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The role of PPARβ/δ in the management of metabolic syndrome and its associated cardiovascular complications

Elisa Benetti[1]*, Nimesh S. A. Patel[2]*, Massimo Collino[1]

*Both authors contributed equally to this work

[1] *Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, Turin, Italy*)

[2] *Centre for Translational Medicine and Therapeutics, Queen Mary University of London, The William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, UK*

Address all correspondence to:

Massimo Collino, Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, via P. Giuria 9, 10125 Torino, Italy. Tel: +39 011 6707955 Fax: +39 011 2367955

E-mail address: massimo.collino@unito.it

ABSTRACT

The association between metabolic syndrome and cardiovascular diseases raises important questions about the underlying pathological processes, especially for designing targeted therapeutic interventions. The Peroxisome Proliferators Activated Receptors (PPARs) are ligand-activated transcription factors that control lipid and glucose metabolism. Accumulating data suggest that PPARs may serve as potential targets for treating metabolic diseases and their cardiovascular complications. PPARs regulate gene expression by binding with RXR as a heterodimeric partner to specific DNA sequences, termed PPAR response elements. In addition, PPARs may modulate gene transcription also by directly interfering with other transcription factor pathways in a DNA-binding independent manner. To date, three different PPAR isoforms, designated α, β/δ and γ, have been identified. PPARα and PPARγ are the most extensively examined and characterized, mainly because they are activated by compounds, such as fibrates and thiazolidinediones, that are in clinical use for the treatment of hypertriglyceridemia and insulin resistance, respectively. In contrast the role of PPARβ/δ in metabolism has been less investigated. The recent availability of specific PPARβ/δ agonists revealed that PPARβ/δ plays a crucial role in fatty acid metabolism in several tissues. Besides, PPARβ/δ activation exerts beneficial effects against organ-related ischemic events, such as myocardial and cerebral infarction, which are among the most critical cardiovascular complications evoked by metabolic dysregulation. This paper reviews the evidence and recent developments relating to the potential therapeutic effects of PPARβ/δ agonists in the treatment of metabolic syndrome and its associated cardiovascular risk factors.

PPAR: an overview

Peroxisome Proliferator-Activated Receptors (PPARs) are members of the nuclear hormone receptor (NHR) superfamily of ligand-activated transcription factors. There are three PPAR subtypes: α, β/δ and γ, named also NR1C1, NR1C2 and NR1C3, respectively, according to the unified nomenclature of nuclear receptors (Nuclear Receptors Nomenclature Committee, 1999). The three isoforms are the products of distinct genes: the human PPARα gene was mapped on chromosome 22 in the general region 22q12–q13.1, the PPARγ gene is located on chromosome 3 at position 3p25, whereas PPARβ/δ has been assigned to chromosome 6, at position 6p21.1–p21.2 [31, 92, 119]. PPARs were originally identified by Isseman and Green [43] after screening the rat liver cDNA library with a cDNA sequence located in the highly conserved C domain of NHRs. The name PPAR is derived from the fact that activation of PPARα, the first member of the PPAR family to be cloned, results in peroxisome proliferation in rodent hepatocytes [22]. Activation of neither PPARβ/δ nor PPARγ, however, elicits this response and, interestingly, the phenomenon of peroxisome proliferation does not occur in humans [106]. The molecular basis for this difference between species is not yet clear. With respect to the PPARγ isotype, alternative splicing and promoter use results in the formation of two further isoforms: PPARγ1 and PPARγ2. In particular, differential promoter usage and alternate splicing of the gene generates three mRNA isoforms. PPARγ1 and PPARγ3 mRNA both encode the PPARγ1 protein product which is expressed in most tissues, whereas PPARγ2 mRNA encodes the PPARγ2 protein, which contains an additional 28 amino acids at the amino terminus and is specific to adipocytes [33]. PPARβ/δ was initially reported as PPARβ in Xenopus laevis and NUC1 in humans [87]. Subsequently, a similar transcript was cloned from mice and termed PPARδ [4]. Though now recognised as homologues for each other, it was not originally certain whether PPARβ from Xenopus was identical to murine PPARδ, hence the terminology PPARβ/δ.

All members of this superfamily share the typical domain organization of nuclear receptors (**Figure 1**). The N-terminal A/B domain contains a ligand-independent transactivation function. In the α and γ isotypes, the activity of this domain can be regulated by mitogen-activated protein kinase (MAPK) phosphorylation [40]. The C domain is the DNA binding domain with its typical two zinc-finger-like motifs, as previously described for the steroid receptors, and the D domain is the co-factor docking domain [89]. The E/F domain is the ligand binding domain, it contains a ligand-dependent trans-activation function (AF)-2 [26], and is able to interact with transcriptional coactivators such as steroid receptor coactivator (SRC)-1 [74] and CREB-binding protein (CBP) [4].

The highest PPARα expression has been found in the liver and in tissues with high fatty acid catabolism, such as the kidney, heart, skeletal muscle, and brown fat [60]. PPARα mainly regulates energy homeostasis, activating fatty acid catabolism and stimulating gluconeogenesis [49]. This increased fatty acid oxidation in response to PPARα activation with a selective agonist, WY14,643, results in lower circulating triglyceride levels and reduction of lipid storage in liver, muscle, and adipose tissue [13], which is associated with improved insulin sensitivity [50]. Consequently, fibrates (fenofibrate, bezafibrate, gemfibrozil), which are synthetic agonists for PPARα, are in wide clinical use for the treatment of dyslipidaemias.

PPARγ is expressed in white and brown adipose tissue, the gut, and immune cells [28]. It is involved in adipocyte differentiation and lipid storage in white adipose tissue [86]. Furthermore, PPARγ is involved in glucose metabolism via an improvement of insulin sensitivity [36]. Therefore, synthetic PPARγ agonists (thiazolidinediones) are in clinical use as insulin sensitizers to treat patients with type-2 diabetes.

PPARβ/δ remained an enigma for almost a decade after its cloning in 1992. It has been reported to be ubiquitously expressed in almost every tissue and, in the past, this widespread tissue expression has suggested a possible "general housekeeping" role for PPARβ/δ [52]. More recently, the use of transgenic mouse models and high-affinity synthetic ligands has led researchers to a better understanding of its physiological role. Specifically, increasing evidence has shown a particular role for PPARβ/δ in insulin sensitivity regulation, lipid metabolism and the inflammation response. However, in contrast to PPARα and γ, PPARβ/δ agonists are not yet in clinical use.

Endogenous and synthetic PPARβ/δ ligands

Although many fatty acids are capable of activating all three PPAR isoforms, some fatty acids are also specific for a particular PPAR isoform. X-ray crystallography studies of PPARβ/δ revealed an exceptionally large ligand-binding pocket of approximately 1,300 $Å^3$, similar to that of PPARγ but much larger than the pockets of other nuclear receptors [116]. The increased dimension is believed to accommodate the binding of various fatty acids or other amphipathic acids to PPARβ/δ via hydrogen bonds and hydrophobic interactions. The long-chain polyunsaturated fatty acids and their oxidized derivatives, especially eicosanoids such as 8-S-hydroxyeicosatetraenoic acid (8-S-HETE), leukotriene B4 (LTB4) and arachidonate monooxygenase metabolite epoxyeicosatrienoic acids have been shown to potently activate PPARα with high affinity [28, 102]. PPARγ can be activated by several prostanoids, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) and 12- and 15-hydroxy-eicosatetraenoic acid (12- and 15-HETE), which are derivatives of arachidonic acid synthesized through the lipoxygenase pathway, as well as modified oxidised lipids, 9- and 13-hydroxyoctadecadienoic acids (9- and 13-HODE) [102, 114]. PPARβ/δ agonists include linoleic acid, oleic acid, arachidonic acid and eicosapentaenoic acid (EPA), which have been shown to co-crystallize within the ligand binding domain of this nuclear receptor [116]. A number of eicosanoids, including prostaglandin (PG)A1 and PGD2, and carbaprostacyclin, a semi-synthetic prostaglandin, have micromolar affinities for PPARβ/δ [29]. Recently, cows milk, ice cream, butter, and yoghurt were described as activators of PPARβ/δ in reporter assays, but a specific common compound was not identified [97].

With respect to the synthetic ligands, while PPARα or γ agonists (fibrates and glitazones) are in clinical use for the treatment of hypertriglyceridemia and insulin resistance, respectively, there are no PPARβ/δ drugs in clinical use yet. However several selective PPARβ/δ ligands have been recently designed, including GW0742, GW2433, GW9578, L-783483, L-165041, or GW501516 [7, 64, 67]. As yet only one selective PPARβ/δ antagonist has been described GSK0660. In skeletal muscle myoblast cells in culture, GSK0660 inhibited GW0742 induction of established PPARβ/δ target genes (carnitine palmitoyltransferase 1A, angiopoietin-like 4 protein and pyruvate dehydrogenase kinase-4)[91].

Molecular mechanisms of PPARβ/δ activation

There are at least three primary mechanisms by which PPARβ/δ can regulate biological functions: transcriptional transactivation, transcriptional transrepression and ligand-independent transrepression (**Figure 2**).

Mechanisms of transcriptional transactivation

PPARβ/δ function as heterodimers with their obligatory partner – the Retinoid X Receptor (RXR). Like other NHRs, the PPAR/RXR heterodimer most likely recruits co-factor complexes - either co-activators or co-repressors - that modulate its transcriptional activity [93]. The PPAR/RXR heterodimer then binds to sequence specific PPAR Response Elements (PPREs), located in the 5'-flanking region of target genes, thereby acting as a transcriptional regulator [75]. The PPRE consists of two direct repeats of the consensus sequence AGGTCA separated by a single nucleotide, which constitutes a DR-1 motif. PPAR binds 5' of RXR on the DR-1 motif and the 5'-flanking sequence conveys the selectivity of binding between different PPAR isotypes [46]. In the absence of a ligand, to prevent PPAR/RXR binding to DNA, high-affinity complexes are formed between the inactive PPAR/RXR heterodimers and co-repressor molecules, such as nuclear receptor co-repressor or silencing mediator for retinoic receptors. In response to ligand binding, PPARβ/δ undergoes a conformational change, leading to release of auxiliary proteins and co-repressors and recruitment of co-activators that contain histone acetylase activity. Acetylation of histones by co-activators bound to the ligand-PPARβ/δ complex leads to nucleosome remodelling, allowing for recruitment of RNA polymerase II causing target gene transcription. The search for PPAR target genes with identified PPREs has led to the identification of several genes involved in lipid metabolism, oxidative stress and the inflammatory response, as widely documented in the literature.

Mechanisms of transcriptional transrepression

PPARβ/δ can also negatively regulate gene expression in a ligand-dependent manner by inhibiting the activities of other transcription factors, such as Activated Protein-1 (AP-1), Nuclear Factor-κB (NF-κB) and Nuclear Factor of Activated T cells (NFAT) (ligand-dependent transrepression). In contrast to transcriptional activation, which usually involves the binding of PPARs to specific response elements in the promoter or enhancer regions of target genes, transrepression does not involve binding to typical receptor specific response elements [76]. Several lines of evidence suggest that PPARs may exert anti-inflammatory effects by negatively regulating the expression of pro-inflammatory genes. To date, several mechanisms have been suggested to account for this activity, but despite intensive investigation, unifying principles remain to be elucidated.

Firstly, competition for limited amounts of essential, shared transcriptional co-activators may play a role in transrepression. *In vitro* studies have revealed a ligand type-specific direct interaction of PPARs with several transcriptional co-activators, such as SRC-1, TIF2, AIB-1, CBP, p300, TRAP220, and DRIP205 [53]. The activated PPAR/RXR heterodimer reduces the availability of co-activators required for gene induction by other transcriptional factors. Thus, without distinct co-factors, transcription factors cannot cause gene expression.

Secondly, PPAR/RXR complexes may cause a functional inhibition by directly binding to transcription factors, preventing them from inducing gene transcription [14]. Ding et al. reported that the PPARβ/δ agonist GW0742 reduced the LPS-induced degradation of the inhibitory protein "Inhibitor of kappa B" (IκB), resulting in the suppression of the transcription factor NF-κB activity [25]. Planavilla et al. reported that PPARβ/δ activation induces the physical interaction between PPARβ/δ and the p65 subunit of NF-κB, thus preventing NF-κB activation [78]. On the other hand,

NF-κB activation down-regulated PPARβ/δ activity during cardiac hypertrophy, possibly through protein-protein interaction between PPARβ/δ and subunit p65 of NF-κB [78]. Therefore, NF-κB and PPARβ/δ have been shown to be negative regulators of each other. There is also evidence that PPARβ/δ can interfere with STAT3 (Signal Transducer and Activator of Transcription 3) [51], thus contributing to the anti-inflammatory activities associated with PPARβ/δ and its ligands

Thirdly, PPAR/RXR heterodimers may also inhibit phosphorylation and activation of several members of the mitogen-activated protein kinase (MAPK) family. In general very little is known about the molecular mechanisms by which PPARβ/δ and its ligands modulate kinase activities. Recently, Meissner and colleagues have shown that PPARβ/δ agonists reduced the phosphorylation of the MAPK isoforms ERK1/2 and p38, which are known to be involved in the increase of cytokine IL-8 mRNA stability, in human endothelial cells [70] and this inhibition resulted in a significant suppression of cytokine expression. PPARβ/δ agonist have been also demonstrated to enhance mouse embryonic stem cells proliferation through p38 MAPK modulation [44] and myocardial ERK1/2 MAPK phosphorylation is affected by PPARβ/δ deficiency in knockout mice subjected to a chronic high-fat diet [63].

Recent studies have suggested another mechanism based on co-repressor-dependent transrepression by PPARs. Evidence has been presented in which PPARβ/δ controls the inflammatory status of macrophages based on its association with the transcriptional repressor BCL-6 (B Cell Lymphoma-6) [58]. BCL-6 belongs to a group of transcription factors that act as a sequence-specific repressor of transcription through recruitment of a silencing mediator for retinoid and thyroid hormone receptors and histone deacetylase-containing complex. The generation of BCL-6 deficient mice has revealed that this factor not only exert a critical role in lymphocyte differentiation, but may also act as a negative regulator of inflammation [20]. Indeed, along with many other immunological defects, BCL-6 deficient mice develop a profound inflammatory disease characterized by tissue infiltration of activated eosinophils, macrophages and T-helper type 2 cells. Besides, expression of genes encoding for chemokines and cytokines has been demonstrated to be regulated by BCL-6, that can suppress their expression either directly and indirectly, by interaction with other transcriptional factors [21]. Recently, BCL-6 overexpression in pancreatic β-cells has been demonstrated to prevent cytokine-induced inflammation in a dose-dependent manner via reduced NF-κB activation, Fas and iNOS expression as well as NO production [41] . BCL-6 expression has also been associated with terminal differentiation, and it has been proposed as antiapoptotic in mouse myocytes [56]. Interestingly, PPARβ/δ, but not PPARα and PPARγ, exhibits BCL-6 binding ability [5, 99]. In the absence of a ligand, PPARβ/δ sequesters BCL-6 reducing its free amount. The PPAR:BCL-6 complex prevents BCL-6 from binding to its response element and therefore inhibits its ability to repress proinflammatory genes [79]. In contrast, in the presence of ligand, PPARβ/δ releases the repressor, which thus exerts its anti-inflammatory effects by repressing transcription from these genes. Ravaux et al., have showed a positive cooperation between PPARβ/δ and BCL-6 in rat vascular smooth muscle cells and in absence of PPAR ligand, this association has been reported to prevent BCL-6 ability to repress phospholipase A2 transcription [79]. Similarly, selective PPARβ/δ ligands have been shown to exert potent anti-inflammatory effects in vascular endothelial cell by reducing the amount of intracellular BCL-6 associated with PPARβ/δ and, thus, increasing the amount of free Bcl-6 to repress the expression of inflammatory genes normally suppressed by Bcl-6. Similar gene expression changes were also seen in mouse peritoneal macrophages where treatment with the selective PPARβ/δ ligand GW0742 resulted in increased amount of free antiinflammatory transcriptional repressor Bcl-6 [99]. In a mouse model of streptozotocin-

induced diabetic nephropathy PPARδ activation has been demonstrated to exert protective effects by increasing the expression of BLC-6, with subsequent suppression of several inflammatory mediators [69]. Overall, these experimental data clearly demonstrate that PPARβ/δ ligands exert synergetic control of different mechanisms of transcriptional transrepression mediated by both PPAR β/δ and BCL-6-activation, thus resulted in marked antiinflammatory effects.

Post-transcriptional modifications include phosphorylation, ubiquitinylation, and sumoylation of PPARs [28]. With respect to PPARβ/δ it is interesting to note that Protein Kinase C (PKC) activation can induce PPARβ/δ expression [100], which plays an important role in inflammation. Regulation of PPARβ/δ by ubiquitinylation seems to depend largely on its expression levels as it has been shown that under conditions of moderate expression, GW501516 (PPARβ/δ ligand) does not significantly influence ubiquitinylation and degradation of PPARβ/δ. Over-expression of the receptor induces its ubiquitinylation and promotes degradation, which is counteracted by GW501516 [82]. This shows an additional control mechanism for PPARβ/δ expression levels and suggests that the synthetic agonist is not only functioning as an activator of the receptor, but also contributes to maintain high expression levels of PPARβ/δ.

Ligand-independent transrepression

PPARs may repress the transcription of direct target genes in the absence of ligands (ligand-independent repression). PPARs bind to response elements in the absence of any ligand and recruit co-repressor complexes that mediate active repression. The co-repressors are capable of fully repressing PPAR-mediated transactivation induced either by ligands or by cAMP-regulated signalling pathways. This suggests co-repressors as general antagonists of the various stimuli inducing PPAR-mediated transactivation. Co-repressors can display different ligand selectivity: the nuclear receptor co-repressor NCoR interacted strongly with the ligand-binding domain of PPARβ/δ, whereas interactions with the ligand-binding domains of PPARγ and PPARα were significantly weaker [55].

Very recently, a team of Harvard Medical School researchers has shown that PPARγ is phosphorylated at Ser273 by cyclin dependent kinase 5 (CDK5) during obesity which results in deregulation of a subset of genes; including a number of key metabolic regulators, such as adipsin, the first fat cell-selective gene whose expression is altered in obesity and adiponectin, a central regulator of insulin sensitivity *in vivo* [12]. Ser273 phosphorylation did not alter the chromatin occupancy of PPARγ, suggesting that other mechanisms, such as differential recruitment of co-regulators, may cause these differences in target gene expression. PPARγ ligands inhibited Ser273 phosphorylation and reversed associated changes in gene expression. Critically, the extent to which PPARγ ligands inhibit CDK5-mediated phosphorylation of PPARγ is not correlated with the extent to which they exert PPAR agonism, suggesting that these compounds have two distinct and separable activities. Whether or not similar mechanisms of receptor phosphorylation lead to changes in gene expression also in the other two PPAR isoforms alpha and beta/delta is a very important question, so far not yet addressed.

The role of PPARβ/δ in the metabolic syndrome: effects in adipose tissue and skeletal muscle

Ever since the metabolic syndrome (MetS) was first described by Reaven in 1988 [80], a number of definitions have been published by organizations including the National Cholesterol Education Program (NCEP) [1] and the World Health Organization [2], among others. Of these, the 2001 Third Report of the NCEP's Adult Treatment Panel has emerged as the most widely used definition, primarily because it provides a relatively simple approach for diagnosing the MetS by employing easily measurable risk factors. Specifically, the NCEP defines the MetS as having 3 or more of the following 5 cardiovascular risk factors: i) central obesity (waist circumference: men >102 cm; women >88 cm); ii) elevated triglycerides ($\geq$150 mg/dl); iii) diminished high-density lipoprotein (HDL) cholesterol (men <40 mg/dl; women <50 mg/dl); iv) systemic hypertension ($\geq$130/$\geq$85 mm Hg); and v) elevated fasting glucose ($\geq$110 mg/dl). In 2004, this NCEP definition was revised (rNCEP) by lowering the threshold for fasting glucose to $\geq$100 mg/dl in concordance with American Diabetes Association criteria for impaired fasting glucose [32]. Also, thresholds for central obesity were lowered from strictly >102 cm in men and 88 cm in women to greater than or equal to these values.

Accumulating evidence demonstrates that PPARβ/δ is an important modulator of the MetS and may be a therapeutic target for treating some of its features, including cardiovascular complications. PPARβ/δ has been identified as a key regulator of adipocyte proliferation and differentiation and cholesterol homeostasis. The first biological effect described for a PPARβ/δ agonist was an increase in serum high density lipoprotein cholesterol (HDLc) in diabetic mice following administration of L165041 [61]. An increase in serum HDLc was also found in obese non-human primates administered the potent PPARβ/δ ligand, GW501516 [73] and in wild-type mice treated with GW610742 [73]. Treatment of insulin-resistant obese rhesus monkeys with GW501516 resulted in a dose-dependent increase in HDLc and decrease in low density lipoprotein cholesterol (LDLc), fasting triglycerides and fasting insulin levels [73]. Obese and nonobese mice similarly develop an increase of up to 50% in HDL cholesterol levels when treated with PPARβ/δ agonists [108]. The mechanism by which PPARβ/δ activation raises HDL cholesterol levels remains to be elucidated,I In mice knockout for the expression of the reverse cholesterol transporter ATP-binding cassette A1 (ABCA1), this increase was not observed, thus supporting an essential role of ABCA1 in this event. Similarly treatment of human macrophages, fibroblasts, and intestinal cells with a selective PPARβ/δ agonist, GW501516, significantly increases ABCA1 level and improves reverse cholesterol transport [61]. In addition PPAR β/δ activation has been shown to reduce intestinal expression of Niemann-Pick C1-like 1 (NPC1L1), a critical element for the uptake of cholesterol across the plasma membrane of the intestinal enterocytes [108]. Studies in obese mice have shown that PPARβ/δ activation increased the expression of enzymes catabolizing fatty acids in skeletal muscle, and significantly decreased serum glucose concentrations [101, 110]. Mice fed a high fat diet exhibited an improvement in insulin sensitivity in response to PPARβ/δ ligand activation, and, interestingly, this effect was not found in PPARβ/δ-null mice [59]. Similarly, PPARβ/δ activation for three months in diabetic db/db mice reduces glycemia in association with improved insulin sensitivity and improved islet function [115]. When dosed for 4 weeks to insulin-resistant obese rhesus monkeys, GW501516 normalized fasting glucose and insulin, increased high-density lipoprotein-cholesterol and reduced low-density lipoprotein-cholesterol [73]. The beneficial effects of PPARβ/δ agonism on lipid metabolism and hepatic fatty acid oxidation was also recently confirmed in mice fed a Western-type high fat diet and treated with a single oral dose of the PPARβ/δ agonist GW501516 [6]. Besides, in vivo experiments in mice with increased PPARβ/δ expression and activity by adenovirus mediated gene delivery further support the pivotal role of PPARβ/δ on energy substrate homeostasis [65]. However, it must be noted that effects of PPARδ agonists on glucose and lipid metabolism can be heterogeneous in different rodent species, as recently suggested by a recent study showing a worsening of muscle insulin resistance in rats but improved insulin action in mice exposed to the same chronic high-fat diet [117]. Several lines of evidence have established that PPARβ/δ plays a central role in the control of lipid metabolism of skeletal muscle, where it is highly expressed [11]. For instance,

fasting upregulates PPARβ/δ expression in mouse skeletal muscle [38] and PPARβ/δ activation in L6 rat myotubes increases the expression of uncoupling protein 3 (UCP3), which is involved in energy metabolism by uncoupling electron transport from adenosine triphosphate synthesis in mitochondria [95]. Several studies have suggested that PPARβ/δ can control skeletal muscle oxidative capacity and slow fiber-type formation in mice [66, 101]. Wang and colleagues have demonstrated that PPARβ/δ is the first transcription factor able to drive the formation of functional type I muscle fibers and that an increase of PPARβ/δ activation, rather than of its expression levels, is an essential element for fiber switching [111]. Mice in which PPARβ/δ is selectively ablated in skeletal muscle myocytes exhibit a functional switch of the skeletal muscle fiber type toward lower oxidative capacity that precedes the appearance of obesity and insulin resistance, thus demonstrating that PPARβ/δ is instrumental in myocytes for the maintenance of oxidative fibres and that fibre-type switching is likely to be the cause and not the consequence of these metabolic disorders [88]. Mice with increased oxidative fibers are resistant to high-fat-induced obesity and have a dramatically improved exercise profile; the combination of ligand activation of PPARβ/δ with exercise increases the number of oxidative myofibers in muscle, resulting in enhanced exercise performance [72]. These observations suggest that PPARβ/δ ligands could be developed to improve athletic performance and this point raises ethical questions regarding the potential for the illicit use of PPARβ/δ agonists. Since January 2009, the list of prohibited substances and methods of doping as established by the World Anti-Doping Agency includes the PPARβ/δ agonist GW501516, which is categorized as a gene doping substance due to its capability to upregulate genes associated with oxidative metabolism and a modified substrate preference that shifted from carbohydrate to lipid consumption [103].

PPARβ/δ activation and cardiovascular complications of the MetS

Individuals with MetS have an increased burden of cardiovascular disease (CVD). Although the presence of any of the five cardinal features of MetS in an individual is a risk factor for cardiac morbidity, the presence of three or more is associated with up to 50% greater risk of major coronary events [30]. In the Kuopio Ischemic Heart Disease study, Lakka et al. [57] reported a 4.26-fold relative risk for mortality due to heart disease and a 1.77 relative risk for all-cause mortality in men with the MetS. In the Botnia study the risk for coronary heart disease (CHD) and stroke was shown to be increased threefold and the risk for cardiovascular mortality was increased sixfold in individuals with MetS [42]. The Hoorn Study examined 615 men and 749 women aged 50 to 75 years without diabetes or a history of CVD at baseline and reported that the NCEP-ATP III definition of MetS was associated with about a twofold increase in age-adjusted risk of fatal CVD in men and nonfatal CVD in women [107].

The pathophysiological mechanism by which the MetS increases cardiovascular risk remains under debate. Several studies have suggested a central role for insulin resistance that progresses toward hyperinsulinemia and hyperglycemia, thus triggering peripheral vasoconstriction and sodium retention. Hepatic production of very low-density lipoprotein also increases, leading to hypertriglyceridemia, low HDL cholesterol, elevated apolipoprotein B, elevated small LDL cholesterol, and consequently, atherosclerosis. As a result of these lipid imbalances, individuals with MetS typically exhibit a prothrombotic state.

Studies published during the past decade have convincingly demonstrated a pathophysiological role for the inflammatory response in the development of both MetS and related CVD [112]. The finding a little over a decade ago

that tumour necrosis factor-α (TNF-α) is overexpressed in the adipose tissue of obese mice provided the first clear link between obesity and chronic inflammation [39]. In obese mouse models a lack of TNF-function results in improved insulin sensitivity and glucose homeostasis, confirming that this inflammatory response has a critical role in the regulation of insulin activity in obesity [105]. TNF-α is also overexpressed in the adipose and muscle tissues of obese humans, and when administered exogenously leads to insulin resistance [54]. In addition to TNF-α, various other inflammatory mediators and cytokines are also overexpressed in adipose and other tissues in experimental mouse models of obesity and in humans. C-Reactive Protein (CRP) is an acute-phase reactant and a very sensitive marker of inflammation and numerous studies have now confirmed that CRP levels are elevated in individuals with MetS. Recently CRP has emerged as one of the most powerful predictors of cardiovascular risk. In a direct comparison of several inflammatory and lipid markers in predicting cardiovascular events in adults, CRP surpassed other classical risk markers, including LDL cholesterol, at least in some, though not in all, studies [81].

Recently published data suggest that PPARβ/δ can regulate inflammatory pathways involved in CVD development through various mechanisms and PPARβ/δ agonist-induced organ protection seems to be specific for injuries in which inflammation is the main cause of cell damage **(Figure 3)**. For instance, PPARβ/δ agonists exert protective effects against the activation of leukocyte-endothelial cell interactions in *in vivo* mouse models of lipopolysaccharide (LPS)-induced pulmonary inflammation and cremasteric microcirculation [77], with a significant reduction in leukocyte rolling, adhesion, and emigration. Interestingly, the decrease in adhesive interactions evoked by the PPARβ/δ agonists was associated with inhibition of adhesion molecule expression. Leukocyte infiltration and the related release of proinflammatory cytokines are known to significantly contribute to the tissue injury evoked by reperfusion of ischemic organs and events of organ-related ischemia/reperfusion (I/R) injury, such as myocardial and cerebral infarction, are among the most critical cardiovascular complications evoked by metabolic disorders. We and others have demonstrated that ligand activation of PPARβ/δ evoke organ protection in experimental models of myocardial, renal, intestinal and lung I/R injury by disrupting multiple levels of the inflammatory cascade [17, 24, 47, 62, 120]. Similarly, selective PPARβ/δ genetic deletion has been demonstrated to result in both increased kidney dysfunction and brain infarction in mice [62, 118]. We have also reported that PPARβ/δ knock-out mice exhibited much greater cardiac dysfunction, renal dysfunction, hepatic injury and lung inflammation caused by endotoxemia than wild-type counterparts [48].

Many of the observed beneficial effects of the PPARβ/δ agonists against an excessive inflammatory response seem to be due mainly to modulation of the pro-inflammatory transcription factor NF-κB **(Figure 3)**. We recently demonstrated that the nuclear translocation of the p65 NF-κB subunit evoked by an endotoxic shock was prevented in the heart of mice treated with the PPARβ/δ agonist GW0742. In addition, endotoxemia resulted in the upregulation of a NF-κB target gene iNOS, which was attenuated by drug treatment. Similarly, PPARβ/δ activation has been reported to inhibit lipopolysaccharide-induced IL-6 expression in mouse adipocytes by preventing NF-κB activation [85] and cytokine release in lung, liver and cecum of rats subjected to polymicrobial sepsis [121]. Furthermore, PPARβ/δ activation inhibits adhesion molecule expression and MCP-1 secretion in endothelial cells by inhibiting NF-κB translocation to the nucleus and significantly reduce the induction of the NF-κB target genes MCP-1 and TNF-α in human cardiac cells stimulated by palmitate and in the heart of mice fed a high-fat diet [3, 84]. NF-κB signaling pathway is one of the most important signal transduction pathways involved in the hypertrophic growth of the myocardium and may also be involved in the down-regulation of fatty acid oxidation. In rat cardiomyocytes, GW0742 [25] and L-165041 [78] inhibit the LPS-induced expression of TNF-α and MCP-1; and this effect was associated with the inhibition of NF-κB. Therefore, activation of PPARβ/δ cardiomyocyte inhibits NF-κB signaling pathway avoiding both hypertrophy and

downregulation of genes involved in fatty acid metabolism. The mechanism of inhibition may involve enhanced protein–protein interaction between this PPAR subtype and the p65 subunit of NF-κB. Cheng et al. indicate that PPARβ/δ is essential for maintaining basal cardiac function, possibly through maintenance of basal myocardial fatty acid oxidation and that ablation of PPARβ/δ in mice caused cardiac dysfunction, progressive myocardial lipid accumulation, cardiac hypertrophy and congestive heart failure with reduced survival [9]. The same authors confirmed that PPARβ/δ is highly expressed in the nuclei of neonatal and adult cardiomyocytes, suggesting that it may play a key role in cardiac energy balance and may serve as a "sensor" of fatty acid of other endogenous ligands in controlling fatty acids oxidation levels in the hearts under normal and pathological conditions [10].

In addition to NF-κB, another transcription factor, STAT3 is known to play an important role in cell survival/apoptosis, proliferation, and inflammatory responses [109, 113] in part at least by inducing the Suppressor Of Cytokine Signalling (SOCS)-3 pathway. SOCS proteins are involved in both inflammation and insulin resistance. This family of proteins is characterized by their ability to cause feedback inhibition of IL-6 and TNF-α activity and the isoform SOCS-3 has been reported to be highly expressed in the livers of obese animals [104]. Furthermore, it has been demonstrated that SOCS-3 binds to the insulin receptor in the liver and prevents the coupling of the Insulin Receptor Substrate-2 (IRS-2) with the insulin receptor, thus reducing insulin sensitivity [27]. In a non-genetic insulin-resistant animal model with rats fed a high cholesterol-fructose diet, we demonstrated that the insulin resistance evoked by the dietary manipulation was accompanied by a drastic increase in hepatic SOCS-3 mRNA levels [15]. In an *in vivo* model of renal I/R injury in streptozotocin-induced diabetic rats, we demonstrated that administration of the selective PPARβ/δ agonist GW0742 attenuated the renal dysfunction, leukocyte infiltration and formation of IL-6 and TNF-α. These effects were due, at least in part, by an increased expression of SOCS-3, thus suggesting that SOCS-3 up-regulation evoked by PPARβ/δ activation may generate a signal that suppresses the production of IL-6 and TNF-α [17].

Reduced inflammatory response evoked by PPARβ/δ activation has been suggested to be also due, at least in part, to a nongenomic activation of the Akt signaling pathway. Akt is a member of the Phosphoinositide 3-kinases (PI3Ks) signal transduction enzyme family, which regulates cellular activation, inflammatory responses, chemotaxis, and apoptosis [8]. More specifically, when phosphorylated by its upstream regulator, PDK, Akt modulates cell survival and growth [68]. Akt activation exerts an important role in inducing the inactivation of GSK-3β by phosphorylation of Ser9 [19, 71]. GSK-3β is a serine/threonine kinase that was originally recognized as a kinase that phosphorylates glycogen synthase. In contrast to most other kinases, GSK-3β is active in a resting cell state; however, it is inactivated upon phosphorylation of Ser9. Several studies have reported an association between GSK-3β and NF-κB activity [16, 18, 37]. For example, mice in which the gene for GSK-3β had been deleted exhibited a phenotype similar to that of mice lacking the genes involved in the activation of NF-κB, such as the p65 subunit and the IκB kinase, a key upstream regulator of the NF-κB pathway [98]. Moreover, treatment of TNF-α stimulated hepatocytes with a specific GSK-3β inhibitor resulted in a decrease of NF-κB-dependent gene transcription [90]. Recently, we and others have reported that selective PPARβ/δ agonists increase Akt phosphorylation and GSK-3β inhibition in experimental models of myocardial I/R injury [47, 120]. This observation was also confirmed in vitro models, including keratinocytes [23], human umbilical vein endothelial cells [45], endothelial progenitor cells [34] and lung cancer cell lines [35]. Overall, these findings support the view that PPARβ/δ activation phosphorylates, and hence, activates the Akt pathway, which in turn phosphorylates, and hence, inhibits GSK-3β, presumably resulting in the inhibition of NF-κB and, in turn, NF-κB dependent proinflammatory gene transcription.

Conclusions: PPARβ/δ and the link between metabolic diseases and cardiovascular complications

Reduced physical activity and poor diet are some of the major risk factors that can lead to what is now a global epidemic of obesity, insulin resistance, and type 2 diabetes mellitus. The treatment options for this global epidemic are lifestyle changes and medications. Most often lifestyle changes cannot treat or even cure metabolic diseases and their associated cardiovascular complications. In these situations pharmacological intervention is required to achieve recommended targets for LDL cholesterol, blood pressure, obesity and glycemic control. Although several pharmacological options exist for individual components of this cluster, effort is being devoted to develop agents with a wider range of beneficial effects against the aforementioned metabolic abnormalities and associated cardiovascular risk factors. Targeting the activation of PPARβ/δ for the treatment of MetS has thus far proved beneficial in pre-clinical studies with rodents. We have presented in this review that treatment with PPARβ/δ agonists may improve atherogenic dyslipidemia by reducing plasma triglyceride levels and enhancing plasma HDL-cholesterol levels. In skeletal muscle, PPARβ/δ ligands may also upregulate fatty acid transport and oxidation, which reduce fatty acid-induced inflammation and insulin resistance, and they prevent the activation of NF-κB in both the heart and liver by reducing the production of inflammatory cytokines through several pro-survival pathways such as the activation of Akt. Besides, PPARβ/δ activation exerts beneficial effects against organ-related ischemic events, such as myocardial and cerebral infarction, which are among the most critical cardiovascular complications evoked by metabolic dysregulation. The ability of PPARβ/δ agonists to target molecular and cellular pathways involved in both metabolic and cardiovascular diseases suggest that these selective ligands have the potential to exert synergistic effects in the control of MetS and its cardiovascular complications. Thus, they could decrease the burden of cardiovascular morbidity and mortality in MetS, although, to date, there are no clinical data to support this concept.

Overall, the relevance of the pre-clinical findings to human pathophysiology remains to be confirmed, although recently clinical efficacy of the selective PPARβ/δ agonist GW501516 has been evaluated in two small cohort studies of lean and overweight subjects. GW501516 (2.5 or 10 mg once daily for 2 weeks) has been demonstrated to reduce circulating TG and to prevent the decline of HDLc and apoA-I levels due to lack of physical activity in lean healthy subjects during metabolic ward conditions treated for two weeks [96]. Similarly, PPARβ/δ activation by GW501516 (10 mg once daily for 2 weeks) led to lowering of fasting and postprandial plasma triglycerides, LDLc and apoB in moderately obese men [83]. Reductions were also seen in liver fat content and urinary isoprostanes, the latter a marker of whole-body oxidative stress. In both studies, no toxicity was observed during the treatment period. Information about genetic polymorphisms in PPARβ/δ have also been provided and positive associations with fasting plasma glucose and BMI have been detected in healthy Korean subjects [94]. More extensive clinical studies are need not only to better clarify the efficacy, but also to see if this pharmacotherapeutic approach is free of unwanted side effects. However, the preliminary clinical data coupled with the positive animal model results point out selective PPARβ/δ agonists as a promising new class of molecules with beneficial effects for the treatment of MetS and the related cluster of multiple cardiovascular risk factors.

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Figure 1. Schematic representation of the domain organization of human PPAR isoforms. The A/B domain contains the Activation Function 1 (AF-1) which has a ligand-independent transcriptional activity. The C domain corresponds to the DNA Binding Domain (DBD). The D domain is the co-factor docking domain. The E/F domain contains the Ligand Binding Domain (LBD) and carries the Activation Function 2 (AF-2), which has a ligand-dependent transcriptional activity. The human chromosome regions in which disting genes encoding for PPAR isoforms are mapped, the percentage of amino acid sequence identity (in comparison with PPARα) and the amino acid number of different isoforms are reported in the Table.

Figure 2. Molecular mechanisms of PPARβ/δ activation. After ligand binding, PPARβ/δ undergoes conformational changes, which lead to recruitment of Retinoid X Receptor (RXR) and coactivators. The resultant heterodimer binds to specific DNA response elements called PPAR response elements, causing target gene transcription (Transactivation). A second mechanism (Transrepression) involves interfering with other transcription-factor pathways by negatively regulating the expression of pro-inflammatory genes. Lastly, PPARβ/δ may repress the transcription of direct target genes in the absence of ligands (ligand-independent Transrepression) recruiting corepressor complexes that mediate active repression.

Figure 3. PPARβ/δ agonism and the metabolic syndrome. The metabolic syndrome results in an elevation of both TNF-α and C-Reactive Protein (CRP) leading to insulin resistance and chronic inflammation. This results in the exacerbation of pro-inflammatory pathways, ie. downregulation of the Akt pathway, and upregualtion of GSK-3β activity, leading to the activation of NF-κB and subsequent translocation of p65 to the nucleus. PPARβ/δ agonists have been shown to upregulate the Akt pathway as well as preventing the degradation of IκB, thus resulting in the indirect inhibition of NF-κB activation. PPARβ/δ agonists may also directly inhibit NF-κB activation in a process known as transrepression. The activation of STAT3 by PPARβ/δ agonists may modulate the production of TNF-α via SOCS3, which may aid in the restoring insulin sensitivity.
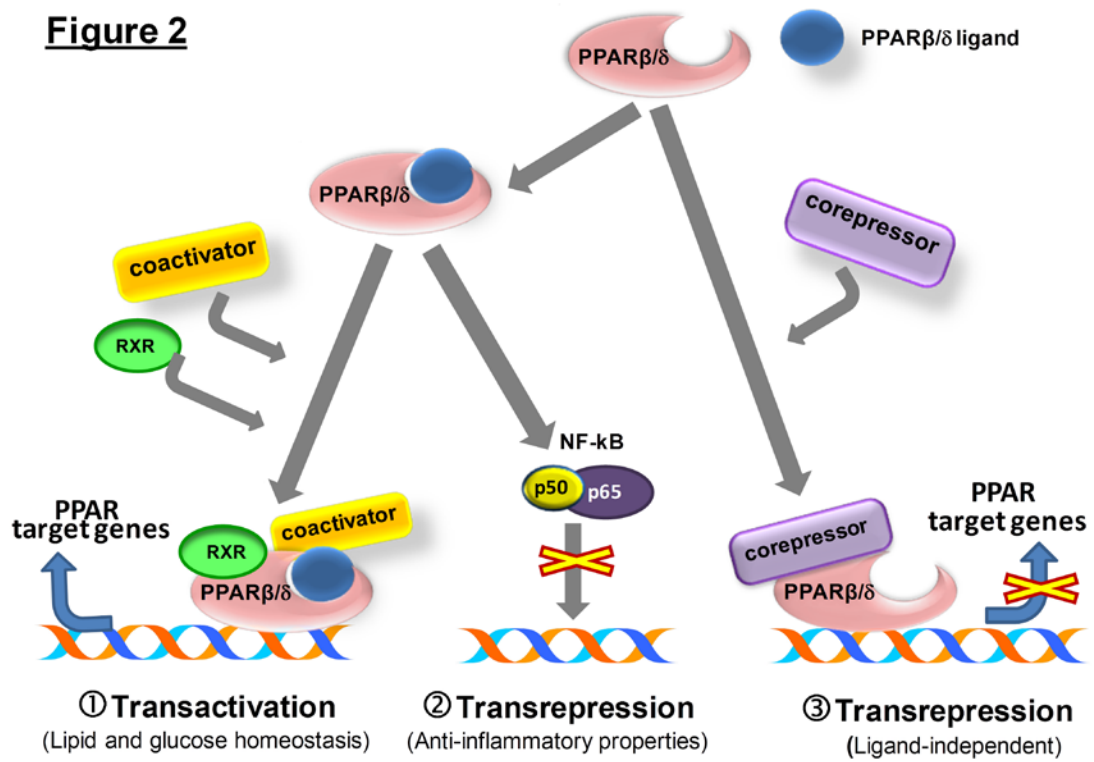
Figure 1

PPAR DOMAIN STRUCTURE

ISOFORMS	ENCODING GENE	AMINO ACID IDENTITY (% vs α)		AMINO ACID NUMBER
		DBD	LBD	
α (NR1C1)	22q12-q13.1	-	-	468
β/δ (NR1C2)	6p21.1-p21.2	86%	70%	441
γ (NR1C3)	3p25	83%	68%	477

Figure 2

Figure 3