mcp1* expression in RAW264.7 macrophages. Effect was not blocked by PPARγ synthetic antagonist	232
		Covalently interact with PPARγ LBD in Cys285 at micromolar concentrations	230
15-keto-PGE2	agonist	Induces adipogenesis of 3T3L1 cells	243
		Abundance of 15-keto-PGE2 was reduced in *Cftr*-null colonic epithelial cells, which exhibit defective PPAR-γ mediated gene expression	112
5-methoxy-indole acetate	agonist	serotonin metabolite that is bound to the AF-2 subpocket of PPAR-γ LBD. PPAR macrophage and stimulate 3T3-L1 adipogenesis.	244
PGF2a	antagonist	Inhibits adipocyte differentiation	250
		Inhibits PPAR-γ activity by inducing phosphorylation of PPAR-γ at serine 112	250
		PPAR-γS112A knock-in mice maintained insulin sensitivity in model of diet-induced obesity	251

Ligand	Activity	Biological Activity	Ref
Cyclic phosphatidic acid	antagonist	Stabilizes PPAR-γ-SMRT interaction	
		Blocks TZD-stimulated adipogenesis and lipid accumulation in macrophages	252
		Production induced by insulin, PMA, LPS, or H_2O_2	

Table 2

PPAR- ligands

Ligand	Activity	Biological Activity	Ref
Congulated linoleic acid (CLA)	agonist	Inhibition of allergic airway inflammation	255
		Suppression of NF-κB activation in dendritic cells, macrophages, and colonic epithelial cells	256-258
		Suppression of inflammation in colitis models	258
16:0/18:1-glycerol-3-phosphocholine	agonist	Mice liver-specific knockout of fatty acid synthase (FAS), responsible for palmitate (16:0) biosysnthesis, have hypoglycemia and steatohepatitis, phenotypes similar to PPAR-α deficiency.	266
		Induces expression of *Acox1* and *Cpt1a*	267
Leukotriene B4	agonist	Induces expression of PPAR-α regulated gene acyl-Co-A oxidase in primary hepatocytes.	
		Implicated in activating its turnover in liver by promoting expression of catabolic genes through PPAR-α. In an ear-swelling model, knockout of PPAR-α prolonged inflammatory phased induced by arachidonic acid or LTB4.	270

Table 3

PPAR-δ ligands

Ligand	Activity	Biological Activity	Ref
Prostacyclin (PGI2)		Proposed ligand.	273, 274
All trans retinoic acid		Proposed ligand	275
5Z,8Z,11Z,14Z, 17Z-eicosapentaenoic acid		Interaction found in crystal structure.	43
15(S)-HETE	agonist	Induce Angptl4 in fibroblasts	279