REVIEW

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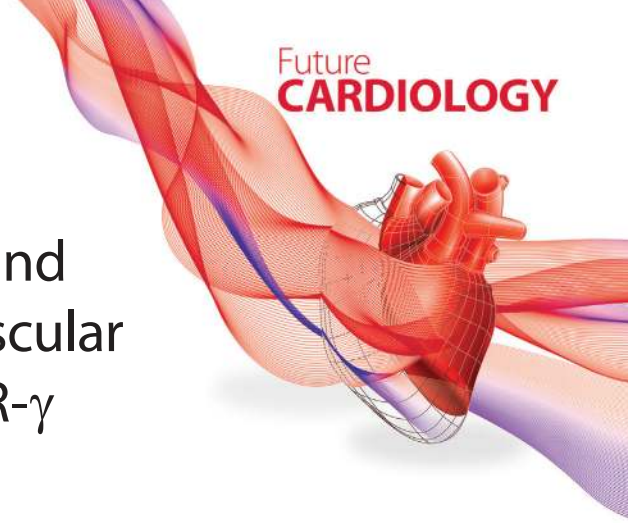

PPARs: regulators of metabolism and as therapeutic targets in cardiovascular disease. Part II: PPAR-β/δ and PPAR-γ

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The PPARs are a subfamily of three ligand-inducible transcription factors, which belong to the superfamily of nuclear hormone receptors. In mammals, the PPAR subfamily consists of three members: PPAR-α, PPAR-β/δ and PPAR-γ. PPARs control the expression of a large number of genes involved in metabolic homeostasis, lipid, glucose and energy metabolism, adipogenesis and inflammation. PPARs regulate a large number of metabolic pathways that are implicated in the pathogenesis of metabolic diseases such as metabolic syndrome, Type 2 diabetes mellitus, nonalcoholic fatty liver disease and cardiovascular disease. The aim of this review is to provide up-to-date information about the biochemical and metabolic actions of PPAR-β/δ and PPAR-γ, the therapeutic potential of their agonists currently under clinical development and the cardiovascular disease outcome of clinical trials of PPAR-γ agonists, pioglitazone and rosiglitazone.

First draft submitted: 31 August 2016; Accepted for publication: 21 March 2017; Published online: 5 June 2017

KEYWORDS
• cardiovascular disease • hyperglycemia • hyperinsulinemia • lipid and glucose metabolism • metabolic syndrome • NAFLD • PPAR-β/δ agonist • PPAR-γ agonist • Type 2 diabetes

Cardiovascular disease (CVD) remains the leading cause of death globally [1]. According to WHO statistics, 17.5 million people die each year from CVD, an estimated 31% of all deaths worldwide [1]. CVD accounts for nearly 801,000 deaths in the USA [2]. The prevalence of obesity has reached to epidemic proportions in both developed countries and in developing countries and, in the USA, during the past few decades there have been significant increases in obesity in both children and adults [3–7]; recent data from US surveys indicate that currently 37.7% of adults and 17.0% children are obese [8]. Obesity is a risk factor for many chronic metabolic diseases such as Type 2 diabetes mellitus (T2DM) [9–12], metabolic syndrome (MetS) [13–15], nonalcoholic fatty liver disease (NAFLD) [16–19], certain cancers [20,21] and CVD [12,22–24], and the prevalence of these clinical conditions are also increasing at a rapid pace. In addition, both MetS and NAFLD independently increase the risk of T2DM and CVD [25–30]. Increasing evidence now also indicates that both MetS and NAFLD are functionally linked and contribute to each other's pathophysiology and clinical manifestation [31–34]. However, currently there are no designated therapies to treat MetS [35] or NAFLD [36].

The PPARs are a subfamily of ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily [37]. The PPAR subfamily consists of three isotypes, PPAR-α (NR1C1), PPAR-β/δ (NR1C2) and PPAR-γ (NR1C3) [37]. PPARs are critically involved in the regulation of a large number of genes that regulate energy homeostasis, glucose triglyceride and

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lipoprotein metabolism, *de novo* lipogenesis, fatty acid uptake, oxidation, storage and export, cell proliferation, inflammation and vascular tissue function [38–59]. Because of their involvement in multiple metabolic processes, PPARs have been implicated in the pathogenesis obesity, MetS, diabetes, NAFLD and atherosclerosis as such they represent important molecular targets for the development of new drugs to treat these metabolic diseases [43,57,60–65]. Two classes of drugs, fibrates and thiazolidinedione (TZD) agonists that selectively activate PPAR-α and PPAR-γ, respectively, are already in clinical use in the management of dyslipidemia and hyperglycemia. Fibrates are used as medication to improve plasma triglyceride levels, HDL-cholesterol (HDL-C) and triglyceride-rich lipoproteins [66]. Fibrates may also reduce LDL-cholesterol (LDL-C) levels and exert long-term cardioprotective effect. Two TZDs, rosiglitazone and pioglitazone, are insulin sensitizers and used as oral hypoglycemic agents to treat patients with T2DM [67,68]. Additional efforts are also underway by many pharmaceutical companies around the globe to develop agonists with multiple (PPAR-α/PPAR-γ, PPAR-α/PPAR-β/δ and PPAR-β/δ/PPAR-γ or PPAR-α/PPAR-β/δ/PPAR-γ) or partial receptor activity with a goal to develop new therapeutic agents for the treatment of MetS, T2DM and associated cardiovascular complications and NAFLD.

This is a second part of two part review articles (part I and part II), which represent an update of a previous article that was written by one of the authors and published in this journal in September 2010 [69]. Here, we focus on the molecular and cellular events connected with the expression and metabolic functions of PPAR β/δ and γ, the involvement of them in the pathophysiology of vasculature and the current developmental status of new single, dual, pan (multiple) and partial PPAR agonists and specific PPAR modulators. We discuss the therapeutic potential of the modulators to treat individual components of MetS, T2DM, CVD and NAFLD including nonalcoholic steatohepatitis.

Molecular characteristics of PPAR-β/δ & PPAR-γ

Human *PPARD* is localized at chromosomal region 6p21.2-21.1 and comprised with nine exons [70], whereas human *PPARG* has nine exons and is localized on chromosome 3p25 [71]. In the case of PPAR-γ, so far a total of seven mRNA transcripts generated as a result of multiple transcription initiation and alternative splicing of five exons have been identified [56]. Similar to other members of the nuclear receptor family and PPAR-α, PPAR-β/δ and PPAR-γ also contain a modular structure consisting of an N-terminal A/B domain, a DNA-binding C domain, a D domain and a C-terminal ligand-binding domain (E/F domain) [69,72]. The central DNA-binding domain recognizes PPAR response elements (PPREs) in the promoter regions of their target genes. PPARs form heterodimers with retinoid X receptors (RXRs, α, β, γ) and bind to a consensus PPRE in the target DNA. Under unliganded state, PPAR/RXR heterodimers are bound to multicomponent repressors thereby inhibiting gene transcription [73,74]. Following stimulation by PPAR activators, PPAR/RXR heterodimers dissociate from corepressors, and recruit coactivators and subsequently bind to PPRE target genes to modulate gene transcription [73].

Metabolic functions of PPAR-β/δ

PPAR-β/δ is ubiquitously expressed in mouse [75–77], rat [76,78] and human tissues [54,76–77,79]. In mouse, highest expression of PPAR-β/δ protein is detected in gastrointestinal tract including small intestine and colon, high-to-moderate levels in skin and brown adipose tissue, liver, kidney, lung and vasculature and low levels in heart, skeletal muscle, brain, thymus and other tissues [76–77,80]. A number of fatty acids and eicosanoids and synthetic single PPAR-β/δ agonists, dual PPAR-α/β(δ) agonist and Pan PPAR-α/β(δ)/γ function as ligands for PPAR-β/δ **(Table 1)**. However, unlike PPAR-α and PPAR-γ, which are therapeutic targets for antihyperlipidemic (fibrates) and antidiabetic drugs (TZDs), respectively, PPAR-β/δ does not appear to be a target of any currently available drugs. Because of the lack of availability of PPAR-β/δ-targeted drugs coupled with its wide expression in many tissues and cells, the metabolic function of PPAR-β/δ is relatively less studied and understood. However, in recent years, the availability of potent synthetic PPAR-β/δ agonists such as GW0742, GW501516, L165041, GW1929 **(Table 1)** and availability of global and tissue-specific PPAR-β/δ transgenic and gene-targeting mice **(Supplementary Box 1)** have generated valuable information, implicating this PPAR in the regulation of insulin sensitivity, adipogenesis, lipid and energy metabolism, inflammation and atherosclerosis [47,51,53–54,57,81–84].

Table 1. Partial list of endogenous and synthetic peroxisome proliferator-activated receptor β/δ agonists.

Endogenous ligand	Synthetic PPAR-β/δ agonists	Dual PPAR-α/βδ agonists	Pan PPAR-α/βδ/γ agonists
Fatty acids			
Palmitic acid	L165041	GFT505	Chiglitazar
Stearic acid	GW501516	–	Netoglitazar
Oleic acid	GW0742	–	Sodelglitazar
Linoleic acid	GW1929	–	Indeglitazar
γ-Linoleic acid	CER-002	–	Sipoglitazar
Dithomo-γ-linolenic acid	HPP593	–	–
	MBX-8025	–	–
Arachidonic acid	Carbaprostacyclin (cPGI)	–	–
Docosahexaenoic acid	–	–	–
Eicosapentaenoic acid	–	–	–
C6–C8	–	–	–
Palmitoleic acid	–	–	–
Eicosanoids			
15d-PGI_2	–	–	–
PGJ_2	–	–	–
PGI_2 (Prostacyclin)	–	–	–
$PGA_{1/2}$	–	–	–
PGB_2	–	–	–

15d-PGI_2: 15-Deoxy-$\Delta^{12,14}$-PGJ_2; GFT505: 2-(2,6-dimethyl-4-(3-(4-(methylthio)phenyl)-3-oxo-1-propenyl)phenyl)-2-methylpropanoic acid; GW0742: [4-[[[2-[3-Fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]thio]-2-methyl phenoxy]acetic acid; GW1929: (2***S***)-((2-Benzoylphenyl)amino-3[4-[2-(methylpyridin-2-ylamino)ethoxy]phenyl)-propionic acid; GW501516: 2-Methyl-4(((4-methyl-2-(4-trifluoromethyl-phenyl)1,3-thiazol-5-yl)methyl)sulfanyl)phenoxy)acetic acid; L165041: (4-[3{4-Acetyl-3-hydroxy-2-propylphenoxy}propoxyl]}phenoxy)acetic acid; MBX-8025: 2-[4-[[2R)-2-ethoxy-3-[4(trifluoromethyl)phenoxy]propyl]thio]-2-methylphenoxy]acetic acid; PGJ_2: Prostaglandin J; $PGA_{1/2}$: Prostaglandin A; PGB_2: Prostaglandin B.

Indeed, many enzymes and proteins that participate in these various metabolic processes have been identified as a direct target of PPAR-β/δ **(Supplementary Box 2)** [57].

• Lipid/lipoprotein metabolism

GW501516 is a potent PPAR-β/δ agonist, which is roughly 1200-times more selective for PPAR-β/δ over the other subtypes [85]. In insulin-resistant middle-aged obese rhesus monkeys, GW501516 treatment caused a dramatic dose-dependent increase in plasma HDL-C and decreased plasma triglyceride, LDL-C and insulin levels [85]. Likewise, GW501516 treatment increased plasma HDL-C, and HDL-associated apoA-I and apoA-II concentrations and increased HDL particle size in African Green/St Kitts vervet monkeys, a nonhuman primate model of atherosclerosis [86]. These nonhuman primate studies provoked the examination of this PPAR-β/δ agonist in human clinical trials for its potential utility in the clinical management of metabolic dysregulation including dyslipidemia [53]. Despite these beneficial actions of GW501516, the further development of this agonist was discontinued because of safety concerns [87].

• Adipose tissue

Although PPAR-γ is a central regulator of adipocyte differentiation (ADD), several reports including cell culture studies suggest that PPAR-β/δ also participates in this process both independently and in concert with PPAR-γ [88–93]. Various studies also demonstrate that PPAR-β/δ regulates the transcription of genes involved in brown or white adipose tissue fatty acid transport, oxidation and thermogenesis [57]. Furthermore, a review of the published scientific literature provides evidence that PPAR-β/δ plays an important role in adipose tissue metabolism as well. Gene deletion studies demonstrated that a small number of surviving PPAR-β/δ knockout (PPAR-β/$\delta^{-/-}$) mice exhibit a lean phenotype, with a significantly reduced fat mass **(Supplementary Box 1)**. PPAR-β/$\delta^{-/-}$ mice, however, showed no significant changes in the size of epididymal white or interscapular brown fat pads. Similarly, adipocyte-specific PPAR-β/δ deletion in mice showed no effect on their fat mass **(Supplementary Box 1)**. In another study, transgenic mice expressing a constitutively active form of PPAR-β/δ (VP16 activation domain fused to the N-terminus of PPAR-β/δ;

VP16-PPAR-β/δ) driven by the aP2 promoter was used (Supplementary Box 1). These transgenic mice exhibited reduced adiposity, with a significant reduction in body fat composition. Interestingly, VP16-PPAR-β/δ mice were protected from high-fat diet (HFD) or leptin receptor deficiency-induced obesity (Supplementary Box 1). Moreover, PPAR-β/δ activation induced genes in brown adipose tissue that participate in fatty acid oxidation including CPT1, ACOX and LCAD and thermogenesis/energy expenditure such as UCP1 and UCP3. Collectively, these data suggest that PPAR-β/δ regulates adiposity by promoting fat combustion.

• Liver

PPAR-β/δ is expressed in all the major liver cell types including hepatocytes and is implicated in the regulation of both hepatic glucose and lipid metabolism. PPAR-β/$\delta^{-/-}$ mice have been reported to be glucose intolerant, whereas pharmacological activation of PPAR-β/δ in diabetic *db/db* and *ob/ob* mice with agonists improves insulin sensitivity [94,95]. Furthermore, it was shown that PPAR-β/δ agonist GW501516 improves hyperglycemia by attenuating hepatic glucose production, promoting glucose disposal and preventing fatty acid release from adipose tissue depots. Gene array analyses suggested that increased glucose metabolism via pentose phosphate pathway, which is to enhance *de novo* fatty acid synthesis (lipogenesis), may be one potential mechanism by which PPAR-β/δ ameliorates hyperglycemia [93]. To more directly examine the role of hepatic PPAR-β/δ in insulin resistance/hyperglycemia, Liu *et al.* (Supplementary Box 1) genetically activated the liver PPAR-β/δ (PPAR-β/δ^{LivTg}) by employing an adenoviral-mediated gene delivery system and used liver-specific PPAR-β/δ-null (PPAR$^{\Delta Liv}$) mice as a control. Evaluation of insulin sensitivity between PPAR-β/δ^{LivTg} and PPAR$^{\Delta Liv}$ led to the conclusion that hepatic PPAR-β/δ serves as an insulin sensitizer. These studies also led to the demonstration that overexpression of PPAR-β/δ in liver is associated with an induction of a hepatic gene expression that contributes to increased glucose utilization and lipogenesis. No such changes were evident in liver of PPAR$^{\Delta Liv}$ mice. Two additional studies suggest that PPAR-β/δ regulated alternative activation of the anti-inflammatory M2 phenotype of resident microphages in liver and adipose tissue is associated with enhanced fatty acid metabolism and improved insulin sensitivity (Supplementary Box 1).

Some evidence also suggests that PPAR-β/δ is involved in the regulation of hepatic lipid metabolism. PPAR-β/δ-null mice on a HFD showed an increased rate of hepatic VLDL production as well as lowered lipoprotein lipase activity in serum compared with wild-type controls. Hepatic expression of gene-encoding angiopoietin-like proteins 3 and 4, which act as inhibitors of lipoprotein lipase, is also increased in response to HFD feeding. A marked increase in the plasma VLDL apoB48, apoE, apoAI and apoAII levels, as well as a reduction in hepatic lipid stores are also observed in PPAR-β/$\delta^{-/-}$ mice (Supplementary Box 1).

• Skeletal muscle & heart

PPAR-β/δ is a key transcription factor involved in the regulation of skeletal muscle fiber types, lipid metabolism, fuel utilization, mitochondrial function and muscle performance [47]. PPAR-β/δ also exerts regulatory effects on cardiac muscle. A number of key genes involved in fatty acid uptake, transport and subsequent catabolism (β-oxidation) have been identified as target genes for PPAR-β/δ (Supplementary Box 2). Given this, PPAR-β/δ modulation of fatty acid metabolism is considered to be the most important regulatory function of this PPAR, which is well documented in cultured muscle cells, isolated skeletal muscle preparations and *in vivo* [47].

The PPAR-β/δ-mediated skeletal muscle function and oxidative metabolism is more clearly delineated using muscle-specific PPAR-β/δ knockout (loss of function) and PPAR-β/δ transgenic (gain of function) mouse models as well as wild-type mice subjected to physiological manipulations. In normal mouse skeletal muscle, PPAR-β/δ is expressed at relatively higher level in soleus muscle [96], which consists of mostly type I muscle fibers and is rich in mitochondria. Soleus muscle predominantly uses oxidative phosphorylation to generate energy (ATP) production. In contrast, it is expressed at low level in gastrocnemius muscle, which mainly consists of type IIA muscle fibers and relies on both oxidative and glycolytic fibers for energy production. PPAR-β/δ expression in skeletal muscle is increased in response to fasting [97] and exercise [98]. Overexpression of either wild-type or an activated form, VP16-PPARδ, of the *PPAR*-β/δ gene in mice demonstrated increased formation of mitochondrial-rich oxidative type I muscle fibers, increased mitochondrial content and genes of

oxidative metabolism, fatty acid catabolism and type I fiber markers (Supplementary Box 1). Mice overexpressing muscle-specific PPAR-β/δ demonstrated increased endurance capacity, and initially nicknamed 'marathon mice'. It was further demonstrated that these transgenic mice show increased expression of lactate dehydrogenase b (*Dub*)/*Ldha* gene expression ratio, an isoenzyme shift that channels glycolysis end product pyruvate to the mitochondria for its oxidation. Furthermore, evidence was provided showing that in skeletal myotubes PPAR-β/δ interacts with activated 5′-AMP-activated protein kinase to synergistically activates *Ldhb* gene transcription in concert with MEF2A (Supplementary Box 1). Additionally, muscle-specific PPAR-β/δ transgenic mice showed increased muscle glycogen accumulation, elevated levels of GLUT4 glucose transporter and enhanced capacity of mitochondrial pyruvate oxidation. It was also observed that these mice persistently oxidized glucose as compared with nontransgenic control mice. The role of PPAR-β/δ in skeletal muscle function was further validated in muscle-specific PPAR-β/δ-null (PPAR-β/$\delta^{-/-}$) mice (Supplementary Box 1). These mice demonstrated a reduction in type I muscle fibers, attenuated expression of genes that participate in fatty acid uptake and transport, oxidation, energy expenditure and oxidative phosphorylation (Supplementary Box 1). Furthermore, these mice developed increased adiposity and insulin resistance/T2DM.

The impact of cardiac muscle-specific genetic alterations in PPAR-β/δ on lipid and energy metabolism and cardiac function has also been evaluated. Mice that were gene ablated for cardiac PPAR-β/δ (cardiac PPAR-β/$\delta^{-/-}$) displayed severe impairments in mitochondrial fatty acid gene expression, reduced rates of fatty acid oxidation, increased myocardial lipid accumulation, cardiac dysfunction, severe cardiomyopathy and congestive heart failure (Supplementary Box 1). Furthermore, chronic feeding of a HFD to cardiac-restricted PPARδ-null (CR-PPAR$\delta^{-/-}$) mice caused a robust induction of genes encoding key fatty acid oxidation enzymes without any corresponding increases in enzyme proteins. CR-PPAR-β/$\delta^{-/-}$ mice also exhibited pathological changes in sarcomere structures and mitochondrial abnormalities when fed either normal chow or HFD. Interestingly, although CR-PPAR-β/$\delta^{-/-}$ mice demonstrated increased expression of PPAR-γ coactivator 1α (PGC1α) and PPAR-α, such increases were not sufficient to overcome PPARδ-deficiency-induced metabolic and pathological abnormalities in the heart. Given that PPAR-α and PPAR-β/δ exhibit functional redundancy in the regulation of cardiac lipid and oxidative metabolism, Liu *et al.* generated cardiomyocyte-restricted PPARδ-deficient (CR-PPAR$\delta^{-/-}$) mice on a PPAR-α-null background (Supplementary Box 1). Loss of whole-body PPAR-α activity had no effect on cardiac PPARδ-deficiency-induced mitochondrial abnormalities, blunted cardiac performance, cardiac hypertrophy and impaired expression of key factors involved in mitochondrial biogenesis and defense. Moreover, combined deficiencies of PPAR-α and PPAR-β/δ had no additional inhibitory effect on the diminished rates of cardiac fatty acid oxidation observed in mice with PPAR-α-deficiency alone. On the other hand, it has been shown that cardiac overexpression of PPAR-β/δ (PPAR-β/δ^{Tg}) results in increased cardiac glucose uptake and oxidation along with increased GLUT4 and PFK (glycolysis) gene expression and also attenuation of ischemia and reperfusion-induced myocardial injury (Supplementary Box 1). In addition, use of transgenic mice constitutively overexpressing cardiac-specific PPAR-β/δ showed increased expression of critical factors involved in mitochondrial biogenesis (PPAR-γ coactivator or PGC1), antioxidant enzymes (SOD1, catalase) and fatty acid and glucose metabolism (CPT1b, CPT II, GLUT4; Supplementary Box 1). Myocardial oxidative metabolism and mitochondrial DNA copy number are also increased along with cardiac performance in the transgenic (PPAR-β/δ^{Tg}) mice. Increased expression of cardiac PPAR-β/δ also improved cardiac function and mice showed resistance to mechanical stress-induced cardiac hypertrophy.

• Vasculature, inflammation & atherosclerosis

During the past 10–15 years, considerable progress has been made in understanding the role of PPAR-β/δ in inflammation, vascular cells and atherosclerosis. PPAR-β/δ is expressed in vasculature with significant expression detected in endothelial cells, vascular smooth muscle cells and monocyte-macrophages and it plays a significant role in the regulation of expression and function of these cell types [42,44,54]. PPAR-β/δ regulates endothelial cell function through several mechanisms (Supplementary Box 3) [54].

Activated PPAR-β/δ promotes endothelial cell and endothelial progenitor cell proliferation, increases the phosphorylation of endothelial cell nitric oxide synthase and release of nitric oxide, upregulates gene expression of antioxidant enzymes, attenuates inflammation and apoptosis and modulates angiogenesis **(Supplementary Box 3)**. PPAR-β/δ also regulates expression and function of smooth muscle cells by inhibiting their proliferation, migration through maintenance of extracellular matrix, attenuating apoptosis and inhibiting senescence by upregulating antioxidant enzyme genes and suppressing inflammation **(Supplementary Box 3)** [54].

Inflammation and dysregulated lipid and lipoprotein metabolism are key determinants of atherosclerosis. Monocyte/macrophages contribute to the pathogenesis of atherosclerosis through the accumulation of cholesterol (in the form of lipid-laden macrophage foam cells) as a result of dysregulated cholesterol homeostasis and abnormal cholesterol metabolism and the production of inflammatory mediators and cytokines [53–54,77,99–100]. Multiple lines of evidence supports that PPAR-β/δ regulates various metabolic processes including lipid/cholesterol metabolism and inflammatory responses in macrophages with relevance to atherosclerosis [54]. In particular, it was demonstrated that macrophage treatment of VLDL- or LDL-derived fatty acids rapidly stimulates foam cell formation [53–54,77,100], induces inflammatory response and causes apoptosis. Interestingly, VLDL-derived fatty acids also activate PPAR-β/δ, which in turn causes the induction of genes involved in macrophage fatty acid catabolism. Considering this, it has been suggested that PPAR-β/δ serves as a sensor in macrophages to prevent excessive lipid accumulation under normal physiological conditions. In contrast, under pathophysiological conditions such as hypertriglyceridemia, PPAR-β/δ-regulated macrophage lipid homeostasis induced by free fatty acids is inadequate to fully neutralize macrophage-delivered atherogenic substrates [53]. However, agonist-mediated activation of PPAR-β/δ in macrophages not only attenuates the VLDL-induced foam cell formation and inflammatory cytokine expression but also activates a transcription gene program that inhibits triglyceride accumulation by promoting increased channeling of fatty acid for their catabolism via β-oxidation. It was further demonstrated that activated PPAR-β/δ-mediated repression of proinflammatory mediators is achieved via modulation of signal transduction pathways involved in macrophage inflammatory response. In addition, some of macrophage inflammatory responses regulated by PPAR-β/δ are mediated by the association or dissociation of PPAR-β/δ with transcriptional corepressor Bcl-6 protein.

Multiple lines of evidence also indicate that PPAR-β/δ may play an antiatherogenic role in the pathogenesis of atherosclerosis. It has been reported that PPARδ$^{-/-}$ bone marrow transplanted into γ-irradiated LDLR$^{-/-}$ mice significantly reduced the atherosclerotic lesion area in mice chronically fed a HFD as compared with wild-type C57 bone-marrow transplanted animals, suggesting a proatherogenic effect of PPAR-β/δ [53]. However, this unexpected finding was interpreted to suggest that PPAR-β/δ is in fact atheroprotective. It was proposed that deletion of *Ppar*δ mimicked the liganded state of the receptor and that ligand activation of receptor may be atheroprotective [53]. Several subsequent studies aimed at delineating the atheroprotective actions of PPAR-β/δ yielded inconsistent results. Likewise treatment of high-cholesterol/high-fat-fed female LDLR$^{-/-}$ with a specific PPAR-β/δ agonist, G0742, at a high dose for 10 weeks decreased lesion area by up to 50%, whereas low doses had no effect on the extent of atherosclerosis [53]. In addition, dietary administration of a high-affinity PPAR-βδ agonist, GW501516, in high-fat, high-cholesterol diet fed LDLR$^{-/-}$ mice attenuated the pre-established fasting hyperlipidemia, hyperglycemia and hyperinsulinemia, as well as glucose intolerance [53]. GW501516 treatment also decreased the aortic sinus lesions and lesion macrophages. In another model of atherosclerosis, HFD-fed apoE$^{-/-}$ mice, treatment with a low dose of GW501516 modestly reduced total aortic lesion area. This antiatherosclerotic action of GW501516 was associated with the modulation of several pathways, including elevation of HDL-C levels, inhibition of chemoattractant signaling in the vessel wall by downregulation of chemokines, induction of expression of regulator of G protein signaling (*RGS*) genes, potent anti-inflammatory effects on the macrophage response to inflammatory atherogenic cytokines and suppression of monocyte transmigration. To investigate the effect of PPAR-β/δ activation on accelerated atherosclerosis, LDLR$^{-/-}$ mice were infused with angiotensin II (AngII) or PBS and fed a HFD, with or without PPAR-β/δ agonist. Agonist treatment significantly reduced AngII-induced atherosclerotic lesion. Likewise,

it was demonstrated that GW501516 activation of PPAR-β/δ ameliorated AngII-induced abdominal aortic aneurism formation via modulation of extracellular matrix and inflammatory responses [53,100].

• Human PPAR-β/δ *(PPARD)* gene polymorphism

According to Giordano and Desvergne [84], 90 single nucleotide polymorphisms have been identified in humans, of which 21 have been studied. It should be noted, however, that none of the polymorphisms identified to date has been localized within the coding sequence; they occur in untranslated regions, promoter sequences, intron sequences or as a synonymous codon [84]. More surprisingly, specific human *PPARD* gene variants show little or no association with CVD. However, a few studies reported that single nucleotide polymorphisms of PPAR-β/δ had variable effects on metabolic disease and lipid profiles.

Molecular & biological functions of PPAR-γ

PPAR-γ is highly expressed in adipose tissue, where it plays an essential role in the regulation of ADD, survival and function, insulin sensitivity, lipogenesis, lipid storage, glucose metabolism and the transcriptional regulation of a number of genes involved in these metabolic processes [52,101–104]. Two PPAR-γ isoforms, PPAR-γ1 and PPAR-γ2, have been identified in mouse, whereas in humans and in monkeys, in addition to PPAR-γ1 and PPAR-γ2, another isoform PPAR-γ4 is also expressed [56,69]. These isoforms are the protein products of seven mRNA transcripts (*PPAR-γ1*, *PPAR-γ2*, *PPAR-γ3*, *PPAR-γ4*, *PPAR-γ5*, *PPAR-γ6* and *PPAR-γ7*) generated through different initiation and alternative splicing of five exons at the 5′-terminal region (A1, A2, B, C and D). *PPAR-γ1*, *PPAR-γ3*, *PPAR-γ5* and *PPAR-γ7* mRNA transcripts translate into the identical PPAR-γ1 protein. *PPAR-γ2* mRNA yields PPAR-γ2 protein, while *PPAR-γ4* and *PPAR-γ6* mRNA transcripts produce identical PPAR-γ4 protein. PPAR-γ1 is expressed at the highest level in brown and white adipose tissues, but low-to-moderate levels also occur in other tissues, including vasculature, where it exerts cell-specific functions. Under normal physiological conditions, the longer PPAR-γ2 isoform (the NH2-terminus of PPAR-γ2 contains additional amino acids, 30 in mouse and 28 in human) is restricted to brown and white adipose tissues only, but its expression is ectopically induced in the liver and skeletal muscle in response to excess calorie intake or genetic obesity. The least studied PPAR-γ4 is expressed in macrophages and adipose tissue [56,69]. A diverse spectrum of naturally occurring endogenous fatty acids and their metabolites, including saturated, monounsaturated and polyunsaturated fatty acids, 15-(S)-hydroxyeicosatetraenoic acid, 9-hydroxyoctadecadienoic acid, 13-hydroxyoctadecadienoic acid and 15-deoxy-$\Delta^{12,14}$-PGJ2, binds to activate PPAR-γ **(Table 2)**. PPAR-γ is also the target of high-affinity synthetic antidiabetic TZDs, rosiglitazone and pioglitazone, which are currently on the market to treat T2DM.

• Regulatory roles of PPAR-γ in metabolism

The two drugs of the TZD class, rosiglitazone (Avandia®) and pioglitazone (Actos®), which function as potent and selective PPAR-γ full agonists, are not only highly effective antidiabetic drugs but have also greatly aided in understanding the underlying mechanisms by which PPAR-γ contributes to the regulation of adipogenesis, lipid and glucose homeostasis and other pathophysiological processes. In humans, pioglitazone and rosiglitazone function as insulin sensitizers and thus, enhance insulin action and improve hyperglycemia in patients with T2DM [62,68,102–109]. Similar beneficial actions of these two TZDs have also been observed in various relevant rodent models [62,68,102–109]. Likewise, several PPAR-γ agonists have been shown to effectively lower elevated plasma free-fatty acid levels, improve excessive lipid accumulation in peripheral tissues such as liver, skeletal muscle and heart and hyperinsulinemia/insulin resistance and modulate the expression of adipokines and inflammatory cytokines that impact hepatic and muscle metabolism and whole-body insulin sensitivity [102]. Besides attenuating hyperglycemia and enhancing insulin action, pioglitazone or rosiglitazone treatment of patients with T2DM is associated with significant improvements in plasma triglycerides, HDL-C, LDL particle concentration and LDL particle size [108–111].

• PPAR-γ regulation of adipocyte metabolism

PPAR-γ is highly expressed in adipocytes and is a primary regulator of adipogenesis, a process by which precursor preadipocytes differentiate to fully mature adipocytes [102,104–105,112]. During this precisely ordered process, the preadipocytes undergo a growth arrest, initiate accumulation of lipid (triglycerides) in the form of lipid

droplets and assume morphologic and biochemical characteristics of mature adipocytes such as hormone-sensitive metabolic processes including lipogenesis, lipolysis and glucose metabolism [102,104–105,112]. The adipocytes also secrete a variety of hormones and factors including cytokines, chemokines and other biologically active molecules commonly referred to as adipokines [113]. Although PPAR-γ is a master regulator of adipogenesis, during ADD, PPAR-γ works in concert with the other major adipogenic transcription protein, CCAAT/enhancer-binding protein (C/EBP) family, C/EBPα, C/EBPβ and CEBPδ [102,104,111]. In addition, PPAR-γ expression and function are also positively regulated by other transcription factors during differentiation, including sterol-regulatory element-binding transcription factor-1 (also known as ADD1), Krüeppel-like factor-5 (KLF5), KLF9 and KLF15, Zinc finger protein-423 and Early B-cell factor-2 [114]. In contrast, KLF2 and GATA-binding proteins, GATA2 and GATA3, negatively regulate PPAR-γ expression during adipogenesis [114]. PPAR-γ is also involved in the regulation of lipogenesis, regulation of insulin sensitivity and adipocyte survival and function [52,102,104–105]. Activation of PPAR-γ in adipose tissue leads to induction of an array of genes whose protein products mediate cellular triglyceride catabolism, and fatty acid uptake, intracellular transport and storage, adipogenesis, lipogenesis and fatty acid oxidation, as well as glucose metabolism **(Supplementary Box 4)**. Genes of several proteins involved in adipose tissue glucose metabolism have also been identified as targets of PPAR-γ including adipocyte PEPCK, glycerol-3-kinase,

Table 2. Partial list of endogenous and synthetic peroxisome proliferator-activated receptor γ agonists.

Endogenous ligand	Synthetic PPAR-γ agonists	Dual PPAR-α/γ agonists	Pan PPAR-α/βδ/γ
Fatty acids			
Palmitic acid	Rosiglitazone	Muraglitazar	Agonists
Erucic acid	Pioglitazone	Tesaglitazar	Chiglitazar
Oleic acid	Troglitazone	Farglitazar	Netoglitazar
Petroselinic acid	Ciglitazone	Ragaglitazar	Sodelglitazar
Linoleic acid	CDDO	Naveglitazar	Indeglitazar
α-Linolenic acid	GW1929	Imiglitazar	Sipoglitazar
γ-Linoleic acid	Indomethacin	Saroglitazar	–
Lauric acid	Fenoprofen	Aleglitazar	–
Arachidonic acid	Ibuprofen	–	–
Docosahexaenoic acid	Flufenamic acid	–	–
Eicosapentaenoic acid	–	–	–
Palmitoleic acid	–	–	–
Eicosanoids			
8-(R)HETE	–	–	–
8-(S)HETE	–	–	–
15-HETE	–	–	–
9-(R/S)HODE	–	–	–
13-(R/S)HODE	–	–	–
13-(S)HpODE	–	–	–
9-oxoODE	–	–	–
13-oxoODE	–	–	–
15d-PGI_2	–	–	–
PGJ_2	–	–	–
$PGA_{1/2}$	–	–	–
PGB_2	–	–	–
azPC	–	–	–

15d-PGI_2: 15-Deoxy-$\Delta^{12,14}$-PGJ_2; azPC: Hexadecyl azelaoyl phosphatidylcholine; CDDO: 2-Cyano-3,12-dioxooleana-1,9-dien-28-oic acid; GW1929: (2*S*)-((2-Benzoylphenyl)amino-3[4-[2-(methylpyridin-2-ylamino)ethoxy]phenyl)-propionic acid; HETE: Hydroxyeicosatetraenoic acid; HODE: Hydroxyoctadecadienoic acid; HpODE: Hydroperoxyoctadecadienoic acid; oxoODE: Oxidized octadecadienoic acid; PGJ2: Prostaglandins J; PGA1/2: Prostaglandin A; PGB2: Prostaglandin B; PPAR: Peroxisome proliferator-activated receptor.

PDK4, which inhibits PDH, GK and PFKFB3. Additionally, PPAR-γ regulates the production of adipokines in adipocytes. Other studies suggest that adipose tissue is a major mediator of PPAR-γ action on insulin sensitivity and is essential for survival of adipocytes **(Supplementary Box 5)**.

• PPAR-γ regulation of metabolic functions in liver, skeletal muscle & heart

In contrast to adipose tissue, liver, skeletal muscle and heart express PPAR-γ protein only at low-to-moderate levels. However, under certain pathophysiologic conditions, the expression of PPAR-γ protein is significantly upregulated in these tissues. Several studies have provided evidence that expression of hepatic PPAR-γ is markedly upregulated in many models of obesity (both lipoatrophy and hyperphagic obesity), insulin resistance and diabetes with varying degree of steatosis. Increased expression of hypoxia-inducible factor (HIF-1α) and PPAR-γ is reported in ventricular biopsy samples of humans and mice with hypertrophic cardiomyopathy [115]. Further evaluation of mouse samples revealed that HIF-1α caused the induction of PPAR-γ gene expression. The cooperative functional interactions between HIF-1α and PPAR-γ subsequently lead to cardiac steatosis, apoptosis and heart failure [116]. In another study, patients with MetS and aortic stenosis was shown to express high levels of cardiac PPAR-γ, which was strongly correlated with the extent of cardiac lipid accumulation and compromised cardiac function [117] . Likewise, it has been reported that PPAR-γ gene expression is upregulated in skeletal muscle of obese subjects with T2DM [118]. To further evaluate the role of PPAR-γ in liver, skeletal muscle and heart, several laboratories generated and examined the metabolic characteristics of tissue-specific knockout and transgenic mouse models. Some of the key findings are summarized in **Supplementary Box 5**.

• Vasculature, inflammation & atherosclerosis

Although PPAR-γ is predominantly expressed in adipocytes, where it plays an essential role in the regulation of adipocyte biology and metabolism, it is also expressed at relatively high levels in various vascular cells, including endothelial cells, smooth muscle cells and monocyte/macrophages [42,44,46,48,100,118–121]. Extensive studies carried out during the past 15–20 years have led to the demonstration that PPAR-γ plays an integral role in the regulation of vascular homeostasis. Significant levels of PPAR-γ have also been detected in atherosclerotic lesions. Many studies have provided evidence that agonist-mediated PPAR-γ activation attenuates activation and inflammation, and this is achieved via several mechanisms **(Table 3)**. A number of studies also provide evidence for an inhibitory role of PPAR-γ in atherosclerosis and that it may exert atheroprotective effects. PPAR-γ agonists have been reported to attenuate atherosclerosis in genetically prone mouse models: $LDLR^{-/-}$ and the $ApoE^{-/-}$ [122–125] or intimal to medial ratio in human patients [126,127]. In another study, troglitazone treatment of male $LDLR^{-/-}$ mice previously maintained on a HFD or high-fructose diet significantly reduced atherosclerotic lesions [124]. None of these PPAR-γ agonists, however, had any significant effect in improving the atherosclerosis in female $LDLR^{-/-}$ mice. Likewise, administration of PPAR-γ agonists, rosiglitazone and GW7845 attenuated atherosclerotic lesions in male LDLR-null mice on the HFD. In keeping with the anti-inflammatory properties of PPAR-γ and TZDs, the aortas from these animals show decreased accumulation of macrophages in lesions and attenuated expression of some inflammatory markers such as TNF-α [122–124]. Further evidence in support of antiatherogenic actions of PPAR-γ came from studies aimed at delineating the effect of rosiglitazone treatment on mechanisms involved in the initial stages of atherosclerosis using high-cholesterol-fed rabbits as a test model [128]. Treatment with rosiglitazone enhanced the downregulated PPAR-γ expression, improved endothelium-dependent vasodilatation, suppressed $gp91^{phox}$ and iNOS expression and inhibited superoxide generation, total NO production and nitrotyrosine formation. It was suggested that endothelial protective effects of PPAR-γ agonists may attenuate leukocyte accumulation in the vascular wall, contributing to its antiatherosclerotic effects. PPAR-γ ligands reduced AngII-accelerated atherosclerosis in $LDLR^{-/-}$ mice [129]. In addition, it was shown that $LDLR^{-/-}$ who received bone marrow irradiation and were transplanted with PPAR-$\gamma^{-/-}$ bone marrow progenitor cells [130] developed more severe atherosclerosis as compared with $LDLR^{-/-}$ mice transplanted with wild-type progenitor cells [131].

• Human PPAR-γ *(PPARG)* gene polymorphism

Currently, two common (P12A and C161T) and a number of rare missense and nonsense

Table 3. Metabolic and vascular actions of peroxisome proliferator-activated receptor γ.

Actions	Positive regulation	Negative regulation
Metabolic actions		
Adipose tissue	↑ Adipogenesis; ↑ Adiponectin; ↑ Fatty acid storage; ↑ Insulin sensitivity; ↑ Lipogenesis; ↑ PAI-1; ↑ Resistin	↓ TNF-α
Liver	↑ Fatty acid storage	
Vascular actions		
VSMCs	↑ Apoptosis ↑ GADD45A ↑ IRF1 ↑ p27 ↑ Phospho-SMAD2 ↑ TGFB1 ↑ TP53	↓ Inflammatory genes ↓ PDGF-induced VSMC migration and proliferation ↓ ETS-1 ↓ NF-κB ↓ IL-1β induction of IL-6 gene expression ↓ Phosphorylation of STAT3 and C/EBP downregulation ↓ TNF-α-induction of *VCAM-1*, *MCP-1* and *CX3CL1* genes ↓ IL-1β-induced gene expression of IL-6 ↓ Proliferation; ↓ ROS
ECs	↑ Apoptosis	↓ Activation and inflammation ↓ AP-1 ↓ CXCL9; ↓ CXCL10; ↓ ICAM-1; ↓ VCAM-1 ↓ E-selectin ↓ EC inflammation; ↓ EC dysfunction ↓ Expression of of chemokine genes such as IP-10, I-TAC ↓ NF-κB and phosphorylation of NF-κB ↓ MHC-II; ↓ Monocyte adhesion ↓ Growth factor receptors Flt-1 and VEGFR-2 (Flk1/KDR)
Macrophages	↑ M2 macrophage markers (CCL17, mannose receptor, arginase-1, CD36) ↑ M2 macrophage activation ↑ Ox-LDL efflux	↓ AP-1 ↓ M1 macrophage activation ↓ M1 macrophage marker (TNF-α, IL-1β, IL-6) ↓ NF-κB ↓ OxLDL accumulation ↓ Pro-inflammatory cytokines TNF-α, IL-1β and IL-6 ↓ STAT1; ↓ STAT6

AP-1: Activator protein-1; CCL17: C–C motif chemokine ligand 17; C/EBP: CCAAT/enhancer binding protein; CXCL9: C-X-C motif chemokine ligand 9; CXCL10: C-X-C motif chemokine ligand 10; EC: Endothelial cell; GADD45A: Growth arrest and DNA damage inducible α; I-TAC: Interferon-inducible T-cell α chemoattractant; IRF1: Interferon regulatory factor 1; KDR: Kinase insert domain receptor; Ox-LDL: Oxidized LDL; PAI-1: Plasminogen activator inhibitor-1; PPAR: Peroxisome proliferator-activated receptor; ROS: Reactive oxygen species; SMAD2: SMAD family member 2; STAT: Signal transducer and activator of transcription; VEGFR2: VEGF receptor 2 for VEGF-A; VSMC: Vascular smooth muscle cells.

mutations in the coding region of the *PPARG* gene have been identified [132–135]. P12A is the most common cytosine to guanine (C→G) base exchange (CCA-to-CCA missense mutation in exon B) resulting in substitution of proline with alanine at position 12 (P12A; rs1801282; Ex4–49C>G) [132–135]. Another common polymorphism is a synonymous CT substitution at nucleotide position 161 in exon 6 (C161T; also referred to as C1431, CAC478CAT, His449His or His447His; rs3856806) [136]. The frequency of the 12Ala allele has been reported to vary from 2 to 28% in individuals of different ethnicity and a number of studies suggest that the P12A mutation is associated with a reduced risk of T2DM and diabetic nephropathy, improved insulin sensitivity, MetS and increased BMI (obesity). A number of studies have examined the effect of the C161T polymorphism primarily in the context of coronary heart disease (CHD) and associated MetS and T2DM. Some studies found no association between the C161T polymorphism and risk of CHD, while other studies provide evidence indicating that this polymorphism is associated with a decreased risk of CHD or plays a protective role in this disease. Another report shows that the C161 polymorphism of the *PPAR-γ* gene is associated with CHD and that CC genotype of this gene may increase the risk of heart disease. In addition, more than a dozen of mutations occurring in the coding region of human PPAR-γ have been described [135]. These familial

partial lipodystrophy type-3 mutations contain a single amino acid substitutions in the ligand-binding domain and the DNA-binding domain or nonsense and frame shift mutations that result in the truncation of the receptor protein [135]. Some of the ligand-binding domain mutants such as V290M, Y355X and P467L function as dominant negative mutants and repress the transcriptional activity of native PPAR-γ [135].

• Post-translational modifications of PPAR-γ receptor protein

It is well established that both endogenous ligands such as prostaglandin 15d-PGJ2 and synthetic antidiabetic TZD drugs such as rosiglitazone and pioglitazone induce transcriptional activity of PPAR-γ. In recent years, it is also becoming clear that PPAR-γ is also subject to regulation by post-translational modifications (PTMs), including phosphorylation, sumoylation, ubiquitination and acetylation [137–141]. Among these PTMs, the phosphorylation of PPAR-γ has been most extensively studied [139]. The activation function (AF1) region of PPAR-γ is phosphorylated by activated MAPKs at Ser82 of PPAR-γ1 and at Ser112 of PPAR-γ2, which in turn inhibits transcriptional activity of PPAR-γ by interfering with ligand binding and altering cofactor requirement [141]. Interestingly, phosphorylation of same serine residues by Cdk7 and Cdk9 activates PPAR-γ activity. In addition, PPAR-γ2 is also phosphorylated at Ser273 by human CDK5 and that CDK5-catalyzed phosphorylation of PPAR-γ2 inversely correlated with TZD-enhanced insulin sensitivity in humans [141]. Limited information further suggests that PPAR-γ can be activated/phosphorylated by activators of protein kinase A, protein kinase C and 5′-AMP-activated protein kinase [137]. Sumoylation of PPAR-γ2 at Lysine 107 in the AF1 region and at lysine 395 in the AF2 region (lysine 77 and lysine 365 in PPAR-γ1, respectively) enhances the PPAR-γ transcriptional activity by preventing the interaction corepressor HDAC3 with PPAR-γ [140]. As expected, ubiquitination of PPAR-γ results in increased degradation of PPAR-γ following its activation by TZDs [140]. PPAR-γ is acetylated by p300 or CBP or deacetylated by SIRT1 [140]. Recently, it was demonstrated that inhibition corepressor HDAC3 promoted ligand-independent activation of PPAR-γ by protein acetylation [140].

• Telmisartan as partial agonist of PPAR-γ

AngII type I (AT1) receptor is a member of the G protein-coupled receptor superfamily. AT1 receptor blockers (e.g., eprosartan, losartan, candesartan, valsartan, telmisartan, olmesartan, irbessartan and azilsartan), which act by selectively blocking the binding of AngII to AT1 receptor, are widely used in the treatment of hypertension [142–144]. Among these, telmisartan also functions as a partial agonist of PPAR-γ [145]. Because of these unique dual properties, a large number of studies have shown that telmisartan exerts beneficial effects on glucose and lipid metabolism in both humans and experimental animals [146–149].

Next-generation PPAR-β/δ & PPAR-γ agonists

Given that PPARs are prime drug targets, two classes of PPAR agonists, fibrates (PPAR-α) and TZDs (PPAR-γ), are in clinical use for several decades as medications to treat dyslipidemia and hyperglycemia in patients with T2DM. However, in view of the wide spread of T2DM, and related metabolic diseases such as MetS and NAFLD, and associated cardiovascular complications contain multiple clinical components, considerable efforts are underway worldwide with a goal to design, synthesize and characterize new highly potent and efficacious single, dual and pan PPAR-β/δ or PPAR-γ agonists. These efforts are aiming at developing these agonists into new drugs that will simultaneously treat two or more components of these metabolic diseases. In part I of this review [72], we described the developmental status of several dual PPAR-α/γ, PPAR-α/β(δ) and a selective PPAR-α modulator (SPPAR-αM). Here, we provide updates about the few PPAR-β/δ and PPAR-γ agonists. Clinical studies have demonstrated that MBX-8025 (CamaBay Therapeutics), a potent and selective agonist of PPAR-β/δ, is effective against homozygous familial hypercholesterolemia, and primary biliary cholangitis, formally referred to as primary biliary cirrhosis. MBX-8025 treatment also reduced the levels of triglyceride and LDL-C, while raising HDL-C. In addition, MBX-8025 impacts other components of MetS, including improvements in insulin sensitivity and trends toward decreased waist circumference and body fat [150–152]. This PPAR-β/δ agonist is currently under development. Another PPAR-β/δ agonist, CER002 (Cerenis Therapeutics,

MI, USA), is in Phase I clinical development for dyslipidemia in the USA. A selective PPAR-β/δ agonist (HPP593) [153] and a dual PPAR-α/β(δ) (GFT505) [152,154–156] are also currently under development. INT131 (formerly T0903131 or AMG131; InteKrin Therapeutics, Inc., CA, USA) is a potent non-TZD selective PPAR-γ modulator (SPPAR-γM) designed to improve glucose metabolism while minimizing the side effects of full PPAR-γ agonists [152,157–161]. Preclinical studies with INT131 demonstrated similar glucose lowering with significantly less fluid retention, weight gain and cardiomegaly than currently available TZDs in similar studies. The INT131 compound is in Phase II development in the USA and Mexico for the treatment of T2DM.

• Clinical trials to test the efficacy of pioglitazone & rosiglitazone in the prevention of CVD

A large number of trials of pioglitazone and rosiglitazone for CVD prevention have been carried out in the past few decades and the results are summarized in **Supplementary Box 6**. Some, but not all clinical evidence presented support a therapeutic potential of TZDs in the clinical management of CVD.

Conclusion

PPAR-α (NR1C1), PPAR-β/δ (NR1C2) and PPAR-γ (NR1C3) PPARs are ligand-activated transcription factors, which form a subfamily of the nuclear receptor superfamily. These PPARs heterodimerize with members of the RXRs and as such regulate target gene expression. PPAR-β/δ is expressed ubiquitously with highest expression of PPAR-β/δ protein in mouse detected in the GI tract including small intestine and colon, high-to-moderate levels in skin and brown adipose tissue, liver, kidney, lung and vasculature and low levels in heart, skeletal muscle, brain, thymus and other tissues. In general, the highest expression of PPAR-γ can be found in adipose tissue and colon followed by the kidney, liver and small intestine, whereas it is barely detectable in skeletal muscle. Of the splice variants, PPAR-γ1 is expressed at the highest level in brown and white adipose tissues, but low-to-moderate levels also occur in other tissues, including vasculature, where it exerts cell-specific functions. Under normal physiological conditions, PPAR-γ2 isoform is restricted to brown and white adipose tissues only, but its expression is ectopically induced in the liver and skeletal muscle in response to excess calorie intake or genetic obesity. The least studied PPAR-γ4 is expressed in macrophages and adipose tissue. Activators of PPAR-β/δ include a variety of endogenously present ligands as well as several synthetic agonists. Likewise, activators of PPAR-γ consist of a large number of endogenous ligands such as free fatty acids, eicosanoids and phosphatidylcholine analog hexadecyl azelaoyl phosphatidylcholine and synthetic agonists, including clinically used TZDs (pioglitazone, rosiglitazone) and telmisartan, an AT1 receptor blocker and partial agonist of PPAR-γ and various synthetic single, dual and pan agonists. Both PPAR-β/δ and PPAR-γ are also regulated by PTM via phosphorylation. In addition, there is experimental evidence that PPAR-γ is also regulated post-transcriptionally by sumoylation, ubiquitination and acetylation.

PPAR-β/δ is critically involved in the regulation of insulin sensitivity, adipogenesis, lipid and energy metabolism, inflammation and atherosclerosis. Many enzymes and proteins that participate in these various metabolic processes have been identified as a direct target of PPAR-β/δ. PPAR-γ plays an essential role in the regulation of ADD, survival and function, insulin sensitivity, lipogenesis, lipid storage, glucose metabolism and the transcriptional regulation of a number of genes involved in these metabolic processes. Currently, two common (P12A and C161T) and a number of rare missense and nonsense mutations in the coding region of the *PPARG* gene have been identified, some of which have been shown to exert modulatory effect on lipid and glucose metabolism. A large number of trials of pioglitazone and rosiglitazone for CVD prevention have been carried out in the past few decades. However, some, but not all clinical evidence presented support to a therapeutic potential of TZDs in the clinical management of CVD. At present, there are no PPAR-β/δ-targeted drugs in the market. However, a selective PPAR-β/δ agonist (HPP593), a dual PPAR-α/β(δ) agonist (GFT505) and an PPAR-α/β(δ)γ pan agonist (chiglitazar) are under various stages of development. Besides TDZs, pioglitazone and rosiglitazone, recently two new PPAR-α/γ dual agonists, saroglitazar and lobeglitazon, have been marketed in India and Korea, respectively, and are now in clinical use.

EXECUTIVE SUMMARY

- PPAR transcription factors, the master regulators of various metabolic pathways, contribute to the pathogenesis of metabolic diseases such as obesity, diabetes, metabolic syndrome (MetS), nonalcoholic fatty liver disease and cardiovascular disease. They also serve as drug targets for these metabolic diseases. Indeed, two classes of drugs, fibrates (PPAR-α agonist) and thiazolidinediones (PPAR-γ agonist), are extensively used in the clinical practice to improve dyslipidemia and hyperglycemia, respectively.
- Additional efforts are underway by many pharmaceutical companies around the globe to develop new single dual or pan PPAR agonists with a goal to develop new therapeutic agents for the treatment of MetS, Type 2 diabetes mellitus and associated cardiovascular complications and nonalcoholic fatty liver disease.

Molecular characteristics of PPAR-β/δ & PPAR-γ

- Human *PPARD* is localized at chromosomal region 6p21.2–21.1 and comprises nine exons, whereas human PPARG has nine exons and is localized on chromosome 3p25.
- PPAR-β/δ and PPAR-γ, like PPAR-α, form heterodimers with retinoid X receptors in the presence of specific ligands, bind to a consensus PPAR response element in the target DNA and modulate gene transcription.

Metabolic functions of PPAR-β/δ

- PPAR-β/δ is ubiquitously expressed in mouse, rat and human tissues.
- Experimental evidence provides evidence that PPAR-β/γ participates in the regulation of insulin sensitivity, adipogenesis, lipid and energy metabolism, inflammation and atherosclerosis.
- Many enzymes and proteins that participate in these various metabolic processes have been identified as a direct target of PPAR-β/δ.
- PPAR-β/δ has important functions in the adipose tissue, liver, skeletal muscle, heart, intestine and vasculature.

Human PPAR-β/δ *(PPARD)* gene polymorphism

- 90 single nucleotide polymorphisms have been identified in humans, of which 21 have been studied.
- These *PPARD* gene variants show little or no association with cardiovascular disease.

Metabolic functions of PPAR-γ

- PPAR-γ is highly expressed in adipose tissue, where it plays an essential role in the regulation of adipocyte differentiation, survival and function, insulin sensitivity, lipogenesis, lipid storage, glucose metabolism and the transcriptional regulation of a number of genes involved in these metabolic processes.
- There are two major isoforms of PPAR-γ: PPAR-γ1 and PPAR-γ2.
- PPAR-γ1 is expressed at highest level in brown and white adipose tissues, but low-to-moderate levels also occur in other tissues, including vasculature.
- Under normal physiological conditions, the expression of PPAR-γ2 isoform is restricted to brown and white adipose tissues only, but its expression is ectopically induced in the liver and skeletal muscle in response to excess calorie intake or genetic obesity.

PPAR-γ regulation of adipocyte metabolism

- PPAR-γ is highly expressed in adipocytes, and it is a primary regulator of adipogenesis. PPAR-γ also regulates adipose tissue lipid metabolism, glucose homeostasis and other metabolic processes.

PPAR-γ regulation of metabolic functions in liver, skeletal muscle, heart vascular tissues

- PPAR-γ also participate in the regulation of multiple metabolic processes in liver, skeletal muscle, heart vascular tissues.

Human PPAR-γ *(PPARG)* gene polymorphism

- Two common (P12A and C161T) and a number of rare missense and nonsense mutations in the coding region of the *PPARG* gene have been identified.
- P12A mutation is associated with a reduced risk of Type 2 diabetes mellitus and diabetic nephropathy, improved insulin sensitivity, MetS and increased BMI (obesity).

EXECUTIVE SUMMARY (CONT.)

Human PPAR-γ *(PPARG)* gene polymorphism (cont.)

- Some studies found no association between the C161T polymorphism and risk of coronary heart disease, while other studies provide evidence indicating that this polymorphism is associated with a decreased risk of coronary heart disease or plays a protective role in this disease.

Post-translational modifications of PPAR-γ receptor protein

- PPAR-γ is regulated post-transcriptionally by several mechanisms, including phosphorylation, sumoylation, ubiquitination and acetylation.

Next-generation PPAR-β/δ & PPAR-γ agonists

- Recently two new PPAR-α/γ agonists, saroglitazar and lobeglitazon, have been marketed in India and Korea, respectively.
- A selective PPAR-β/δ agonist (HPP593), a dual PPAR-α/β(δ) agonist (GFT505) and a PPAR-α/β(δ)γ pan agonist (chiglitazar) are under various stages of development.

Future perspective

Although PPARβ/δ is ubiquitously expressed in humans, this PPAR isotype is least studied in terms of its genetic and metabolic actions. Future efforts should be directed at using metabolic and genetic approaches to identify novel targets that can be exploited in the development of new PPARβ/δ single, double or pan agonists with high selectivity and sensitivity. This strategy may also help to minimize off-target activity. Besides, efforts should be taken to critically evaluate the events connected with the post-translational modification of PPARβ/δ and the information generated should be exploited in the development of PPARβ/δ agonists as new drugs to treat metabolic diseases. PPARγ is a target of the glitazone class of antidiabetic drugs, however, the use of these drugs to activate PPARγ is associated with weight gain, fluid retention and bone fracture and there is immediate need to develop new highly effective and specific PPARγ drugs. Given that PPARγ regulates a complex array of metabolic pathways through its interaction with coactivator and corepressor proteins, future efforts should be aimed at examining and exploiting cofactor biology to develop new PPARγ agonists with high efficacy and safety profiles. PPARγ, like PPARα, is also subject to post-translational modifications, including phosphorylation, sumoylation, ubiquitination and acetylation; future efforts should be devoted to develop new efficacious PPARγ agonists based on manipulating these post-translational modifications.

Acknowledgements

The authors thank K Morrow for his assistance in creating illustrations.

Financial & competing interests disclosure

This work was supported by the Office of Research and Development, Medical Service, Department of Veterans Affairs, and grants from the NIH (HL033881 and HL092473). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fca-2017-0019

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