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Abstract

Peroxisome proliferator-activated receptors (PPARs) regulate energy metabolism and are, as such, therapeutic targets in metabolic diseases such as type 2 diabetes and non-alcoholic fatty liver disease. While they share anti-inflammatory activities, the PPAR isotypes distinguish themselves by differential actions on lipid and glucose homeostasis. In this review, we discuss the complementary and distinct metabolic effects of the PPAR isotypes together with the underlying cellular and molecular mechanisms, as well as the synthetic PPAR ligands used in the clinic or under development. We will highlight the potential of new PPAR ligands with improved efficacy and safety profiles in the treatment of complex metabolic disorders.

Introduction

Metabolic syndrome (MetS) is a pathophysiological condition characterized by increased visceral adiposity, dyslipidemia, prediabetes and hypertension. This cluster of risk factors predisposes to type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) and increases the risk of microvascular complications and cardiovascular (CV) events. With the global increase in obesity, the prevalence of MetS has reached epidemic proportions. The pathophysiology of MetS and its co-morbidities is complex due to multiple alterations in lipid and glucose metabolism accompanied by inflammation occurring simultaneously in several tissues; therefore, current treatments address the individual components (1).

Over the last decades, the peroxisome proliferator-activated receptors (PPARs), which are members of the nuclear receptor superfamily of transcription factors (TFs), have been targeted to fight MetS and its complications. PPARs regulate many metabolic pathways upon activation by natural ligands, such as fatty acids (FA) and derivatives, or synthetic agonists, which bind to the ligand-binding domain of the receptor triggering a conformational change. Subsequent recruitment of coactivators to the PPAR/retinoid-X-receptor (RXR) heterodimer assembled at specific DNA response elements – PPAR response elements or PPREs – ultimately results in transactivation of target genes. In addition, PPAR activation attenuates the expression of pro-inflammatory genes, mostly through a transrepressive mechanism (2).

Three PPAR isotypes with different tissue distribution, ligand specificity and metabolic regulatory activities exist in mammals: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). Fibrates are synthetic PPARα ligands used to treat dyslipidemia. Thiazolidinediones (TZDs) or glitazones, synthetic PPARγ ligands, are anti-diabetic drugs with potent insulin-sensitizing effects. There are currently no synthetic PPARβ/δ ligands in use clinically. Potency and safety issues have undermined the use of certain PPAR ligands, underscoring the need for new, safer, and more selective ways to target PPARs for combating metabolic diseases (2).

Natural PPAR ligands

PPARs are activated by FA and their derivatives, and the level of physiological receptor activation depends on the balance between ligand production and inactivation. Natural PPAR ligands originate from three main sources: diet, *de novo* lipogenesis (DNL) and lipolysis, alternating processes which depend on the integration of nutritional status and circadian rhythms (3). PPARs control these metabolic processes to maintain metabolic flexibility, a prerequisite for the preservation of health.

Dietary lipids are important regulators of PPAR activity, as evidenced by the increased target gene expression of PPARα in liver (4) and PPARβ/δ in skeletal muscle (SKM) (5) upon high-fat diet (HFD) feeding in mice. Tissue-specific deficiency of fatty acid synthase (FAS) – a key enzyme in FA synthesis – impairs PPARα activity, identifying DNL as another source of PPAR ligands (6)(7). PPARα ligands originating from DNL are not only simple FA but include more complex molecules, such as phosphatidylcholines (8). A third source of natural PPAR activators is through lipolysis. Angiopoietin-like proteins are secreted glycoproteins which inhibit lipoprotein lipase (LPL), hence controlling the plasma lipid pool according to lipid availability and cellular fuel demand. Angptl4 expression is induced in several tissues including adipose tissue, liver and SKM by circulating FA via PPARs, leading to inhibition of LPL and decreasing plasma TG-derived FA uptake, thus forming a negative feedback loop (9). Intracellular lipolysis also provides PPAR ligands. Indeed, deficiency of adipose triglyceride lipase (ATGL), which lipolyses TG in lipid droplets, decreases PPAR target gene expression in various tissues (10)(11)(12)(13). Ligand availability is also modulated by FA degradation in peroxisomes, whose genes are regulated by PPARs as part of a feedback mechanism (14). PPAR activity hence relies on a careful balance between ligand production and degradation in order to meet the fluctuating energy demand.

Contrasting metabolic effects of ligand-activated PPARα and PPARγ

Although they share some similarities in function and mechanism of action, it has become increasingly clear that both PPAR isotypes display important physiological and pharmacological differences. This section discusses the clinical and genetic evidence of contrasting PPARα and PPARγ effects, and sheds light on the cellular and molecular mechanisms underlying these differences.

Clinical effects of PPARα and PPARγ activation

Except for the weak pan-agonist bezafibrate, all clinically used fibrates are specific activators of PPARα. Fibrate outcome trials such as the Helsinki Heart Study (HHS) (15), Veterans Affairs-High density lipoprotein cholesterol Intervention Trial (VA-HIT) (16), Bezafibrate Infarction Prevention (BIP) (17), Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) (18), and Action to Control Cardiovascular Risk in Diabetes (ACCORD) (19) consistently show beneficial effects on plasma lipids, particularly in normalizing the typical dyslipidemia of MetS characterized by the so-called 'atherogenic lipid triad' (high low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG), low high density lipoprotein-cholesterol (HDL-C)). Fibrate therapy significanty decreases TG and increases HDL-C, whereas LDL-C generally decreases except in patients with severe hypertriglyceridemia and low baseline LDL-C. Fibrate therapy, however, does not change circulating FA concentrations (20). Although both the FIELD and ACCORD trials showed a trend towards decreased CV risk (primary endpoint) in T2D, post-hoc and meta-analysis revealed that dyslipidemic patients (high TG and low HDL-C) show the highest CV reduction (21)(22). Fibrates do not improve glucose homeostasis in T2D patients (23)(18)(19); however, PPARα activation improves glucose homeostasis in prediabetic patients (24), and may thus prevent conversion of prediabetes to overt T2D. Fibrates exert few adverse effects. Most compounds induce mild hypercreatininemia and hyperhomocysteinemia, but these effects appear to be pharmacodynamic markers of PPARα activation rather than indicators of renal dysfunction (25). Hepatic carcinogenesis has been observed in rodents treated with fibrates but

not in humans and non-human primates likely due to lower levels of peroxisomes and peroxisomal β-oxidation in human liver (26).

TZDs are PPARγ ligands that exert strong insulin-sensitizing effects, resulting in long-term glycemic control in T2D patients (27). However, their clinical use has been challenged due to side effects including body weight gain, edema, and bone fractures (2). The increase in body weight upon TZD administration is due to PPARγ-dependent adipocyte expansion in WAT (28) as well as fluid retention caused by PPARγ activation in the collecting ducts of the kidney (29). The increased fracture risk in TZD-treated patients results from a PPARγ-driven rebalancing of bone remodelling in favour of net bone loss. Indeed, PPARγ activation in bone marrow stimulates mesenchymal progenitor differentiation into the adipocyte lineage, thereby reducing osteoblast production thus suppressing bone formation (30). In this context, protein phosphatase PP5 controls mesenchymal differentiation towards adipocytes and osteoblasts through reciprocal regulation of PPARγ and RUNX2, respectively (31). Conversely, pharmacological PPARγ activation promotes osteoclast formation thereby increasing bone resorption (32), although this effect may not occur in physiological conditions (33).

Rosiglitazone and pioglitazone increase plasma levels of the insulin-sensitizing adipokine adiponectin (2). They also increase HDL-C and reduce circulating FA levels (34), but have differential effects on TG and LDL-C as well as on CV risk. Pioglitazone, a full PPARγ agonist with moderate PPARα activating properties (35), lowers TG, increases HDL-C and reduces CV outcomes in T2D (36) as well as insulin-resistant patients (37). In contrast, the pure PPARγ agonist rosiglitazone does not decrease CV risk in T2D, while increasing both HDL-C and LDL-C (38). Hence, the beneficial effects of pioglitazone on TG levels and CV events are likely due to combined PPARα and PPARγ activation. A recent Mendelian randomization study refuted a causal role for adiponectin in CV disease (39), providing a plausible explanation for the neutral effect of rosiglitazone on CV outcome despite the rise in adiponectin.

In summary, activation of PPARα improves the lipid profile, whereas activation of PPARγ rather improves glycemic control and insulin sensitivity.

Genetic evidence of contrasting PPARα and PPARγ functions

The different phenotypes observed in patients carrying single nucleotide polymorphisms (SNPs) and mutations in PPARα or PPARγ coding sequences further highlight the contrasting functions of the two isotypes. *PPARA* variants are associated with perturbations of lipid metabolism (40) and CV risk (41). The STOP-NIDDM trial revealed an association of *PPARA* SNPs with conversion from impaired glucose tolerance to T2D (42). These findings corroborate data showing that *PPARA* gene variation influences age of onset and progression of T2D (43). On the other hand, dominant-negative mutations in the ligand-binding domain of PPARγ result in severe insulin resistance (44). Accordingly, rare variants in *PPARG* with decreased adipogenic properties are associated with increased risk of T2D (45). Genome-wide association studies also revealed an association between *PPARG* SNPs and T2D, although not all studies concur (46)(47). A recently developed functional assay has permitted to identify new *PPARG* variants with altered PPARγ function (48). Moreover, SNPs found within DNA recognition motifs for PPARγ or cooperating factors and which alter PPARγ recruitment to chromatin modulate the response to anti-diabetic drugs (49). In addition, these SNPs in PPARγ binding sites are highly enriched among SNPs associated with TG and HDL-C levels in genome-wide association studies (49).

Altogether, these genetic data confirm the functional dichotomy between PPARα and PPARγ in humans, underscoring PPARα's effects on lipid metabolism and conversion from impaired glucose tolerance to T2D, as opposed to the role of PPARγ in T2D and the regulation of glucose homeostasis.

Cellular and molecular mechanisms underlying PPARα and PPARγ functions

PPARα's function (**Figure 1**) is best characterized in the liver, where it regulates genes involved in lipid and plasma lipoprotein metabolism during the nutritional transition phases (50)(51). During fasting, PPARα increases hepatic uptake and transport to mitochondria of FA originating from

adipose tissue lipolysis through transcriptional upregulation of FA transport proteins and carnitine palmitoyltransferases. In the mitochondria, enhanced expression of acyl-CoA dehydrogenases by PPARα stimulates hepatic FA oxidation (FAO), resulting in increased acetyl-CoA production which upon prolonged fasting is preferentially converted into ketone bodies to provide energy for extra-hepatic tissues. Mitochondrial HMGCS, a rate-limiting enzyme of ketogenesis, is also upregulated by PPARα (52)(53). Moreover, glucagon receptor and PPARα-signaling cooperate to control metabolic pathways during fasting (54). The hepatic IRE1α/XBP1 pathway also participates in the adaptation to fasting by promoting FAO and ketogenesis through upregulation of PPARα (55). In the fed state, on the other hand, PPARα coordinates different pathways of DNL to supply FA which will be stored as hepatic TG for periods of starvation. A crucial step in DNL is the citrate-malate shuttle which controls the efflux of acetyl-CoA from the mitochondria to the cytosol, where it serves as a precursor for FA synthesis. Citrate carrier (CIC), an essential component of this shuttle system, is a direct PPARα target gene in hepatocytes (56). In addition, PPARα increases protein levels of the lipogenic factor SREBP1c by promoting proteolytic cleavage of its precursor (57), and stimulates transcription of SREBP1c target genes including Fas, Scd1 and Acc1 (58). In these postprandial conditions, mTORC1 is activated through the insulin-dependent PI3K pathway, resulting in NCoR1-dependent inhibition of PPARα-mediated hepatic ketogenesis (59). Adjustment of PPARα activity to nutritional status also involves phosphorylation of the receptor by several kinases (see next section). PPARα thus contributes to the maintenance of metabolic flexibility by adapting fuel utilization to fuel availability, and its expression decreases in conditions of metabolic 'inflexibility', such as during NAFLD progression (60). Mechanisms contributing to dysregulated PPARα signaling in NAFLD may include microRNA-10b (61), microRNA-21. (62) and JNK (63). Indeed, these pathways repress hepatic PPARα signalling and are upregulated in NAFLD.

Reduction of plasma TG-rich lipoproteins upon PPARα activation is linked to enhanced FA uptake and oxidation as well as increased activity of LPL which hydrolyses lipoprotein TG. PPARα stimulation of LPL enzymatic activity is both direct through PPRE-dependent activation of the *LPL* gene (64) as well

as indirect through decreasing the levels of the LPL inhibitor and pro-atherogenic APO-CIII (65)(66) and increasing the levels of the LPL activator APO-AV (67). Reduced VLDL production contributes to the TG-lowering effects of PPARα activation mainly in rodents and, likely to a lesser extent, in humans. Indeed, reduced VLDL production, as observed in patients carrying a SNP in the *TM6SF7* gene, is associated with lower circulating TG levels but more severe hepatic steatosis (68), an effect not observed in PPARα agonist-treated patients (69). In MetS patients, fenofibrate treatment increases the fractional catabolic rate of VLDL-APOB, IDL-APOB and LDL-APOB, without affecting VLDL-APOB production (70). On the other hand, the rise in plasma HDL-C upon PPARα activation is linked to increased synthesis of major HDL-C constituents, apolipoproteins APO-AI and APO-AII (71) as well as induction of PLTP (72). Of note, differences between rodents and humans with respect to apolipoprotein regulation exist, as APO-AI and APO-AV are direct positive PPARα target genes in human but not murine liver (50). Through FAO, PPARα activation leads to energy dissipation not only in the liver but also in SKM (73) and white adipose tissue (WAT) (74). In brown adipose tissue (BAT), PPARα stimulates lipid oxidation as well as thermogenesis in synergy with PPARγ coactivator 1α (PGC1A) (75). Interestingly, while PPARα activation reduces weight gain in rodents (74), there is no evidence of PPARα effects on body mass in humans (18)(19).

The inability of fibrates to improve glucose homeostasis in T2D patients (18)(19) may result from several mechanisms. First, glucose handling in liver and peripheral tissues is reduced as a consequence of increased FAO (76). Next, PPARα activation reduces PK expression and induces PDK4 in the liver, leading to decreased glycolysis and enhanced gluconeogenesis (77). By contrast, as discussed above, clinical and genetic data revealed a role for PPARα in preventing conversion from impaired glucose tolerance to overt T2D. This effect might stem from PPARα-induced protection of pancreatic β-cells from lipotoxicity (78) as well as PPARα-dependent decrease in insulin clearance through repression of CEACAM1, a membrane glycoprotein that promotes hepatic insulin endocytosis and targeting to the degradation process (79).

PPARγ is highly expressed in WAT, where it controls FA uptake and lipogenesis. Target genes contributing to this activity include fatty acid binding protein-4 (FABP4) and the fatty acid translocase CD36 (80). In addition, PPARγ is a master regulator of white adipocyte differentiation. Multiple TFs including the glucocorticoid receptor (GR) and STAT5A team up to induce PPARγ during adipogenesis (28), while other TFs such as C/EBPα cooperate with PPARγ to stimulate genomic binding and transcription of target genes (81), thereby regulating both house-keeping and adipocyte-specific functions (82). These PPARγ-mediated changes in gene expression are preceded by chromatin remodelling involving both adipocyte-specific TFs such as C/EBPβ (83) as well as ubiquitous TFs such as CCCTC-binding factor (CTCF) (84). Interestingly, promotion of adipogenesis by the mTORC1 complex occurs through stimulation of PPARγ translation (85) and transcriptional activity (86), which contrasts with the inhibitory effect of mTORC1 on PPARα discussed above (59).

In contrast to WAT, PPARγ target genes in BAT encode thermogenic proteins and inducers of mitochondrial biogenesis such as PGC1A and uncoupling protein 1 (UCP1, also known as thermogenin). PPARγ promotes differentiation of brown adipocytes, but activation of additional TFs including PPARα is required to switch on their thermogenic program (75).

PPARγ enhances whole body insulin sensitivity through multiple mechanisms (**Figure 2**). By augmenting WAT expandability, PPARγ shifts lipids from liver and SKM to WAT, thereby indirectly increasing glucose utilization in liver and peripheral tissues. As a result of this 'lipid steal' process, lipotoxicity, which impairs insulin signalling, is alleviated. PPARγ also regulates the expression of adipocyte hormones such as adiponectin and leptin, which modulate liver and SKM insulin sensitivity (2). PPARγ induces adiponectin, an effect which likely contributes to PPARγ-mediated increase in glucose tolerance (87). Leptin expression is inhibited by PPARγ activation (88), which may contribute to TZD-induced appetite and body weight gain. Finally, PPARγ activation also improves pancreatic β-cell function and survival by preventing FA-induced impairment of insulin secretion (78) and enhancing the unfolded protein response (89). Thus, whereas PPARα activation leads to energy

dissipation, activation of PPARγ stimulates energy storage in WAT, thereby sensitizing liver and peripheral tissues to insulin.

The contrasting mechanisms of action of PPARα and PPARγ are also illustrated by their opposite function on hepatic lipid metabolism. Reduced hepatic steatosis due to increased FAO in hepatocytes occurs upon PPARα activation in rodent models of NAFLD (90)(91). In contrast, PPARγ activation in rodents increases liver fat accumulation by enhancing hepatic expression of PPARγ-dependent genes involved in lipogenesis (80), as confirmed by liver-specific PPARγ deletion (92). Interestingly, hepatic PPARγ expression levels determine liver steatosis. Indeed, mice with low hepatic PPARγ expression are resistant to diet-induced development of fatty liver when treated with rosiglitazone, whereas liver steatosis is exacerbated in obese mice with high hepatic levels of PPARγ (93). The dimeric AP-1 protein complex modulates NAFLD via differential regulation of hepatic PPARγ expression depending on the dimer composition. Whereas the FOS-like AP-1 transcription factor subunits FRA1 and FRA2 inhibit the PPARγ pathway and reduce hepatic lipid content, other AP-1 proteins such as c-Fos and JunD induce hepatic PPARγ signalling and lipid accumulation (94). In NAFLD patients, PPARγ expression is, however, unaltered (60) and TZD treatment rather lowers hepatic steatosis, likely due to decreased FA flux from WAT to liver (95)(96).

In addition to the 'classical' metabolic organs, energy homeostasis is also regulated by inter-organ communications involving the brain and the gut. Neuronal PPARγ deletion in mice diminishes food intake and energy expenditure resulting in reduced weight gain upon HFD feeding, suggesting that brain PPARγ exerts hyperphagic effects and promotes obesity (97). Similarly, central activation of PPARα may also increase food intake (6), although not all studies concur (98). In the intestine, PPARα activation suppresses the transient postprandial hyperlipidemia by enhancing intestinal epithelial cell FAO (99). In addition, activation of intestinal PPARα reduces cholesterol esterification, suppresses chylomicron production and increases HDL synthesis by enterocytes (100).

Molecular basis for differential activities of PPARα and PPARγ

The exact mechanisms via which the different PPAR isotypes – which share similar DNA binding motifs – bind and regulate different genes, remain to be fully established. Nevertheless, several explanations and hypotheses can be put forward. First, PPARα is predominantly expressed in the liver, whereas PPARγ expression is highest in WAT (2). The different PPARs emerged during evolution from gene duplications, but subsequent sequence variations of their promoter and 3'UTR have contributed to acquisition of differential expression patterns and functions (101). Tissue-specific chromatin and TF environments also play a deterministic role by restricting PPAR recruitment to selective enhancers and therefore specifying PPAR target genes (28). This is illustrated by the tissue-specific PPARγ cistromes in white adipocytes and macrophages, both expressing high levels of PPARγ. The macrophage-specific PPARγ cistrome is defined by the pioneer TF PU.1 (102), which induces nucleosome remodelling and histone modifications, promoting the recruitment of additional TFs (103). In white adipocytes, however, these macrophage-specific binding regions are marked with repressive histone modifications, disabling PPARγ binding (104). Furthermore, PPARγ cistromes differ between different types of white adipocytes (epididymal vs. inguinal) and are associated with depot-specific gene expression patterns (105).

Nutritional status is another contributor to differential PPAR regulation. PPARα is a metabolic sensor, switching its activity from coordination of lipogenesis in the fed state to promotion of FA uptake and oxidation during fasting (50). PPARα activation during fasting involves the induction of the coactivator PGC1α by the fasting-induced TF EB (TFEB) (106). In addition to PPARα itself (107), circadian transcription of genes encoding acyl-CoA thioesterases coordinates cyclic intracellular production of FA ligands (108). The TF CREBH, a recently identified circadian regulator of hepatic lipid metabolism, rhythmically interacts with PPARα and regulates its activity (109). Adjustment of PPARα transcriptional activity to nutritional status is also controlled by kinases phosphorylating PPARα or its coregulators. In the fed state, PPARα activity is enhanced through insulin-activated MAPK and glucose-activated PKC, while glucagon-activated PKA as well as AMPK increase PPARα signaling in

fasting (50). Moreover, the fasting response is co-controlled by interaction of PPARα with GRα, which show extensive chromatin colocalization and cooperate to induce lipid metabolism genes upon prolonged fasting through genomic recruitment of AMPK (110). By contrast, GRβ antagonizes glucocorticoid-induced signaling during fasting via inhibition of GRα and PPARα, increasing inflammation and hepatic lipid accumulation (111).

PPARγ activity is higher in the fed state, in line with its major role in the regulation of lipid synthesis and storage. PPARγ activity in WAT is repressed during fasting via mechanisms involving SIRT1 (112) or AMPK (113). In mice, the amplitude of hepatic circadian clock gene expression is reduced by HFD feeding (114), whereas circadian rhythmicity of PPARγ and PPARγ-binding site-containing genes is induced (115). Thus, the HFD-induced transcriptional reprogramming relies at least in part on changes in expression, pattern of oscillation and chromatin recruitment of PPARγ. Gut microbiota, which also exhibit circadian activity (116), are drivers of this hepatic transcriptional reprogramming by PPARγ upon HFD in mice (117).

Nutritional status also links PPARs to FGF21 signaling. Indeed, fasting increases PPARα-dependent FGF21 expression in liver, further enhancing FAO and ketogenesis (118). In WAT, PPARγ induces the expression of FGF21 (119), where it acts as an autocrine factor in the fed state, regulating PPARγ activity through a feedforward loop mechanism (120). The related family member FGF1 is also induced by PPARγ in WAT, and this PPARγ-FGF1 axis is critical for maintaining metabolic homeostasis and insulin sensitization (121). In the pancreas, PPARγ agonism reverses the high glucose-induced impairment of islet function by enhancing FGF21 signaling (122).

Combating inflammation: a shared function of PPARα and PPARγ

Besides differentially regulating lipid and glucose metabolism, PPARα and PPARγ also display a shared function, i.e. countering inflammation. However, the anti-inflammatory effects of PPARα and PPARγ activation are likely distinct due to differences in tissue and cell-type expression.

Anti-inflammatory effects

MetS is accompanied by a low grade inflammatory state in different metabolic tissues – termed meta-inflammation – characterized by increased secretion of pro-inflammatory chemokines and cytokines, many of which (including TNFα, IL-1 and IL6) influence lipid metabolism and insulin resistance (123).

In WAT, fenofibrate as well as rosiglitazone reduce the expression of several pro-inflammatory mediators by white adipocytes, including IL-6 and the chemokines CXCL10 and MCP1 (124). PPARγ also inhibits pro-inflammatory cytokine production by WAT-resident macrophages, and modulates macrophage polarization (125). Although innate immune cells such as macrophages have long been considered as the drivers of WAT inflammation and metabolic dysregulation, recent reports argue for an important role of the adaptive immune system, including WAT regulatory T cells (Tregs) (126). PPARγ acts as a crucial molecular orchestrator of WAT Treg accumulation, phenotype, and function (127)(128). Indeed, the WAT Treg transcriptome alterations in obese mice are dependent on PPARγ phosphorylation by cyclin-dependent kinase 5 (CDK5) (127). In addition, PPARγ expression in WAT Tregs is necessary for complete restoration of insulin sensitivity in obese mice upon pioglitazone treatment (128). PPARγ is also implicated in the metabolic reprogramming of CD4+ T-cells. Indeed, T-cell activation leads to mTORC1-dependent PPARγ induction and increased expression of genes involved in FA uptake, enabling the rapid proliferation of these T-cells, a prerequisite for an optimal immune response (129).

In liver as well as isolated hepatocytes, IL-1-mediated induction of pro-inflammatory genes is repressed by pretreatment with PPARα agonists (130). In the vascular wall, PPARα and PPARγ modulate the recruitment of leukocytes to endothelial cells, stimulate cholesterol efflux from macrophage-derived foam cells, and regulate inflammatory cytokine production by smooth muscle cells (131).

Molecular mechanisms

Inhibition of pro-inflammatory gene expression is the main process underlying the anti-inflammatory properties of PPARα and PPARγ. Several mechanisms have been proposed for transcriptional repression by PPARs that are not mutually exclusive. These include direct physical interaction of PPARα or PPARγ with several pro-inflammatory TFs including AP-1 and NF-κB (132)(133). Repression of inflammation independently of direct DNA binding of PPARα results in anti-inflammatory and anti-fibrotic effects in a mouse model of non-alcoholic steatohepatitis (NASH) (134). In addition to this PPRE-independent transrepression mechanism, interaction between NF-κB and PPRE-bound PPARα also occurs, leading to repression of TNF-α-mediated upregulation of complement C3 gene expression and protein secretion during acute inflammation (135). Moreover, simultaneous activation of PPARα and GRα increases the repression of NF-κB-driven genes, thereby decreasing cytokine production (136). Transcriptional repression of pro-inflammatory genes by PPARγ may include ligand-activated PPARγ sumoylation, which targets the receptor to corepressor complexes assembled at inflammatory gene promoters. This prevents promoter recruitment of the proteasome machinery that normally mediates the inflammatory signal-dependent removal of corepressor complexes required for gene activation. As a result, these complexes are not cleared from the promoters and inflammatory genes are maintained in a repressed state (137).

In addition to downregulating the expression of pro-inflammatory genes, PPARα (138) as well as PPARγ (139) also suppress inflammation by direct upregulation of genes with anti-inflammatory properties such as IL-1Ra, suggesting a possible cooperation between PPAR-dependent transactivation and transrepression to counter inflammation.

PPARα and PPARγ in human NAFLD

The anti-inflammatory properties of PPARα likely contribute to the improved lobular inflammation and hepatocellular ballooning observed in NAFLD patients treated with pioglitazone (140) or elafibranor (141), a dual PPARα/β(δ) agonist. Pioglitazone reduces hepatic steatosis in NAFLD patients (140), likely due to PPARγ activation. In line, the pure PPARγ agonist rosiglitazone also lowers liver fat in humans (96), whereas the pure PPARα agonist fenofibrate does not (69). Treatment of dyslipidemic patients with fenofibrate lowers plasma atypical deoxysphingolipids (142), whose levels increase upon transition of simple steatosis to NASH (143). Thus, both PPARα and PPARγ activation appear beneficial in human NAFLD, although the underlying mechanisms clearly differ. Whereas effects of PPARα agonism on inflammation and ballooning are due to direct PPARα activation in the liver, PPARγ's effects on hepatic steatosis are likely mediated by indirect mechanisms, such as suppression of FA flux to the liver, in line with the low expression and absence of PPARγ induction in human fatty liver (60).

PPARβ/δ, the clinically enigmatic third PPAR

Selective synthetic PPARβ/δ agonists are not yet clinically available. However, beneficial effects of PPARβ/δ activation on various components of the MetS, displaying both differences and similarities with PPARα and PPARγ, have been reported.

PPARβ/δ shares metabolic effects with both PPARα and PPARγ

PPARD variants are associated with cholesterol metabolism (144) as well as insulin sensitivity (145) and T2D risk (146). In addition, several SNPs in *PPARD* associate with CV risk (41). In obese men, administration of the synthetic PPARβ/δ agonist GW501516 lowers liver fat content and plasma levels of insulin, FA, TG, and LDL-C (147). These beneficial effects on plasma lipids are also observed in overweight patients treated with MBX-8025, a novel PPARβ/δ agonist (148). Thus, PPARβ/δ

agonism combines the metabolic effects of PPARα and PPARγ activation on lipid metabolism and glucose homeostasis, respectively. Preclinical studies support this conclusion, as GW501516 administration to overweight monkeys (149) or obese rats (150) lowers serum LDL-C and raises HDL-C, while improving insulin sensitivity.

Cellular and molecular mechanisms

PPARβ/δ activation protects from diet- or genetically induced obesity in mice by increasing energy expenditure (151). In BAT, activation of PPARβ/δ induces the expression of thermogenic genes including UCP1 as well as genes involved in FAO (151). In addition, PPARβ/δ agonism promotes FAO in SKM (152), WAT (153), and liver (154). PPARβ/δ in brain also controls energy expenditure, since neuron-specific deletion of PPARβ/δ increases the susceptibility to diet-induced obesity (155). Thus, similar to PPARα, activation of PPARβ/δ induces energy dissipation. Interestingly, both isotypes exhibit crosstalk in liver, where PPARβ/δ stimulates the production of the PPARα ligand 16:0/18:0-phosphatidylcholine as well as PPARα expression and DNA-binding activity, thereby increasing hepatic FAO (156). Enhanced FAO upon PPARβ/δ activation contributes to its plasma lipid-lowering effects, together with decreased cholesterol absorption (157) and increased trans-intestinal cholesterol efflux (158). PPARβ/δ also raises HDL-C by increasing hepatic expression of apolipoprotein AII (APO-AII) (159) and phospholipid transfer protein (PLTP) (160).

Countering inflammation is also a hallmark of PPARβ/δ agonism. Similar to PPARα and PPARγ, ligand-activated PPARβ/δ inhibits pro-inflammatory cytokine production (161) and regulates macrophage polarization in WAT (162) and liver (163). Inhibition of NF-κB is one anti-inflammatory mode of action of PPARβ/δ (164).

PPARβ/δ agonism improves insulin sensitivity through several mechanisms (**Figure 3**). In SKM, PPARβ/δ activation favours fiber-type switching from type II fast-twitch glycolytic fibers towards type I slow-twitch oxidative fibers (165) via mechanisms involving PGC1α (166) and an estrogen-related

411 receptor γ (ERRγ)/miRNA regulatory circuit (167), thereby improving glucose handling (168). The type
412 I fiber fraction is reduced in T2D patients (169), which may contribute to altered glucose
413 homeostasis. Myocyte-selective PPARβ/δ knockout mice exhibit a fiber-type switching towards fewer
414 type I fibers that precedes the development of a diabetic phenotype (166). PPARβ/δ also improves
415 glucose handling and insulin sensitivity in the liver. GW501516 treatment suppresses hepatic glucose
416 output and enhances glucose disposal by increasing glucose flux through the pentose phosphate
417 pathway (170). Liver-restricted PPARβ/δ overexpression reduces fasting glucose levels and stimulates
418 hepatic glycogen production via upregulation of glucose utilization pathways (171). In addition,
419 stress-induced JNK signalling is reduced, contributing to improved hepatic insulin sensitivity (171).
420 PPARβ/δ agonism promotes pancreatic β-cell mitochondrial function and ATP production, thus
421 improving glucose-stimulated insulin secretion (172). In addition, PPARβ/δ increases intestinal
422 production of the incretin glucagon-like peptide 1 (GLP1), an insulin secretagogue (173).
423 In summary, the mechanisms underlying the metabolic effects of PPARβ/δ resemble those of PPARα,
424 which promotes energy dissipation, as opposed to PPARγ, which promotes energy storage. PPARβ/δ
425 normalizes plasma lipids through enhanced FAO in several tissues, coupled to actions on hepatic
426 apolipoprotein metabolism and intestinal cholesterol homeostasis. However, in contrast to PPARα
427 and similar to PPARγ, activation of PPARβ/δ enhances insulin sensitivity. The mechanisms underlying
428 PPARβ/δ-mediated improvement in glucose handling are not similar to PPARγ, but involve PPARβ/δ-
429 specific actions on SKM fiber type distribution, hepatic glucose metabolism, and pancreatic islet
430 function.
431
432 **Where do we stand?**
433
434 Currently used PPAR agonists display weak potencies (PPARα) or are associated with important side
435 effects (PPARγ). Optimization of therapeutic efficacy may be achieved through the development of
436 selective PPAR modulators that retain the beneficial effects of PPAR activation while diminishing

437 unwanted side effects (174). Pemafibrate (K-877), a promising candidate among the selective PPARα
438 agonists (175), displayed greater lipid modifying efficacy than fenofibrate in a phase II trial, with little
439 or no effect on serum creatinine and homocysteine levels (176). This compound is currently
440 undergoing a phase III clinical cardiovascular prevention trial, PROMINENT, in patients with high TG
441 and low HDL-C. Administration of LY518674, another potent and selective PPARα agonist, to MetS
442 patients increased cholesterol efflux capacity without changing steady-state HDL-C or APO-AI levels
443 (177). Use of the PPARγ agonists rosiglitazone and pioglitazone is restricted due to safety concerns
444 related to congestive heart failure and bone fracture. Better insights into the molecular mechanisms
445 of PPARγ activity may lead to new compounds with fewer side effects. As an example, PPARγ
446 phosphorylation at serine 273 by CDK5 modifies interaction with thyroid hormone receptor-
447 associated protein 3 (THRAP3) and alters regulation of a large number of genes whose expression is
448 changed in obesity including adiponectin (178)(179). These findings spawned the development of a
449 new class of anti-diabetic drugs that inhibit CDK5-mediated phosphorylation of PPARγ while lacking
450 classical agonist activity. Along these lines, Gleevec blocks CDK5-mediated PPARγ phosphorylation,
451 exhibiting potent anti-diabetic effects in obese mice without triggering fluid retention or weight gain
452 (180). Whether this concept will eventually result in novel, clinically useful compounds is still unclear.
453 The non-TZD PPARγ modulator INT131 improves glucose tolerance in T2D patients to a similar extent
454 as rosiglitazone without adverse effects on body weight or hemodilution (181). The PPARβ/δ agonist
455 GW501516, whose development has been abandoned because of preclinical safety issues (182), and
456 MBX-8025 have shown efficacy in decreasing plasma TG and increasing HDL-C as well as improving
457 insulin sensitivity and liver function (148).
458 Alternatives to selective agonists are dual (activating two PPAR isotypes) and pan-agonists (activating
459 the three PPARs), aiming to combine the beneficial effects of each receptor isotype. The pan-agonist
460 chiglitazar (CS038) improves the lipid profile and insulin sensitivity without increasing body weight in
461 animal models of obesity, and is currently in phase III clinical development (183). IVA337, a pan-
462 agonist which prevents and reverses fibrosis in skin (184), is currently entering phase II for the

treatment of NASH. Many dual PPARα/γ agonists, also termed glitazars, showed improved efficacy on glucose and lipid metabolism in clinical trials, although safety concerns have halted further development (185). Substantial preclinical and clinical evidence suggests that a large part of adverse events are drug-specific, off-target actions and hence PPAR-independent, although identifying the mechanisms is often challenging in the in vivo setting as off-target actions are superimposed on target-mediated effects (186). Two phase III trials with saroglitazar showed improved glucose and lipid profiles in patients with diabetic dyslipidemia compared to pioglitazone (187) or placebo (188). In contrast to the other PPARγ-dominant glitazars, saroglitazar predominantly activates PPARα with only moderate PPARγ agonism, which may explain the lack of typical PPARγ side effects. The non-TZD dual PPARα/γ agonist DSP-8658, reportedly in phase I development, normalizes blood glucose and plasma lipids in obese mice without increasing adipogenesis (189). The dual PPARβ(δ)/γ agonist DB959 regulates glucose, TG, and HDL in preclinical models of T2D and dyslipidemia (190). Elafibranor (GFT505), a dual PPARα/β(δ) agonist, demonstrated protective effects against hepatic steatosis, inflammation, and fibrosis in animal models of NAFLD/NASH (90). In phase IIa trials, elafibranor improved the lipid profile and enhanced insulin sensitivity in dyslipidemic and prediabetic patients (191) as well as in obese individuals (192). The GOLDEN-505 phase IIb study in NASH patients showed that elafibranor treatment induces resolution of NASH without worsening fibrosis in a higher proportion of patients compared to placebo. The drug was well tolerated and improved glucose homeostasis and the patients' CV risk profile (141).

PPARs are still valuable targets for metabolic diseases

Over the last decades, market withdrawals and failed drug development programs have cast doubts on the clinical value of PPAR-activating compounds. However, this issue is not black and white. The pure PPARγ agonist rosiglitazone as well as dual PPAR agonists with predominant PPARγ activating properties all displayed important adverse effects, leading to restricted use or halted development.

However, most of these side effects were either drug-specific and hence off-target, or related to excessive PPARγ activation. Several fibrate trials, including FIELD and ACCORD, failed to meet the primary endpoint of reduced CV risk. However, such negative outcomes are likely linked to inappropriate patient selection, since subgroup analyses revealed significant CV risk reduction in those patients with marked dyslipidemia upon fenofibrate treatment (21). In addition, in several of these fibrate trials, including BIP and FIELD, the proportion of patients receiving statin therapy was unbalanced between the placebo and treatment groups. Correction for this non-randomized statin drop-in in the FIELD study estimated that fenofibrate induces a 19% relative CV risk reduction (193).

To date, the mechanism of action of endogenous ligands is not fully elucidated. Therefore, one needs to rely on knockout mice and synthetic ligands to study PPAR functions, resulting in limitations in our understanding of the physiological functions of PPARs. Nevertheless, it has become increasingly clear that PPARα and PPARγ agonism display contrasting metabolic effects with different mechanisms of action. Whereas PPARβ/δ agonism is more related to PPARα, subtle differences exist (e.g. in regulation of glucose homeostasis). These findings are in line with the enhanced metabolic actions and improved safety profiles of novel compounds such as dual PPARα/β(δ) ligands, which target both the lipid abnormalities (via PPARα and PPARβ/δ), as well as the glucose alterations (via PPARβ/δ) in MetS patients without displaying PPARγ-related adverse effects. Altogether, we are convinced that modulating PPARs in metabolic disorders remains a valuable and promising approach with a future ahead.

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Figure legends

Figure 1. PPARα activation stimulates fatty acid and triglyceride metabolism. During fasting (yellow), fatty acids (FA) released from white adipose tissue (WAT) are taken up by the liver and transported to mitochondria where FA oxidation (FAO) takes place to produce acetyl-CoA (AcCoA) which can be further converted to ketone bodies serving as fuel for peripheral tissues. In the fed state (green), AcCoA is shuttled to the cytosol where de novo lipogenesis (DNL) takes place. Effects of PPARα activation and PPARα target genes are indicated in pink. FAO is also stimulated by PPARα in WAT and skeletal muscle (SKM). By regulating hepatic apolipoprotein synthesis, PPARα activation decreases plasma triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C) and increases high density lipoprotein-cholesterol (HDL-C) levels. PPARα also acts on brown adipose tissue (BAT), gut and pancreas, whereas central effects are unclear. Blue brackets indicate PPARα actions restricted to mice and not (peroxisome proliferation, reduced liver fat content) or to a lesser extent (reduced APO-B production) seen in humans

Figure 2. PPARγ activation increases whole body insulin sensitivity. In white adipose tissue (WAT), PPARγ activation (effects are indicated in pink) enhances fatty acid (FA) uptake and storage, lipogenesis and adipogenesis (lipid steal action). PPARγ activation lowers circulating FA levels, alleviating lipotoxicity and increasing insulin sensitivity. PPARγ agonism induces adiponectin production by WAT further enhancing insulin sensitivity and lowering blood glucose. PPARγ also exerts metabolic effects on brown adipose tissue (BAT), brain and pancreas. Increased hepatic steatosis upon PPARγ activation occurs in mice but not in humans (blue brackets), who in contrast display increased hepatic insulin sensitivity due to reduced FA flux from WAT.

Figure 3. PPARβ/δ activation enhances glucose and lipid homeostasis. In skeletal muscle (SKM), PPARβ/δ activation (effects are indicated in pink) favours fiber-type switching towards type I

oxidative fibers, which have a higher glucose-handling capacity compared to type II fibers. PPARβ/δ also augments fatty acid oxidation (FAO) in SKM, liver and white adipose tissue (WAT) and enhances hepatic glucose metabolism and pancreatic β-cell function. PPARβ/δ activation decreases fatty acids (FA), triglycerides (TG) and low density lipoprotein-cholesterol (LDL-C) and increases high density lipoprotein-cholesterol (HDL-C) levels in blood. Metabolic effects of PPARβ/δ agonism also take place in brain and gut.