

## Peroxisome Proliferator-Activated Receptor (PPAR) $\beta/\delta$ : A New Potential Therapeutic Target for the Treatment of Metabolic Syndrome

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**Abstract:** Metabolic syndrome is defined as the clustering of multiple metabolic abnormalities, including abdominal obesity, dyslipidemia (high serum triglycerides and low serum HDL-cholesterol levels), glucose intolerance and hypertension. The pathophysiology underlying metabolic syndrome involves a complex interaction of crucial factors, but two of these, insulin resistance and obesity (especially visceral obesity), play a major role. The nuclear receptors Peroxisome Proliferator-Activated Receptors (PPAR) $\alpha$  and PPAR $\gamma$  are therapeutic targets for hypertriglyceridemia and insulin resistance, respectively. Evidence is now emerging that the PPAR $\beta/\delta$  isotype is a potential pharmacological target for the treatment of disorders associated with metabolic syndrome. PPAR $\beta/\delta$  activation increases lipid catabolism in skeletal muscle, heart and adipose tissue and improves the serum lipid profile and insulin sensitivity in several animal models. In addition, PPAR $\beta/\delta$  ligands prevent weight gain and suppress macrophage-derived inflammation. These data are promising and indicate that PPAR $\beta/\delta$  ligands may become a therapeutic option for the treatment of metabolic syndrome. However, clinical trials in humans assessing the efficacy and safety of these drugs should confirm these promising perspectives in the treatment of the metabolic syndrome.

**Keywords:** PPAR, fatty acid, obesity, type 2 diabetes mellitus, metabolic syndrome.

### INTRODUCTION

As the prevalence of obesity has reached epidemic proportions in Western societies, a proportional increase has been observed in obesity-associated metabolic disorders, including glucose intolerance, insulin resistance, hyperlipidemia and hypertension, which are major risk factors for atherosclerotic cardiovascular disease. Extensive research has demonstrated that it is not coincidence that several of these cardiovascular risk factors are clustered. It is widely held that their simultaneous development is associated with a greater risk of atherosclerotic cardiovascular disease than that generated by any of the individual components. Recently, this disorder, formerly termed "syndrome X" by Reaven [1], has become known as metabolic syndrome and is defined as the clustering of multiple metabolic abnormalities, including abdominal obesity, dyslipidemia (high serum triglycerides and low serum HDL-cholesterol levels), glucose intolerance and hypertension [2, 3]. The prevalence of metabolic syndrome is increasing worldwide and is a growing threat to global health, since it accounts for 6-7% of all-cause mortality. This is because subjects with metabolic syndrome have a five-fold greater risk of developing type-2 diabetes mellitus, if it is not already present [4], and they are twice as likely to die from a heart attack or stroke, and three times more likely to have a heart attack or stroke than subjects without metabolic syndrome [5]. At least five different definitions have been put forward for metabolic syndrome (Table 1) [6-10]. They include cut-off points for visceral obesity, blood pressure, blood glucose, plasma triglyceride and HDL-cholesterol concentrations. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), the prevalence of metabolic syndrome in U.S. adults (>20 years of age) is approximately 24% [11] and reaches 43.5% for those people 60-69 years old.

The pathophysiology underlying metabolic syndrome involves a complex interaction of multiple factors. Two of these, insulin resistance and obesity (especially visceral obesity), which are closely and reciprocally interrelated, play an important role [2, 12]. Insulin resistance is characterized by hyperinsulinemia, enhanced hepatic gluconeogenesis and impaired insulin-stimulated glucose uptake in fat and skeletal muscle. There is considerable evidence that visceral intra-abdominal fat is closely linked to insulin re-

sistance. Adipose tissue secretes signaling molecules that are involved in the pathogenesis of insulin resistance. Among these molecules, free fatty acids (FFA) are candidates for linking obesity to insulin resistance. Thus, several studies have consistently demonstrated that rises in plasma FFA cause insulin resistance in diabetic patients and in non-diabetic subjects [13-16]. Increased levels of circulating FFA in obesity enhance lipid accumulation in insulin target tissues and contribute to reducing their sensitivity to insulin. Evidence for the role of lipids in insulin resistance has been found in studies with NMR spectroscopy, which show a strong relationship between the accumulation of intramyocellular triglyceride content and insulin resistance [17-19]. In addition, adipose tissue-derived inflammation and adipocytokine secretion (leptin, adiponectin, interleukins, etc.) may also contribute to the development of insulin resistance [20].

Since insulin resistance leads to impaired fatty acid metabolism in adipose tissue, its development contributes, together with the presence of visceral intra-abdominal fat, to an increased release of fatty acids into the circulation. This, in turn, leads to multiple abnormalities in the lipoprotein profile, resulting in what is known as atherogenic dyslipidemia of the metabolic syndrome. This consists of rises in lipoproteins containing apolipoprotein B (apoB), including very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), increased triglycerides and VLDL remnants and increased small dense LDL, whereas the levels of HDL-cholesterol are reduced. Hypertension is a frequent disorder in metabolic syndrome and is more frequent in those suffering obesity and insulin resistance [1]. The presence of hyperglycemia, dyslipidemia and hypertension in those suffering metabolic syndrome results in endothelial dysfunction and impairs atherogenesis. Within the vascular wall, recruitment of monocyte-derived macrophages and uptake of oxidized-LDL result in the formation of foam cells, the hallmark of atherosclerotic lesions. These cells secrete inflammatory mediators and matrix remodeling enzymes, leading to a chronic inflammatory process and the formation of atherosclerotic plaque through interactions with and recruitment of vascular cells, T cells and additional macrophages [11].

### THE PPAR FAMILY

Although there is as yet no single treatment for metabolic syndrome, it is well established that lifestyle modifications, including weight reduction through exercise and dietary modification, form the first-line strategy of intervention. When the lifestyle approach

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**Table 1. Criteria for Diagnosis of Metabolic Syndrome**

Clinical Features Minimum Criteria for Diagnosis	WHO (1999) <sup>6</sup> Impaired Glucose Regu- lation/Insulin Resistance and $\geq 2$ other Criteria	EGIR <sup>a</sup> (1999) <sup>10</sup> Insulin Resistance Plus Two other Criteria	NCEP ATPIII (2001) <sup>7</sup> $\geq 3$ of the Criteria Below	AACE (2003) <sup>9</sup> Clinical Judgment Based on all Features	IDF (2005) <sup>8</sup> Central Adiposity Plus Two other Features
Glucose/Insulin abnormal- ity	Type 2 diabetes or im- paired fasting glycemia (fasting plasma glucose $\geq 110$ mg/dl) or impaired glucose tolerance or glu- cose uptake below lowest quartile under hyperinsu- linemic, euglycemic condi- tions	Insulin resistance: hyperin- sulinemia (non-diabetic fasting insulin in top 25%) and impaired fasting glu- cose (fasting plasma glu- cose $\geq 110$ mg/dl)	Fasting plasma glucose $\geq$ 110 mg/dl	Glucose intolerance (fas- ting plasma glucose $\geq 110$ mg/dl) o 2 h postprandial glucose $> 140$ mg/dl	Glucose intolerance (fas- ting plasma glucose $\geq 100$ mg/dl) or pre-existing diabetes
Abdominal visceral obesity	Waist-to-hip ratio $>0.9$ (M) or $>0.85$ (W) and/or BMI $>30$ kg/m <sup>2</sup>	Waist circumference $\geq 94$ cm (M), $\geq 80$ cm (W)	Waist circumference $> 102$ cm (M), $> 88$ cm (W)	BMI $\geq 25$ kg/m <sup>2</sup>	Waist circumference: European $\geq 94$ cm (M) or 80 cm (W); South Asian and Chinese $\geq 90$ cm (M) or $\geq 80$ cm (W); Japanese $\geq 85$ cm (M) or 90 cm (W)
Hypertriglyceridemia	$\geq 150$ mg/dl	$> 176$ mg/dl	$\geq 150$ mg/dl	$\geq 150$ mg/dl	$\geq 150$ mg/dl
Low HDL-c levels	$<35$ mg/dl (M) $<39$ mg/dl (W)	$<39$ mg/dl	$<40$ mg/dl (M) $<50$ mg/dl (W)	$<40$ mg/dl (M) $<50$ mg/dl (W)	$<35$ mg/dl (M) $<50$ mg/dl (W)
Hypertension (with or without drugs)	$\geq 140/90$ mm Hg	$\geq 140/90$ mm Hg	$\geq 130/85$ mm Hg	$\geq 130/85$ mm Hg <sup>b</sup>	$\geq 130/85$ mm Hg
Other	Microalbuminuria ( $\geq 20$ $\mu$ g/min or albu- min/creatinine ratio $\geq 30$ mg/g)	-	-	-	-

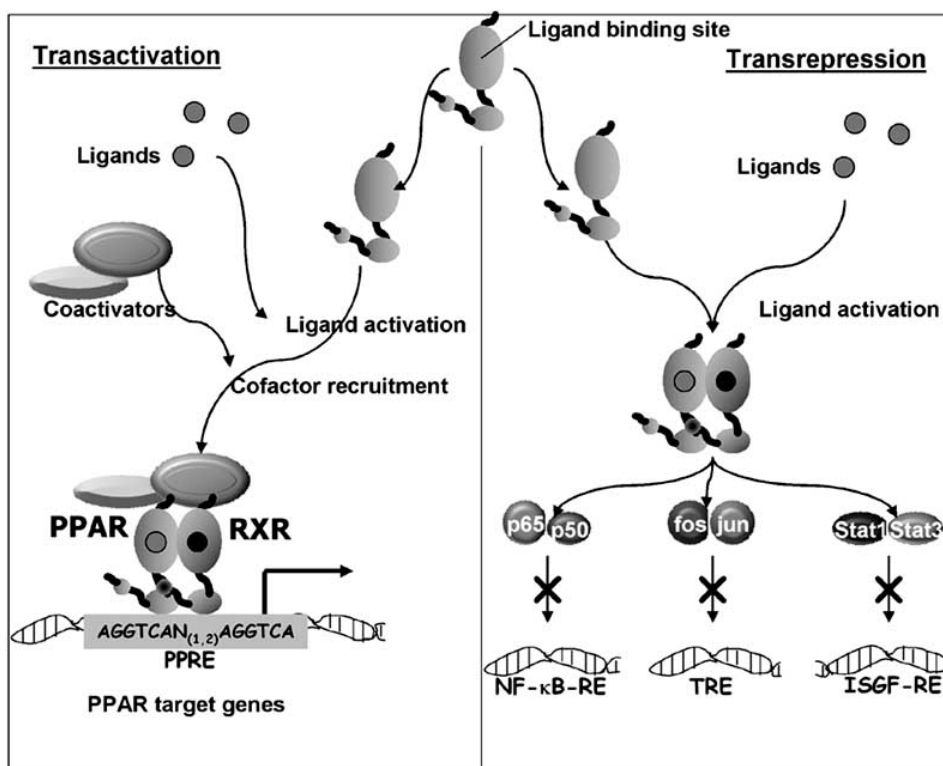
<sup>a</sup>Individuals without diabetes only. <sup>b</sup>Without medication only. Abbreviations: AACE, the American College of Endocrinology; EGIR, European Group for the Study of Insulin Resistance; IDF, International Diabetes Federation; M, Men; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III; W, Women; WHO, World Health Organization.

appears to be inadequate and cannot reverse existing metabolic risk factors, the individual components have to be treated.

Although, at present, there are several pharmacological treatments available for the treatment of metabolic syndrome (statins, fibrates, thiazolidinediones and metformin), the disorder is often refractory to these drugs. Intensive efforts have been made to develop new drugs with new mechanisms for treating metabolic syndrome. Nuclear receptors are novel targets for the development of therapeutic agents for the treatment of numerous diseases, including type 2 diabetes mellitus, obesity, dyslipidemia, atherosclerosis and metabolic syndrome. Nuclear receptors are transcription factors that act as intracellular receptors, activated through the binding of hydrophobic ligands, including fatty acids, hormones, bile acids and oxysterols. During the last decade it has been extensively demonstrated that, within the family of nuclear receptors, Peroxisome Proliferator-Activated Receptors (PPARs) are effective molecular targets for treating some aspects of metabolic syndrome, mainly hypertriglyceridemia (fibrates) and type 2 diabetes mellitus (thiazolidinediones). PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors that regulate the expression of genes involved in fatty acid uptake and oxidation, lipid metabolism and inflammation [21]. To be transcriptionally active, PPARs need to heterodimerize with the 9-*cis* retinoic acid receptor (RXR) (NR2B) (Fig. (1)). PPAR-RXR heterodimers bind to DNA-specific sequences called peroxisome proliferator-response elements (PPREs), consisting of an imperfect direct repeat of the consensus binding site for nuclear hormone receptors (AGGTCA) separated by one nucleotide (DR-1). These sequences have been characterized within the promoter regions of PPAR target genes. The binding occurs in such a way that PPAR is always oriented to the DNA's 5'-end, while RXR is to the 3'-end. In the absence of ligand, high-affinity complexes are formed between PPAR-RXR

heterodimers and nuclear receptor co-repressor proteins, which block transcriptional activation by sequestering the heterodimer from the promoter. Binding of the ligand to PPAR induces a conformational change resulting in dissociation of co-repressor proteins, so that the PPAR-RXR heterodimer can then bind to PPREs. Moreover, once activated by the ligand, the heterodimer recruits co-activator proteins that promote the initiation of transcription [22]. As a consequence of these changes in transcriptional activity, binding of ligands to the receptor results in changes in the expression level of mRNAs encoded by PPAR target genes. In a specific cellular context, the activity of PPARs regulating the transcription of their target genes depends on many factors (relative expression of the PPARs, the promoter context of the target gene, the presence of co-activator and co-repressor proteins, etc.).

However, the regulation of gene transcription by PPARs extends beyond their ability to transactivate specific target genes in an agonist-dependent manner. PPARs also regulate gene expression independently of binding to PPREs. They cross-talk with other types of transcription factors and influence their function without binding to DNA, through a mechanism termed receptor-dependent *trans*-repression [23]. Most of the anti-inflammatory effects of PPARs are probably explained by this mechanism [24, 25]. Thus, through this DNA-binding independent mechanism, PPARs suppress the activities of several transcription factors, including nuclear factor  $\kappa$ B (NF- $\kappa$ B), activator protein 1 (AP-1), signal transducers and activators of transcription (STATs) and nuclear factor of activated T cells (NFAT). There are three main *trans*-repression mechanisms by which ligand-activated PPAR-RXR complexes negatively regulate the activities of other transcription factors. First, *trans*-repression may result from competition for limiting amounts of shared co-activators. Under conditions in which the levels of specific co-activators are rate-limiting, activation of PPAR may



**Fig. (1). Molecular mechanisms of Peroxisome Proliferator-Activated Receptors (PPARs).** PPARs are ligand-activated transcription factors that regulate gene expression through two mechanisms: *trans*-activation and *trans*-repression. In *trans*-activation PPAR-RXR heterodimers bind to DNA-specific sequences called peroxisome proliferator-response elements (PPREs), which are located in the promoter regions of genes involved in glucose and fatty acid metabolism. PPARs may also regulate gene expression through a DNA-independent mechanism called *trans*-repression. Through this mechanism, PPARs inhibit the activity of several transcription factors, such as Nuclear Factor- $\kappa$ B, leading to anti-inflammatory effects. STAT denotes signal transducers and activators of transcription; IS-GFRE interferon-stimulated gene factor responsive element; and TRE, TPA responsive element, where TPA is a phorbol ester.

suppress the activity of other transcription factors that use the same co-activators [26, 27]. In the second mechanism, activated PPAR-RXR heterodimers are believed to act through physical interaction with other transcription factors (for example AP-1, NF- $\kappa$ B, NFAT or STATs). This association prevents the transcription factor from binding to its response element and thereby inhibits its ability to induce gene transcription [28]. The third *trans*-repression mechanism relies on the ability of activated PPAR-RXR heterodimers to inhibit the phosphorylation and activation of certain members of the mitogen-activated protein kinase (MAPK) cascade [29], preventing activation of downstream transcription factors.

The PPAR family consists of three members, PPAR $\alpha$  (NR1C1 according to the unified nomenclature system for the nuclear receptor superfamily), PPAR $\beta/\delta$  (NR1C2) and PPAR $\gamma$  (NR1C3) [30]. PPAR $\alpha$  was the first PPAR to be identified and is the molecular target of the fibrate hypolipidemic class of drugs. This PPAR isotype is expressed primarily in tissues with a high level of fatty acid catabolism such as liver, brown fat, kidney, heart and skeletal muscle [31]. PPAR $\gamma$  has a restricted pattern of expression, mainly in white and brown adipose tissues and macrophages, whereas other tissues such as skeletal muscle and heart contain limited amounts. The  $\gamma$  isotype is the molecular target for the anti-diabetic drugs, the thiazolidinediones. PPAR $\beta/\delta$  is ubiquitously expressed and for this reason was initially thought to be a "housekeeping gene" [32]. However, studies with knockout mice [33-35] and the development of specific and high-affinity ligands for this receptor have shown that PPAR $\beta/\delta$  is a potential molecular target for prevention or treatment of several disorders of the metabolic syndrome. In this review we will highlight the role of PPAR $\beta/\delta$  in those metabolic processes with potential for therapeutic intervention in metabolic syndrome.

### PPAR $\beta/\delta$ -SPECIFIC FEATURES AND LIGANDS

The crystal structure of the ligand-binding domain of the PPAR $\beta/\delta$  isotype, which was first cloned in *Xenopus laevis* [36], revealed an exceptionally large pocket of approximately 1300 Å<sup>3</sup>. This pocket is similar to that of PPAR $\gamma$ , but much larger than the pockets of other nuclear receptors [37, 38], which may explain, at least in part, the great variety of natural and synthetic ligands that bind to and activate this nuclear receptor. Saturated (14 to 18 carbons) and polyunsaturated (20 carbons in length) fatty acids have affinities for PPAR $\beta/\delta$  in the low micromolar range [38-41]. In addition, all-*trans*-retinoic acid (vitamin A) [42] and fatty acids derived from VLDL [43] can activate PPAR $\beta/\delta$ . Finally, the availability of three synthetic ligands (GW501516, GW0742 and L-165041) that activate PPAR $\beta/\delta$  at very low concentrations both *in vivo* and *in vitro* with high selectivity over other PPAR isotypes [44] led to a huge increase in experimental studies on the role of PPAR $\beta/\delta$  in cellular processes. The EC<sub>50</sub> for these compounds assessed with recombinant human PPAR $\beta/\delta$  were 1.0 nM for GW0742, 1.1 nM for GW501516 and 50 nM for L-165041 [44, 45].

### ROLE OF PPAR $\beta/\delta$ IN LIPOPROTEIN METABOLISM

Among the disorders of the metabolic syndrome, particular attention should be paid to dyslipidemia. Treatment of the atherogenic dyslipidemia associated with metabolic syndrome requires lowering triglycerides, increasing HDL-cholesterol and increasing the size of the LDL-cholesterol particle. Treatment of obese rhesus monkeys, a model for human obesity and its associated metabolic disorders, with the PPAR $\beta/\delta$  agonist GW501516 increased HDL-cholesterol (79%), and decreased triglycerides (56%), LDL-

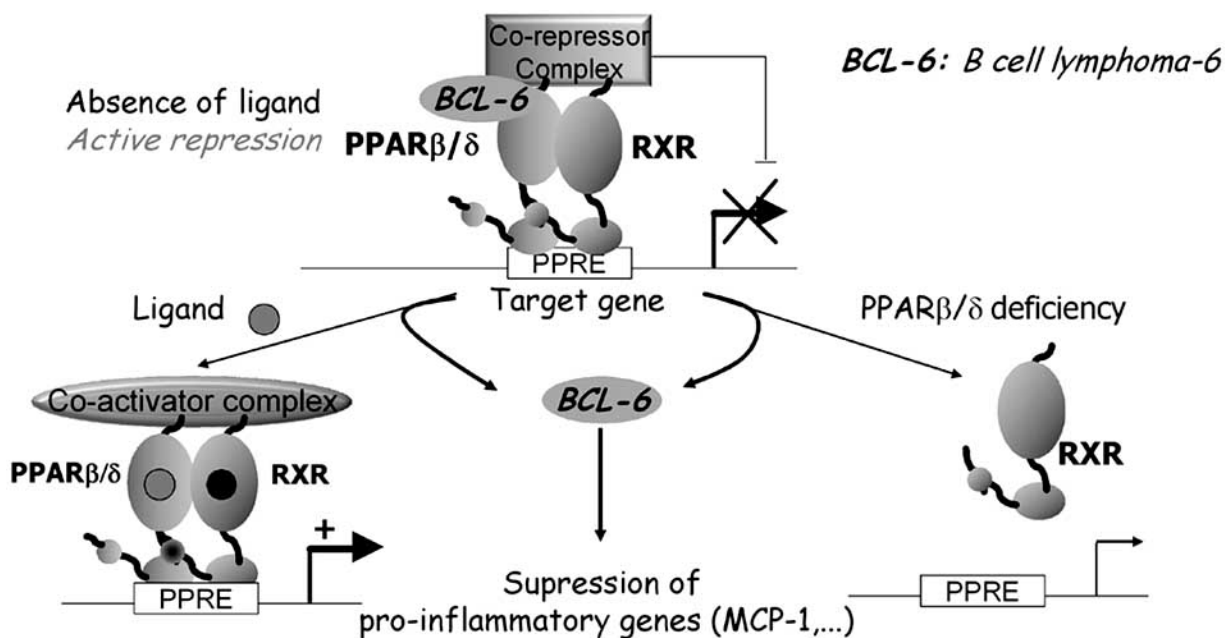
cholesterol (29%) and fasting insulin levels (48%) [46]. A decrease in small dense LDL was also observed in treated animals [46]. It has been suggested that the increase in HDL-cholesterol levels after PPAR $\beta/\delta$  treatment is caused by enhanced cholesterol efflux stimulated by a higher expression of the reverse cholesterol transporter ATP-binding cassette A1 (ABCA1) in several tissues, including human and mouse macrophages and intestinal cells and fibroblasts [47, 48]. Apart from these beneficial effects of PPAR $\beta/\delta$  activation on HDL levels, treatment with this compound also increased HDL particle size in primates [49], an effect which is thought to be protective against the progression of coronary artery disease in humans [50]. In addition, PPAR $\beta/\delta$  activation reduces cholesterol absorption through a mechanism that may involve, at least in part, reduced intestinal expression of Niemann-Pick C1-like 1 (*Npc1l1*), the proposed target for the inhibitor of cholesterol absorption ezetimibe [47]. It remains to clearly demonstrate that the effects of these drugs are mediated through PPAR $\beta/\delta$  activation. In mice, deletion of PPAR $\beta/\delta$  led to enhanced LDL and triglyceride levels [51]. Administration of a PPAR $\beta/\delta$  agonist to obese and diabetic *db/db* mice modestly increased HDL particles, without affecting triglyceride levels [48], whereas in a shorter treatment with GW501516 a reduction in plasma free fatty acids and triglyceride levels was observed in *db/db* mice, but not in mice exposed to a high fat diet [52]. In humans, there are conflicting reports as to whether PPAR $\beta/\delta$  polymorphisms are associated with changes in plasma lipoproteins. Thus, while some studies found an association between a PPAR $\beta/\delta$  polymorphism and plasma lipids [53], this was not confirmed in other studies [54]. These discrepancies could be caused by differences in gender or the influence of gene-environment interactions, since a recent study reported that the association between the PPAR $\beta/\delta$  -87T>C polymorphism and plasma HDL-cholesterol might be sex-specific, women showing a stronger association, and that this association was only observed in subjects consuming a low-fat diet [55]. The authors of this study conclude that the presence of the PPAR $\beta/\delta$  -87T>C polymorphism, which may result in enhanced PPAR $\beta/\delta$  activity, is associated with lower risk of suffering metabolic syndrome and that this association depends on the amount of fat consumed. In summary, the findings available at present on the effects of PPAR $\beta/\delta$  activation on lipopro-

tein metabolism were so promising that PPAR $\beta/\delta$  drugs are now in clinical trials for the treatment of human dyslipidemia.

### PPAR $\beta/\delta$ EFFECTS IN ATHEROSCLEROSIS

There is a strong relationship between circulating lipoproteins and atherosclerosis, since modified LDL-cholesterol particles are taken up by monocyte-derived macrophages, which leads to the formation of what are known as foam cells in the arterial intimal wall. Further, these macrophages contribute to the inflammatory reaction by the production and secretion of numerous pro-inflammatory cytokines [56]. Activated macrophages express the three PPAR isotypes and, whereas the roles of the PPAR $\alpha$  and  $\gamma$  isotypes in macrophage cholesterol homeostasis are well established, the role of PPAR $\beta/\delta$  remains controversial. Oliver *et al.* [46] showed that treatment of THP-1 human monocytes with the PPAR $\beta/\delta$  ligand GW501516 enhanced the expression of the reverse cholesterol transporter ABC1 and induced apoA1-specific cholesterol efflux. Opposite findings were reported by Vosper *et al.* [57]. They found that treatment with a different PPAR $\beta/\delta$  ligand promoted lipid accumulation in human macrophages (THP-1) exposed to oxidized LDL by increasing the expression of the class A and B scavenger receptors (SR-A and CD-36) and the lipid storage-related genes *ap2* and *adipophilin* [57]. Finally, two studies showed that cholesterol efflux or accumulation was not affected by PPAR $\beta/\delta$  depletion or by PPAR $\beta/\delta$  ligands in murine macrophages [58, 59]. Collectively, these findings suggest that PPAR $\beta/\delta$  does not affect cholesterol metabolism in mouse. However, additional studies are needed to establish the role of this nuclear receptor in human macrophage cholesterol metabolism.

It is accepted that inflammation links dyslipidemia to atherosclerotic plaque formation [60]. A role for PPAR $\beta/\delta$  in inflammation during the atherosclerotic process has been described in several studies. For instance, atherosclerosis-prone LDL receptor-null mice transplanted with bone marrow from PPAR $\beta/\delta$ -deficient mice and fed a high-cholesterol diet showed smaller vascular lesions (50% reduction) than wild-type recipient controls [58], whereas no significant differences between the two groups were found for plasma



**Fig. (2).** PPAR $\beta/\delta$  as a molecular switch in inflammation. PPAR $\beta/\delta$  regulates an inflammatory switch by binding or releasing the anti-inflammatory transcriptional suppressor protein B cell lymphoma-6 (BCL-6). In the absence of ligand, PPAR $\beta/\delta$  sequesters BCL-6, leading to inflammation. However, in the presence of ligand, PPAR $\beta/\delta$  releases BCL-6, which then represses inflammatory gene expression. Similarly, deletion of PPAR $\beta/\delta$  also releases BCL-6, leading to anti-inflammatory effects.

cholesterol levels. However, PPAR $\beta/\delta$ -null macrophages had lower expression of the inflammation markers monocyte chemoattractant protein (MCP1), interleukin 1 $\beta$  (IL-1 $\beta$ ) and metalloproteinase 9 (MMP-9) [58]. In contrast, PPAR $\beta/\delta$  ligands suppressed the expression of these inflammation markers in wild-type macrophages. These findings led to the suggestion that PPAR $\beta/\delta$  regulates an inflammatory switch by binding or releasing the anti-inflammatory transcriptional suppressor protein B cell lymphoma-6 (BCL-6) [61, 58] (Fig. 2). In the absence of ligand, PPAR $\beta/\delta$  sequesters BCL-6, leading to inflammation. However, in the presence of ligand, PPAR $\beta/\delta$  releases BCL-6, which then represses inflammatory gene expression. Similarly, deletion of PPAR $\beta/\delta$  also releases BCL-6, leading to anti-inflammatory effects. Therefore, this mechanism may explain how both PPAR $\beta/\delta$  activation and the deletion of this nuclear receptor result in a similar reduction of the inflammatory process. Whether additional mechanisms may contribute to the anti-inflammatory effect of the PPAR $\beta/\delta$  isotype needs to be studied. It should be noted that part of the anti-inflammatory effects of PPAR $\gamma$  agonists used at high concentrations in macrophages has been attributed to the activation of PPAR $\beta/\delta$  [62], since at high concentrations PPAR $\gamma$  ligands may activate both PPAR isotypes. Although these findings may suggest that PPAR $\beta/\delta$  activation is beneficial in the treatment of atherosclerosis, *in vivo* studies are contradictory. Thus, Li *et al.* [59] reported no effect of the PPAR $\beta/\delta$  agonist GW0742 on atherosclerotic lesion size in male LDL<sup>-/-</sup> mice fed an atherogenic diet (1.25% cholesterol). In contrast, Graham *et al.* [63] reported that administration of GW0742 to female LDL<sup>-/-</sup> mice fed an atherogenic diet (0.25% cholesterol) reduced atherosclerosis by 30%. Differences between these two studies (cholesterol supplementation or drug doses) may explain these contradictory results. Overall, these data suggest that PPAR $\beta/\delta$  agonists have anti-inflammatory effects *in vivo*, but this seems not to be sufficient to inhibit the development of atherosclerosis in extreme hypercholesterolemic animal models of this disease. Additional studies will be needed before the role of PPAR $\beta/\delta$  in modulating the development of atherosclerosis can be determined.

### ROLE OF PPAR $\beta/\delta$ IN ADIPOSE TISSUE

Adiposity correlates with insulin resistance and weight loss reduces all of the metabolic risk factors [64], demonstrating that obesity, especially excess visceral fat, is one contributing cause of metabolic syndrome. At present, however, as the drugs available for treating obesity are of limited effectiveness [65], there is great interest in the development of weight-loss drugs.

PPAR $\beta/\delta$  is involved in adipogenesis, where it strengthens PPAR $\gamma$ -induced adipocyte differentiation in cell cultures [66, 67], contributing to the expansion of adipose tissue. Consistent with this role, PPAR $\beta/\delta$ -null mice are smaller with less epididymal or gonadal adipose tissue [34]. However, adipose tissue-specific deletion of PPAR $\beta/\delta$  does not alter fat mass [33], suggesting that part of its actions are mediated *via* other tissues. Consistent with this hypothesis, transgenic mice overexpressing a constitutively active PPAR $\beta/\delta$ -VP16 fusion protein in adipose tissue on a standard chow diet showed a 20% reduction in body weight, a 40% reduction in inguinal fat pad masses, decreased adipocyte triglyceride accumulation and lower plasma free fatty acids and triglycerides, compared with their control littermates [68]. Moreover, PPAR $\beta/\delta$ -VP16 overexpression protected against obesity in high-fat diet feeding. Transcriptional analysis showed that, in the brown fat rather than in white adipose tissue, there was an increased expression of genes involved in fatty acid  $\beta$ -oxidation, lipolysis and energy uncoupling in the mitochondria [68]. Interestingly, systemic treatment of genetically obese mice (*db/db* mice) with the PPAR $\beta/\delta$  ligand GW501516 restores oxidative capacity in brown adipose tissue, reducing intracellular triglyceride accumulation in this tissue and in liver [68]. Moreover, PPAR $\beta/\delta$  ligands also enhance  $\beta$ -oxidation in 3T3-L1 preadipocytes [68] and retard weight gain in animal models

of high-fat diet-induced obesity [68]. However, these findings in murine models contrast with those observed in rhesus monkeys in which a short treatment (4 months) with a PPAR $\beta/\delta$  ligand did not affect body weight [46].

### ROLE OF PPAR $\beta/\delta$ IN SKELETAL MUSCLE

Although insulin-resistant subjects have chronic increases in plasma FFA, due in part to excess lipolysis [69, 70], their rates of fatty acid oxidation in skeletal muscle are lower [71, 72]. As a result of these changes, fatty acids accumulate in skeletal muscle of insulin-resistant subjects, which leads to higher levels of triglycerides [73], fatty acyl CoA [18], diacylglycerol [18] and ceramides [74]. It is worth noting that a positive correlation has been reported between increased intramyocellular triglyceride content and insulin resistance [75, 76], suggesting that prevention of lipid accumulation in skeletal muscle may improve insulin resistance.

Recent studies have shown that PPAR $\beta/\delta$  plays an important role in controlling fatty acid metabolism in skeletal muscle cells [52, 68, 77, 78]. This PPAR $\beta/\delta$  subtype induces the expression of target genes involved in fatty acid utilization and oxidation in myocytes. It has been suggested that PPAR $\alpha$  and PPAR $\beta/\delta$  share similar functions in myotubes regarding fatty acid metabolism. In agreement with this hypothesis, Muoio *et al.* [79] showed that fatty acid oxidation was not impaired in skeletal muscle of PPAR $\alpha$ <sup>-/-</sup> mice, probably because PPAR $\beta/\delta$  compensates for the lack of PPAR $\alpha$  in these mice. Therefore, impaired PPAR $\beta/\delta$  signaling may be involved in lipid accumulation in skeletal muscle. We recently reported that exposure of skeletal muscle cells to palmitate reduces the expression of PPAR $\beta/\delta$ -target genes involved in fatty acid utilization by a mechanism that may involve NF- $\kappa$ B activation and that these changes were prevented by treatment with a PPAR $\beta/\delta$  activator [80]. Interestingly, palmitate exposure led to increased protein-protein interaction between the p65 subunit of NF- $\kappa$ B and PPAR $\beta/\delta$ . These findings are consistent with the results reported by Westergaard *et al.* [81], who showed that PPAR $\beta/\delta$  physically interacts with p65 in psoriatic lesions. Furthermore, they showed p65-dependent repression of PPAR $\beta/\delta$ , but not of PPAR $\alpha$  or PPAR $\gamma$ . Surprisingly, the interaction between p65 and PPAR $\beta/\delta$  was elevated in skeletal muscle cells not exposed to palmitate, suggesting that the levels of endogenous agonists in these cells were low or that NF- $\kappa$ B regulates the basal activity of PPAR. This latter point is consistent with a previous study showing that inactivation of basal NF- $\kappa$ B activity enhanced the expression of the PPAR $\alpha$ -target gene apoA-I [82], since most of the genes under the control of PPAR $\alpha$  are also regulated by PPAR $\beta/\delta$ . Perhaps activation of NF- $\kappa$ B by high plasma FFA may result in a reduction in PPAR signaling, leading to a fall in fatty acid utilization and oxidation that may favor its accumulation in skeletal muscle in the form of triglycerides and diacylglycerol. Furthermore, accumulation of these lipid species may enhance the inflammatory process in skeletal muscle. Since insulin resistance correlates closely with intramyocellular lipid levels in skeletal muscle [18, 19, 83], the reduction in the activity of NF- $\kappa$ B in diabetic patients by PPAR $\beta/\delta$  agonists may be a therapeutic target for the treatment of this pathology. In support of this hypothesis, it has been reported that activation of PPAR $\beta/\delta$  induces fatty acid  $\beta$ -oxidation in skeletal muscle and attenuates metabolic syndrome, in which insulin resistance plays a pivotal role in rodents [52] and humans [84]. Moreover, muscle-specific over-expression of an activated form of PPAR $\beta/\delta$  (VP16-PPAR $\delta$ ) resulted in resistance to diet-induced obesity, higher metabolic rate and lipid utilization, and lower intramyocellular triglyceride levels [68]. The resistance to body weight gain is caused by decreases in both visceral and subcutaneous adipose depots. In addition, there is an increase in the number of type-I muscle fibers [68]. Skeletal muscle is composed of heterogeneous myofibers that differ in their metabolic and contractile properties, including oxidative slow-twitch fibers (type I), mixed oxida-

tive/glycolytic fast-twitch ones (type IIA), and glycolytic fast-twitch (type IIB) forms [85]. Oxidative myofibers mainly express enzymes involved in fatty acid oxidation and contain low levels of contractile proteins, whereas glycolytic myofibers preferentially utilize glucose and contain fast contractile protein isoforms [86, 87]. The PPAR $\beta/\delta$  isotype is expressed in skeletal muscle at higher levels than PPAR $\alpha$  and PPAR $\gamma$ , respectively, and it is located mainly in oxidative rather than glycolytic myofibers [31, 68]. PPAR $\beta/\delta$ -mediated reprogramming of muscle fibers may have several physiological implications. Thus, a greater number of type-I muscle fibers may reduce muscle fatiguability. In fact, mice with muscle-specific VP-16 PPAR $\delta$  transgenes run twice as long and far as wild-type mice [68]. In addition, obesity and insulin resistance are linked to a decrease in the number of oxidative slow-twitch fibers in skeletal muscle [88-92]. This is consistent with the fact that muscle-specific VP16-PPAR $\delta$  transgenic mice, with more oxidative slow-twitch fibers in skeletal muscle, are resistant to high-fat diet-induced obesity [68]. It is known that endurance training increases the number of type-I fibers, and increased PPAR $\beta/\delta$  expression in rodent skeletal muscle has been reported after endurance training with 3 weeks of swimming [93]. Interestingly, a single exhaustive bout of cycling increases PPAR $\beta/\delta$  expression in humans [94, 95].

The reported actions of PPAR $\beta/\delta$  on muscle fiber formation and fatty acid  $\beta$ -oxidation may rely on modulation of the expression of PPAR $\gamma$  Coactivator 1 $\alpha$  (PGC1- $\alpha$ ). This coactivator of various transcription factors is known to play an important role in slow muscle fiber formation, mitochondrial biogenesis and fatty acid utilization. Its expression has been related to insulin sensitization. PPAR $\beta/\delta$  controls PGC-1 $\alpha$  gene transcription through a PPAR-response element in the PGC-1 $\alpha$  promoter [96]. Mice in which PPAR $\beta/\delta$  was selectively ablated in skeletal muscle showed reduced PGC-1 $\alpha$  expression and a muscle fiber-type switch toward lower oxidative capacity that preceded the development of obesity and diabetes [97]. Moreover, since PGC-1 $\alpha$  co-activates PPAR $\beta/\delta$  transcriptional activity, a positive self-regulating loop of control is established following PPAR $\beta/\delta$  activation.

## ROLE OF PPAR $\beta/\delta$ IN CARDIAC MUSCLE

Constant pump function of the heart is a high-energy demand, which is mainly satisfied by fatty acids and glucose. The oxidation of fatty acids and glucose covers, respectively, 65% and 30% of the energy demand of the adult heart [98]. The heart, unlike other tissues such as the brain, adapts its metabolism to substrate availability. For example, during cardiac hypertrophy and congestive heart failure, increased glucose utilization and decreased fatty acid oxidation are observed [99-101]. Similar to the role of PPAR $\beta/\delta$  in skeletal muscle, this nuclear receptor is also involved in the control of fatty acid oxidation in heart. We recently reported that the levels of both PPAR $\alpha$  and PPAR $\beta/\delta$  are reduced in pressure-overload cardiac hypertrophy [102]. Therefore, it can be argued that the fall in the expression of both PPAR subtypes during the development of cardiac hypertrophy seems necessary to down-regulate the expression of genes involved in fatty acid metabolism. Interestingly, the changes caused by cardiac hypertrophy in the expression of genes involved in fatty acid metabolism were not observed when NF- $\kappa$ B activity was inhibited [102]. Indeed, a recent study demonstrated that inhibition of NF- $\kappa$ B increased the expression of the well-known PPAR $\alpha$ -target gene, apoA-I [82], confirming the negative cross-talk between NF- $\kappa$ B and PPAR $\alpha$ . Therefore, we evaluated in an additional study whether, in addition to the reported reduction in the expression of PPARs during cardiac hypertrophy [103], other mechanisms, such as protein-protein interaction between NF- $\kappa$ B and PPAR, contribute to the changes in the expression of genes involved in cardiac fatty acid metabolism. Using both *in vitro* and *in vivo* models of cardiac hypertrophy, we studied the contribution of NF- $\kappa$ B activation during this process to the down-regulation of

fatty acid oxidation. Stimulation of rat neonatal cardiomyocytes with phenylephrine (PE), which leads to NF- $\kappa$ B activation [104], caused cardiac hypertrophy that was accompanied by a fall in the expression of pyruvate dehydrogenase kinase 4 (*Pdk4*), a PPAR $\beta/\delta$  target gene involved in fatty acid metabolism [52], and palmitate oxidation. Further, the fall in the expression of *Pdk4* and fatty acid oxidation observed in PE-stimulated rat neonatal cardiomyocytes was restored by NF- $\kappa$ B inhibitors. These data pointed to the involvement of NF- $\kappa$ B in the down-regulation of fatty acid oxidation during the development of cardiac hypertrophy. A recent study confirmed this, showing that cardiomyocyte-restricted PPAR $\beta/\delta$  deletion in mouse heart of mice reduced myocardial fatty acid oxidation and the expression of genes involved in this process, such as *Pdk4*, and led to cardiomyopathy [105]. The mechanism by which activation of NF- $\kappa$ B results in reduced expression of PPAR $\beta/\delta$  target genes seems to involve reduced interaction of this PPAR subtype with its *cis*-regulatory element, since NF- $\kappa$ B activation caused a dramatic reduction in the binding of PPAR $\beta/\delta$  protein to the PPRE probe. This reduction was partially reversed by co-incubation of the cells with NF- $\kappa$ B inhibitors, confirming the involvement of this transcription factor in the changes observed. Therefore, the reduced binding activity of PPAR $\beta/\delta$  seems to be related to the activation of NF- $\kappa$ B in cardiac cells. However, it remains to be established through what mechanism NF- $\kappa$ B activation reduced the interaction of PPAR $\beta/\delta$  with its response element. NF- $\kappa$ B is present in the cytoplasm as an inactive heterodimer, consisting of the p50 and p65 subunits. However, after activation, this heterodimer translocates to the nucleus and regulates the expression of genes involved in inflammatory and immune processes. Our results indicate that, once the p65 subunit of NF- $\kappa$ B reaches the nucleus, it interacts with PPAR $\beta/\delta$ . This association prevents PPAR $\beta/\delta$  from binding to its response element and thereby inhibits its ability to induce gene transcription, leading to a reduction in the expression of *Pdk4*. In addition, a recent study reported that PPAR $\beta/\delta$  agonist administration, as well as over-expression of this nuclear receptor, suppresses myocardial inflammatory responses, such as the lipopolysaccharide-mediated production of TNF $\alpha$ , thus exerting beneficial effects in animals who had suffered ischemia/reperfusion injury or cardiac hypertrophy [106].

## ROLE OF PPAR $\beta/\delta$ IN LIVER

Insulin resistance is characterized by an increase in hepatic glucose production, among other alterations. Recently, it has been reported that PPAR $\beta/\delta$  may also control glucose metabolism and insulin sensitivity through its role in liver. Lee *et al.* [107] showed that PPAR $\beta/\delta$ <sup>-/-</sup> mice are glucose-intolerant, whereas diabetic *db/db* mice treated with a PPAR $\beta/\delta$  agonist show improved insulin sensitivity in all major insulin-responsive tissues. These authors demonstrated that PPAR $\beta/\delta$  activation reduces hepatic glucose output by increasing glycolysis and the pentose phosphate shunt and promoting fatty acid biosynthesis in the liver. In summary, this new mechanism of the PPAR $\beta/\delta$  activators may contribute, in addition to their effects on fatty acid oxidation, to the control of insulin sensitivity.

## CONCLUDING REMARKS

Treatment and prevention of metabolic syndrome requires lifestyle changes, including weight reduction, increased physical activity and better diet. However, as many patients cannot control the pathology with lifestyle modification, there is a need for drugs to manage the metabolic syndrome. Drugs activating PPAR $\beta/\delta$  may become a pharmacotherapy for metabolic syndrome, since they target multiple components of the syndrome through tissue- and cell-specific effects. Thus, activation of this nuclear receptor improves atherogenic dyslipidemia by reducing plasma triglyceride levels and enhancing plasma HDL-cholesterol levels. PPAR $\beta/\delta$  also

regulates the availability of BCL-6, an inflammatory suppressor protein released upon ligand binding to PPAR $\beta/\delta$ , thereby behaving as an “anti-inflammatory switch” to control macrophage-elicited inflammation and atherogenesis. In skeletal muscle, PPAR $\beta/\delta$  ligands may also upregulate fatty acid transport and oxidation and stimulate formation of slow-twitch muscle fibers. In adipose tissue, they activate thermogenesis and fatty acid oxidation, effects that may result in weight reduction. In addition, PPAR $\beta/\delta$  activation in the heart may enhance contractile function and improve cardiomyopathy. Finally, activation of PPAR $\beta/\delta$  in the liver suppresses glucose production by upregulating the pentose phosphate shunt.

As with any drug designed for human therapy, a great deal of research will be needed to determine the efficacy and safety of PPAR $\beta/\delta$  activators, before they can be used clinically. For instance, whereas the ability of PPAR $\beta/\delta$  activators to raise HDL-cholesterol levels was seen in both rodents and primates, the ability of these drugs to prevent obesity in rodents was not observed in primates. This suggests that weight reduction caused by PPAR $\beta/\delta$  ligands in mice depends on their effects on thermogenesis, which is a minor mechanism for energy expenditure in humans and primates. Safety issues regarding the connection between PPAR $\beta/\delta$  ligands and carcinogenesis, especially in animal models, have also been raised [108-112]. However, we should bear in mind that synthetic PPAR $\gamma$  and  $\alpha$  ligands induce carcinogenesis in rodents, but they do not do so in humans [113, 114]. In short, clinical studies are required to solve all these questions concerning the efficacy and safety of PPAR $\beta/\delta$  ligands.

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#### ABBREVIATIONS

ABCA1	=	ATP-binding cassette A1
AP-1	=	Activator protein 1
ApoB	=	Apolipoprotein B
BCL-6	=	Protein B cell lymphoma-6
FFA	=	Free fatty acids
IL-1 $\beta$	=	Interleukin 1 $\beta$
LDL	=	Low-density lipoprotein
MCP-1	=	Monocyte chemoattractant protein
MMP-9	=	Metalloproteinase 9
NFAT	=	Nuclear factor of activated T cells
NF- $\kappa$ B	=	Nuclear factor $\kappa$ B
Npc111	=	Niemann-Pick C1-like 1
PKD4	=	Pyruvate dehydrogenase kinase 4
PE	=	Phenylephrine
PGC1- $\alpha$	=	PPAR $\gamma$ Coactivator 1 $\alpha$
PPAR	=	Peroxisome Proliferator-Activated Receptor
PPRE	=	Peroxisome proliferator-response element
RXR	=	9- <i>cis</i> retinoic acid receptor

STAT = Signal transducers and activators of transcription

VLDL = Very-low-density lipoprotein

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