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# Unravelling the effects of PPARbeta/delta on insulin resistance and CVD

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| **Abstract:** | Insulin resistance precedes dyslipidemia and type 2 diabetes mellitus (T2DM) development. Preclinical evidence suggests that peroxisome proliferator-activated receptor (PPAR)b/dactivators may prevent and treat obesity-induced insulin resistance and T2DM, while clinical trials highlight their potential utility in dyslipidemia. Here, I review recent mechanistic insights into the antidiabetic effects of PPARb/d activators, including their anti-inflammatory actions, ability to inhibit endoplasmic reticulum stress and hepatic lipogenesis and to improve atherogenesis and insulin sensitivity, and their capacity to activate pathways also stimulated by exercise. Findings from clinical trials are also examined. Dissecting the effects of PPARb/d ligands on insulin sensitivity and atherogenesis may provide the basis for the development of therapies for the prevention and treatment of T2DM and cardiovascular disease. |

**Trends**

Deciphering the role of the antidiabetic effects of PPARβ/δ ligands may provide the basis for the development of medications for enhanced prevention and treatment of insulin resistance, T2DM and CVD.

PPARβ/δ ligands elicit antidiabetic effects in adipose tissue, liver, skeletal muscle, β-cells and the cardiovascular system.

The antidiabetic effects of PPARβ/δ ligands result from their anti-inflammatory effects, ability to inhibit ER stress and hepatic lipogenesis, mimic the adaptive responses to severe endurance training in skeletal muscle fiber composition, and improve atherogenesis and insulin secretion in insulin resistant states.

Some of the beneficial effects of current antidiabetic drugs may result from the activation of PPARβ/δ.

Clinical trials are exploring the effects of PPARβ/δ ligands in insulin resistance, NAFLD/NASH, dyslipidemia and CVD.

# UNRAVELLING THE EFFECTS OF PPARβ/δ ON INSULIN RESISTANCE AND CARDIOVASCULAR DISEASE

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**Keywords:** PPARβ/δ; Insulin resistance; Type 2 diabetes mellitus; Inflammation.

**Abstract**

Insulin resistance precedes dyslipidemia and type 2 diabetes mellitus (T2DM) development. Preclinical evidence suggests that peroxisome proliferator-activated receptor (PPAR)β/δ activators may prevent and treat obesity-induced insulin resistance and T2DM, while clinical trials highlight their potential utility in dyslipidemia. Here, I review recent mechanistic insights into the antidiabetic effects of PPARβ/δ activators, including their anti-inflammatory actions, ability to inhibit endoplasmic reticulum stress and hepatic lipogenesis and to improve atherogenesis and insulin sensitivity, and their capacity to activate pathways also stimulated by exercise. Findings from clinical trials are also examined. Dissecting the effects of PPARβ/δ ligands on insulin sensitivity and atherogenesis may provide the basis for the development of therapies for the prevention and treatment of T2DM and cardiovascular disease.

**Insulin resistance is a major determinant of type 2 diabetes mellitus**

Type 2 diabetes mellitus (T2DM), is a chronic disease affecting at least 250 million people worldwide, a figure that is set to more than double by 2030, with India, China, and the USA presenting the largest numbers of patients [1]. Insulin resistance, a hallmark of T2DM, encompasses defect in glucose uptake into insulin target tissues, suppression of hepatic glucose output and aberrant regulation of lipolysis. [2]. Insulin resistance precedes and predicts the development of T2DM, although the onset of T2DM is ultimately determined by a failure of beta-cell function and the resulting inability to compensate insulin resistance [3]. Obesity is strongly associated with insulin resistance; in fact, more than 90% of type 2 diabetic patients are overweight or obese. However, up to one-third of obese subjects appear to be metabolically healthy, and present neither insulin resistance nor **atherogenic dyslipidemia** (see Glossary), although this is not a completely benign condition [4]. While the factors underlying an 'healthy' vs. 'unhealthy' obesity are not well understood, it has been suggested that inter-individual variations in the ability of adipose tissue (AT) to expand in visceral AT (VAT) depots ultimately results in insulin resistance [5], whereas the expansion of subcutaneous fat depots (SAT) has no metabolic consequences [6]. Since the absolute numbers of adipocytes remain relatively stable in adults due to a constant turnover, expansion of AT results in the presence of hypertrophic adipocytes, which are characterized by a pro-inflammatory, insulin resistant phenotype, whereas small adipocytes are characterized by an anti-inflammatory, insulin sensitive phenotype [7] (see Box 1). In addition, VAT is unable to store excessive lipids appropriately, which leads to "lipid overflow" and the subsequent accumulation of lipids in target tissues, such as the liver, the heart, the skeletal muscle and the pancreas —a phenomenon described as ectopic fat deposition (lipotoxicity) —, which induces insulin resistance

[8]. Overall, most of these mechanisms ultimately result in a chronic, low-grade inflammatory process, which is a major driver of insulin resistance.

Obesity-induced insulin resistance begins insidiously, progresses over time and may culminate in T2DM. To reverse or delay this, early and aggressive lifestyle therapy, including weight reduction, increased physical activity and a healthy diet, are recommended [9]. Despite the awareness and practice of this non-pharmacological therapy, many patients cannot control these pathologies with lifestyle modifications [10]. In addition, once T2DM is established, the drugs currently available for its management have limited efficacy, limited tolerability and significant mechanism-based side effects and, as a result, only a small percentage of all patients with T2DM achieve adequate disease control [11]. Therefore, there is a need for new drugs to prevent and treat T2DM. Among the new pharmacological strategies for treating obesity-induced insulin resistance, peroxisome proliferator-activated receptor (PPAR) β/δ agonists show promise.

**PPARβ/δ: the basics**

PPARβ/δ is a member of the nuclear receptor (NR) superfamily of ligand-inducible transcription factors (TFs). PPARβ/δ forms an obligate heterodimer with retinoic acid receptor (RXR or NR2B), binds to peroxisome proliferator response elements (PPREs) and initiates transcription of target genes (Box 2). The receptor is almost ubiquitously expressed, although it is most abundant in metabolically active tissues, especially in organs/cells associated with fatty acid (FA) metabolism, such as skeletal and cardiac muscle, hepatocytes and adipocytes, and in macrophages. There, it regulates the

expression of many genes involved in lipid metabolism and glucose homeostasis, as well as inflammation [12,13]. The PPARβ/δ activation status depends on the presence of tissue specific ligands and the recruitment of co-activators or co-repressors.

X-ray crystallography studies have revealed that the ligand-binding pocket of all three PPARs is much larger than those of other NRs [12], which may explain, at least in part, the great variety of natural and synthetic ligands that bind to and activate these receptors. Saturated (SFAs) and polyunsaturated FAs (PUFAs) bind all PPAR isoforms, showing little selectivity. The development of high affinity and specificity synthetic ligands (GW501516, GW0742 and L-165041) that activate only the PPARβ/δ at very low concentrations both *in vivo* and *in vitro* [12] has helped further elucidate the PPARβ/δ functions. Although three PPARβ/δ agonists have reached clinical trials, MBX-8025 (Metabolex) [14], KD-3010 (Kalypsys) [15], and CER-002 (Cerenis), currently no clinically available drugs targeting PPARβ/δ exist. The PPARβ/δ antagonist GSK0660 has also been used to study the functions of this receptor [16], although its poor bioavailability has hindered *in vivo* studies. GSK3787, another potent antagonist, has good bioavailability and has been used in animal studies [17,18].

## PPARβ/δ as a major regulator of metabolism and inflammation in the setting of insulin resistance and T2DM

### *PPARβ/δ effects in adipocytes*

The expansion of abdominal fat during obesity is a risk factor for the development of insulin resistance through different processes; infiltration of macrophages into AT and their polarization towards the pro-inflammatory M1 phenotype promotes inflammation

and correlates with the degree of insulin resistance [19]. Macrophages from lean animals that infiltrate AT show an M2 alternative anti-inflammatory phenotype [19] that is induced by $Th_2$ cytokines, such as IL-4 and IL-13. PPARβ/δ is considered a crucial signaling molecule controlling the phenotypic switch between M1 and M2, in rodents, and thus insulin sensitivity [20,21]. Adipocyte-derived $Th_2$ cytokines, IL-13 and IL-4, induce macrophage *Pparβ/δ* expression, which in turn activates polarization toward the M2 phenotype (Figure 1). In agreement with this model, myeloid specific PPARβ/$\delta^{-/-}$ mice show adipocyte dysfunction, insulin resistance and hepatosteatosis [20]. Moreover, AT macrophage infiltration and inflammation and glucose intolerance are aggravated in fructose-fed PPARβ/δ-null mice via nuclear factor E2-related factor 2 (Nrf2) [22].

Although AT is not traditionally thought of as oxidative, PPARβ/δ activation increases total oxidative metabolism in white AT (WAT) [23] and this may explain why transgenic mice with tissue-specific over-expression of PPARβ/δ in WAT are resistant to both high fat diet (HFD)-induced and genetically predisposed obesity, and have reduced adipocyte triglyceride accumulation, circulating non-esterified FAs (NEFAs) and circulating triglyceride (TG) [24].

Little is known about the functions of PPARβ/δ in human WAT. PPARβ/δ expression is lower in morbidly obese patients than non-obese subjects [25], and TNF-α reduces PPARβ/δ transcriptional activity in adipocytes [26] (Table 1), suggesting that inflammation may exacerbate dysregulation of WAT by reducing the activity of the receptor in humans.

As VAT (which is very sensitive to lipolytic stimuli) increases, so does the rate of lipolysis. This increases FA mobilization and raises the levels of circulating SFAs, which induce insulin resistance through activation of the IκB kinase β (IKKβ)/nuclear factor (NF)-κB and c-Jun N-terminal kinase 1 (JNK1)/ activator protein-1 (AP-1) inflammatory pathways, and ultimately the increase in the levels of cytokines in WAT and other tissues [19]. Part of the pro-inflammatory effects of SFAs are mediated by the activation of toll-like receptor 4 (TLR4) and require the presence of its endogenous ligand, fetuin A, as removing either of them prevents FA-induced inflammation and insulin resistance in adipocytes [27]. TLR4 is ubiquitously expressed in human cells, and binds a plethora of exogenous and endogenous ligands, including lipopolysaccharide (LPS). LPS, a component of the cell walls of gram-negative bacteria, is produced by gastrointestinal-tract microflora. Obesity [28] and HFD [29] drive alterations in gut microbiota composition that result in leakage of LPS into the systemic circulation, promoting inflammation and insulin resistance. The PPARβ/δ agonist GW501516 inhibits LPS-induced cytokine expression and secretion by preventing NF-κB activation in adipocytes via MAPK–extracellular signal–regulated kinase (ERK)1/2 (MEK1/2) activation [30] (Figure 1) (Table 1). Indeed, inhibition of this pathway reduces LPS-induced cytokine production in adipocytes [31]. Moreover, IL-1β, IL-6, ERK1/2 and NF-κB have been shown to account for some of the differences seen in VAT of morbidly obese individuals with insulin resistance, compared to VAT from morbidly obese that are insulin-sensitive [32]. In fact, the authors of that study suggested that these are critical effectors that mediate the inflammatory effects promoting insulin resistance.

Among the inflammatory mediators released by WAT, increased levels of IL-6 correlate most strongly with obesity and insulin resistance and predict the development of T2DM [33]. IL-6 signals through glycoprotein gp130, which activates Janus tyrosine kinases (Jak1, Jak2, Tyk2), which in turn phosphorylate signal transducer and activator of transcription 3 (STAT3) and ultimately attenuates the insulin signaling pathway (Figure 1) [33]. GW501516 binding to and activation of PPARβ/δ prevents IL-6-induced insulin resistance via the STAT3 pathway in 3T3-L1 adipocytes, whereas this pathway is over activated in WAT of PPARβ/δ-null mice compared with that of wild-type mice [34] (Table 1). PPARβ/δ activation also blocks the interaction of STAT3 with the chaperone heat shock protein 90 (Hsp90), which contributes to STAT3 activation, whereas the association of these two proteins is greatly enhanced in the WAT of PPARβ/δ-null mice.

*PPARβ/δ effects in skeletal muscle cells*

Skeletal muscle accounts for most insulin-stimulated glucose utilization, and is the primary site of insulin resistance in obesity and T2DM [7]. The induction of insulin resistance in skeletal muscle is the result of changes in the levels of plasma NEFAs and adipokines caused by obesity. Increased SFA levels induce insulin resistance in skeletal muscle cells through TLR-dependent and -independent mechanisms [19] (Figure 2), explaining an important component of SFA induced "lipotoxicity" seen in insulin resistance. For instance, in addition to TLR-dependent mechanisms, the presence of high plasma NEFAs increases FA flux in skeletal muscle cells, exceeding their β-oxidation capacity, which then leads to the accumulation of FA derivatives such as diacylglycerol (DAG) and ceramide [35] that ultimately attenuate insulin signaling.

DAG activates protein kinase Cθ (PKCθ) in skeletal muscle [36], which in turn activates IKKβ-NF-κB. Both kinases phosphorylate IRS-1 on serine residues, attenuating the insulin signaling pathway. PPARβ/δ has been shown to transcriptionally regulate oxidative metabolism in muscle [12,13], providing a therapeutic strategy to prevent FA-induced insulin resistance. The rate-limiting step in β-oxidation of long-chain FAs is their transport into mitochondria via carnitine palmitoyltransferase-1β (CPT-1β). CPT-1β activity is inhibited by malonyl-CoA, a product of acetyl-CoA carboxylase, which, in turn, is inhibited by AMP-activated protein kinase (AMPK), a metabolic sensor that detects low ATP levels and increases oxidative metabolism by reducing the levels of malonyl-CoA [37]. Pharmacological activation of PPARβ/δ prevents palmitate-induced insulin resistance in skeletal muscle cells by enhancing the expression of two PPARβ/δ-target genes involved in β-oxidation, *Cpt-1* and pyruvate dehydrogenase kinase 4 (*Pdk-4*), and by increasing AMPK phosphorylation [38]. Thus, PPARβ/δ activation prevents palmitate-induced increases in DAG, activation of PKCθ and NF-κB and the reduction in insulin sensitivity, effects that are abolished by the CPT-1 inhibitor etomoxir, thereby implicating increased β-oxidation as a likely factor in the changes observed. PPARβ/δ-mediated increase in skeletal muscle FA uptake requires diurnal hepatic PPARβ/δ activity through the synthesis of the PPARα endogenous ligand 18:0/18:1-phosphatidylcholine [39]. Different compounds, such as the antihypertensive drug telmisartan [40] and the myokine β-aminoisobutyric acid [41], have been reported to improve insulin sensitivity in skeletal muscle cells by activating PPARβ/δ since their effects were not observed when this nuclear receptor was antagonized or knockdown.

Endoplasmic reticulum (ER) stress is involved in the association between SFA-induced inflammation and insulin resistance [42,43]. Part of the antidiabetic effects of PPARβ/δ activation in skeletal muscle cells exposed to palmitate involve the inhibition of ER stress and are dependent on AMPK activation [44] (Figure 2). This mechanism seems to involve a negative crosstalk between AMPK and ERK1/2 and may also contribute to the effects observed, since ERK1/2 inhibition was found to restore the AMPK and Akt pathways, and to reverse ER stress-induced insulin resistance in skeletal muscle cells [45].

Skeletal muscle is an important consumer of FAs and PPARβ/δ provokes a switch in skeletal muscle fiber composition toward a higher oxidative capacity, through an estrogen-related receptor γ (ERRγ)/miRNA circuit [46], that helps prevent obesity and T2DM [12,13] (Figure 2). It is worth noting that the changes induced by PPARβ/δ are comparable to changes in the skeletal muscle of mice undergoing long-term exercise that promotes increases in PPARβ/δ expression in the muscle. Moreover, experiments in transgenic mouse over-expressing PPARβ/δ in skeletal muscle, that have enhanced exercise performance, showed an interaction between PPARβ/δ and the exercise-inducible kinase AMPK, which promotes glucose uptake, FA oxidation, mitochondrial biogenesis, insulin sensitivity and supranormal performance [47].

PPARβ/δ may also ameliorate fructose-induced insulin resistance by increasing in muscle the expression of fibroblast growth factor 21 (*Fgf21*) [48], a hormone able to induce favorable metabolic effects. In agreement with this, circulating FGF21 levels are increased in humans in response to pharmacological PPARβ/δ activation [49].

*PPARβ/δ effects in hepatocytes*

The liver is a central organ in metabolic control and alterations in liver function are frequently observed in obesity and T2DM. In fact, more than 40% of type 2 diabetic patients present hepatic steatosis, that if not treated it results in nonalcoholic steatohepatitis (NASH), cirrhosis, and eventually liver failure [50]. Lipid accumulation in the liver during insulin resistance is due to unregulated lipogenesis and reduced FA oxidation, with sterol regulatory element-binding protein-1 (SREBP-1) and AMPK, being the pivotal regulators of these processes in hepatocytes, respectively. In fact, aberrant expression and activity of SREBP-1c is associated with obesity and fatty liver, whereas AMPK activation provides a potential mechanism for the attenuation of hepatic steatosis [51]. Recent findings also suggest that increased FA flux to the liver is a major contributor to hepatic steatosis [52]. Qin et al. [53] demonstrated that PPARβ/δ prevented the proteolytic processing and activity of SREBP-1 and improved hepatic steatosis in obese diabetic db/db mice, possibly via the induction of insulin-induced gene (*Insig*)-1, a protein that prevents SREBP activity by keeping the SREBP cleavage-activating protein (SCAP) / SREBP complex longer in the ER (Figure 3).

GW501516 administration to mice fed an HFD enhanced hepatic β-oxidation, nuclear lipin 1 protein accumulation and increased the levels of the hepatic endogenous ligand for PPARα, 16:0/18:1-phosphatidylcholine, leading to amplification of the PGC-1α-PPARα pathway. Moreover, GW501516 activated AMPK probably though an increase in the AMP:ATP ratio in hepatocytes [54]. Similar findings have been obtained more recently in mice deficient in the LDL receptor (*Ldlr*$^{-/-}$ mice) fed a high-fat, high-cholesterol diet, were enhanced β-oxidation, reduced lipogenesis and improved insulin sensitivity were observed, following treatment with GW501516 [55] (Table 1).

Adenoviral delivery of PPARβ/δ in the liver and activation also confirmed that targeting PPARβ/δ reduces fasting glucose levels in chow- and HFD-fed mice and indirectly activates AMPK, likely contributing to its glucose-lowering activity [56]. Furthermore, PPARβ/δ activation reduces hepatic glucose production by increasing the level of the pentose phosphate shunt and by promoting monounsaturated FA synthesis in the liver [57].

Liver inflammation has also been implicated in hepatic insulin resistance [58]. PPARβ/δ activation in HepG2 cells attenuated IL-6-induced insulin resistance through inhibition of STAT3 [58]. Moreover, PPARβ/δ mediated alternative M2 activation of the hepatic resident macrophages (Kupffer cells) ameliorated HFD-induced insulin resistance, while PPARβ/δ deficiency in Kupffer cells led to hepatic dysfunction and systemic insulin resistance [21], in obese mice. Some PPARβ/δ agonists may also show antifibrotic effects [15], suggesting a protective effect of these drugs in NASH.

In humans, PPARβ/δ agonists reduce hepatic fat content and improve plasma markers of liver function (γ-glutamyltransferase and alkaline phosphatase) [59,60]. Although clinical data are still scarce these findings suggest that PPARβ/δ ligands show promise for the treatment of chronic liver disease in humans.

*PPARβ/δ effects in the cardiovascular system*

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality for patients with T2DM. A major cause of CVD in T2DM is atherogenic dyslipidemia [61], initiated by hepatic overproduction of TG rich lipoproteins; very low-density lipoprotein (VLDL) overproduction leads to a reduction in high-density lipoprotein

(HDL)-cholesterol and an increase in small, dense low-density lipoprotein (LDL) particles [61]. The main factor influencing hepatic triglyceride secretion is FA availability. The increase in hepatic β-oxidation in liver following PPARβ/δ activation likely helps prevent hypertriglyceridemia in insulin resistant states by reducing the availability of FAs and subsequent hepatic VLDL secretion [54]. Moreover, PPARβ/δ activation inhibits human macrophage foam cell formation and inflammation induced by VLDL [62]. Likewise, PPARβ/δ agonist administration inhibits aortic inflammation and attenuates the progression of pre-established atherosclerosis in *Ldlr*$^{-/-}$ mice [63] and in other animal models of atherosclerosis [64] (Table 1). This anti-atherosclerotic effect involves the ability of PPARβ/δ to repress inflammatory gene expression by releasing the inflammatory suppressor protein B cell lymphoma-6 (BCL-6) in macrophages [12,13].

PPARβ/δ activation also exerts favorable effects on HDL levels [for review see 64 and 65], a lipoprotein with protective effects on endothelial function and atherosclerosis [66]. A recent study demonstrated that the PPARβ/δ agonist GW0742 also regulates the expression of hepatic phospholipid transfer protein, which contributes to the maintenance of HDL levels in plasma and generates preβ-HDL [67].

The available *in vitro* and *in v*ivo data prompted the evaluation of PPARβ/δ agonists in small-scale clinical trials, mainly for the treatment of atherogenic dyslipidemia and metabolic dysregulation [64,65]. In healthy volunteers, GW501516 increased HDL-cholesterol levels and improved postprandial triglyceride clearance [68]. In overweight volunteers, GW501516 treatment stimulated moderate weight loss, decreased plasma

triglycerides, apoB, NEFAs, total and LDL-cholesterol and improved insulin sensitivity [59]. In subjects with low HDL-cholesterol, GW501516 increased the plasma levels of this lipoprotein, reduced VLDLs, NEFAs, ApoA1, ApoB and LDL-cholesterol, whereas it increased the number of large LDL particles, indicating the transition toward a less atherogenic lipoprotein profile [69]. In dyslipidemic patients with visceral obesity, GW501516 treatment decreased plasma triglyceride, NEFAs, ApoB100 and ApoB48 and increased plasma HDL-cholesterol associated with increased ApoA2 production and reduced cholesteryl ester transfer protein activity [69]. Although the development of GW501516 was discontinued due to preclinical adenocarcinoma [12], its interesting effects led to the development of new compounds such as MBX-8025 and CER-002. MBX-8025 has been evaluated in monotherapy or in combination with atorvastatin in dyslipidemic overweight patients [60,71]. MBX-8025 administration reduced plasma triglycerides and NEFAs, and increased HDL-cholesterol. It also improved insulin sensitivity, VLDL particle number and the presence of small LDL particles, and reduced the number of patients meeting diagnostic criteria for the metabolic syndrome [60,71]. Moreover, MBX-8025 significantly reduced high-sensitivity C-reactive protein (hsCRP) among those subjects with elevated hsCRP at baseline [60]. Recently, CER-002, a new PPARβ/δ agonist that increases HDL-cholesterol levels and halts the progression of atherosclerosis in pre-clinical studies, has completed Phase I clinical trial for treatment of cardiovascular disease (http://www.cerenis.com/en/). Moreover, elafibranor (GFT505), a dual PPARα - β/δ agonist shows both insulin sensitizing and hepatoprotective effects in humans and its efficacy in NASH is currently being investigated in a phase IIb clinical trial [72,73].

Increased risk of CVD in type 2 diabetic patients has also been attributed to the presence of endothelial dysfunction [74]. Under normal conditions, stimulation of the insulin receptor results in Akt phosphorylation, endothelial nitric oxide (NO) synthase phosphorylation, and vasodilation. However, insulin resistance in the vascular system impairs the endothelium-dependent response to insulin [75]. PPARβ/δ activation protects endothelial function in diabetes through several mechanisms; including increased levels of antioxidant genes, anti-inflammatory effects, regulation of angiogenesis and apoptosis inhibition [see reference 76 for review]. More recently, some novel mechanisms for the vascular protective effect of PPARβ/δ agonists have been described, such as a decrease in VLDL receptor expression and VLDL uptake via the induction of miR-100 [77], the prevention of hyperglycemia-induced endothelial dysfunction through PDK4 activation [78] or lipid-induced endothelial dysfunction through up-regulation of CPT-1 [79]. Moreover, the beneficial effects of metformin in endothelial function in obese and diabetic mice have been attributed to inhibition of ER and oxidative stress, and an increase in NO availability via AMPK/PPARβ/δ activation [80].

Insulin resistance and T2DM in the heart can promote the development of diabetic cardiomyopathy, which is a major cause of morbidity and mortality in diabetic patients [81]. Cardiomyocyte insulin resistance is characterized by increased FA uptake and decreased glucose uptake [81]. The increase in the rate of FA uptake provokes the accumulation of FA-derived complex lipids, such as DAG, promoting lipotoxicity, and inflammation and further attenuating insulin signaling. Although the proteins involved in FA transport and oxidation are under the transcriptional control of PPARβ/δ, cardiac

overexpression of PPARβ/δ in mice did not result in myocyte lipid accumulation or cardiomyopathy, even when fed an HFD, whereas PPARα cardiac overexpression mimicked insulin resistance [13]. Moreover, activation of PPARβ/δ prevented activation of NF-κB in the heart of mice fed an HFD and in human cardiac cells exposed to palmitate, through a mechanism that seems to involve an increase in the physical interaction between PPARβ/δ and the p65 subunit of NF-κB [82].

One of the underlying mechanisms responsible for the progression of diabetic cardiomyopathy to heart failure is ER stress [81]. The PPARβ/δ agonist GW501516 attenuated palmitate-induced ER stress in human cardiac cells through a mechanism that might involve the induction of autophagy [83].

*PPARβ/δ effects in β-cells*

Both insulin resistance and impaired insulin secretion due to the progressive decline in pancreatic β-cell function and mass contribute to the development of T2DM [84]. The gut hormone incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) can preserve the morphology and function of pancreatic β-cells [82]. In addition, GIP and GLP-1 stimulate insulin secretion and inhibit glucagon secretion in a glucose-dependent manner. Daoudi et al. [85] reported that PPARβ/δ activation potentiated GLP-1 production by the small intestine and suggested that pharmacologic targeting of PPARβ/δ might be a promising approach in the treatment of patients with T2DM, especially in combination with dipeptidyl peptidase IV inhibitors. In addition, PPARβ/δ and its endogenous ligand 4-hydroxy-2E-nonenal amplified the adaptive insulin secretory response of β-cells upon exposure to

increasing glucose and FA concentrations [86,87]. PPARβ/δ is also crucial for the maintenance of mitochondrial function, and thus ATP production, required for glucose-stimulated insulin secretion (GSIS) [88]. Activation of PPARβ/δ in β-cells is afforded by the lipolytic action of the major triacylglycerol hydrolase ATGL (adipose triglyceride lipase). ATGL-catalyzed lipolysis controls TG and FA content and utilization in islet β-cells. FAs provided by the action of ATGL activate PPARβ/δ, which in turn increases the expression of genes involved in mitochondrial function, and ATP production required for GSIS. In fact, GW501516 administration to mice with β-cell specific ATGL deletion improved glucose tolerance, suggesting that GW501516 can substitute for ATGL [88]. This is interesting, since HFD feeding, which is associated with insulin resistance and β-cell dysfunction, decreases ATGL levels in islets, and pharmacological PPARβ/δ activation could compensate for this reduction. Overall, these findings suggest potential mechanisms by which PPARβ/δ agonist reduces blood glucose levels, in association with improved insulin sensitivity and pancreatic islet function [89].

**Concluding remarks and future perspectives**

A substantial body of preclinical evidence supports the hypothesis that PPARβ/δ activation represents an attractive therapeutic strategy for the treatment and prevention of obesity-induced insulin resistance, T2DM and CVD. Thanks to this potential interest, PPARβ/δ activators have reached clinical trials. However, the number of clinical trials performed to study the efficacy and safety of PPARβ/δ ligands in humans remains scarce and some questions need to be answered (see outstanding questions). Future studies are needed to confirm the therapeutic efficacy and potential deleterious effects

of these drugs. Safety issues have been raised regarding the role of PPARβ/δ ligands in carcinogenesis, with conflicting studies indicating that PPARβ/δ activation can both inhibit and promote tumorigenesis, the latter particularly in animal models [90]. This is of interest, given that PPARs are known to be expressed at lower levels in human cells than they are in rodent cells, and gene expression is also differentially regulated by PPARs in human *versus* rodent cells [13]. For instance, the PPARγ ligand pioglitazone was associated with a higher incidence of bladder cancer in preclinical studies, whereas in humans, although conflicting results have been reported, the latest large-scale studies indicate that pioglitazone use was not associated with the incidence of bladder cancer [91,92]. Similarly, synthetic PPARα ligands induce carcinogenesis in rodents. However, human subjects receiving fibrates for the treatment of dyslipidemia are resistant to the carcinogenic effects of these PPARα ligands [93].

In summary, while several concerns remain regarding the future therapeutic use of PPARβ/δ agonists, these drugs have demonstrated their efficacy in the treatment of T2DM and CVD in preclinical studies and in a few short clinical studies in humans. Although these data are promising, additional studies must be performed to confirm the efficacy and the safety of these drugs in humans.

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**Glossary**

**Adipokine:** any protein (cytokine, growth factor, hormone, etc.) secreted from adipocytes.

**Atherogenic dyslipidemia:** the presence of high levels of triglycerides, small-dense low-density lipoprotein (LDL) and low levels of high-density lipoprotein cholesterol (HDL). It is often observed in patients with metabolic syndrome, obesity, insulin resistance and T2DM.

**Endothelial dysfunction:** a key event in the development of atherosclerosis that compromises the normal function of endothelial cells leading to the inability of arteries and arterioles to dilate fully in response to an appropriate stimulus.

**Hypertrophic adipocytes:** larger adipocytes that are prone to hypoxia, fibrosis, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, generation of reactive oxygen species (ROS), altered adipokine secretion and infiltration and activation of inflammatory/immune cells, all resulting in insulin resistance

**Lipotoxicity:** a process that results from the accumulation of lipid intermediates in non-adipose tissues.

**Low-grade inflammatory process:** Obesity-induced inflammation differs from inflammation in classical immunity in that it is a low-grade inflammation that produces much lower levels of circulating cytokines. It is also a chronic inflammation because it requires relatively long high fat diet treatments (>8 weeks in animal models) before inflammation is observed in AT. Obesity-induced inflammation is the result of exposure to nutrient excess, resulting in adipocyte hypertrophy-macrophage infiltration and polarization-activation of inflammatory pathways.

**Macrophage polarization:** To fulfill their functionally distinct roles, macrophages are capable of polarizing toward a spectrum of phenotypes, which include classical (pro-inflammatory, M1) and alternative (anti-inflammatory, M2) activation states.

**Small dense LDL particles:** LDL particles are heterogeneous in sizes, density, and lipid composition. Small dense LDL (diameter ≤25.5nm) are highly atherogenic as a result of their higher penetration into the arterial wall, their lower binding affinity for the LDL receptor, a prolonged plasma half-life, and lower resistance to oxidative stress, compared to buoyant LDL.

**Unfolded protein response (UPR):** adaptive (defensive) ER stress response that involves the activation of a signaling pathway so as to restore the folding capacity. If ER homeostasis cannot be restored apoptosis is induced**.**

**Table 1.** EC50 and main reported antidiabetic effects of synthetic PPARβ/δ ligands.

| PPARβ/δ ligand | $EC_{50}$ | Main reported antidiabetic effects on different cell types and systems | REF |
|---|---|---|---|
| GW501516 | ~ 1 nM | **Adipocytes:** | |
| | | Prevents the expression of inflammatory markers and the reduction in the expression of PPARβ/δ-target genes in human adipocytes stimulated with TNF-α. | [26] |
| | | Inhibits LPS-induced cytokine expression and secretion by preventing NF-κB activation. | [30] |
| | | Prevents IL-6-induced insulin resistance via the STAT3 pathway in adipocytes. | [34] |
| | | **Skeletal muscle cells:** | |
| | | Prevents lipid-induced inflammation and insulin resistance in skeletal muscle cells by reducing the DAG-PKCθ-NF-κB pathway and ER stress through an AMPK-dependent mechanism. | [38,44] |
| | | **Hepatocytes:** | |
| | | In liver of mice fed an HFD, activates AMPK and increases LIPIN 1 protein levels and the hepatic levels of the endogenous ligand for PPARα, 16:0/18:1-phosphatidylcholine, potentiating the PGC-1α-PPARα signaling system and increasing hepatic FA oxidation. | [54] |
| | | In $Ldlr^{-/-}$ mice fed a high-fat, cholesterol-containing diet, normalizes fasting hyperinsulinemia and selective hepatic insulin resistance and reverses the polarization of the liver toward a M1 pro-inflammatory state. | [55] |
| | | Ameliorates hyperglycemia by increasing glucose flux through the pentose phosphate pathway and enhancing fatty acid synthesis. | [56] |
| | | Despite the increased lipid accumulation, PPARβ/δ-regulated lipogenic program may protect against lipotoxicity. | [57] |
| | | Attenuates IL-6-induced insulin resistance through inhibition of STAT3. | [58] |
| | | **Cardiovascular system:** | |
| | | Attenuates diet-induced aortic inflammation, insulin resistance, and atherosclerosis in $Ldlr^{-/-}$ mice and in other animal models of atherosclerosis. | [63,64] |
| | | Promotes the release of BCL-6, which in turn can suppress the expression of pro-inflammatory cytokines and chemokines in macrophages. In dyslipidemic patients, overall effects include reductions in plasma triglyceride, NEFA, apoB100, apoB48 and small, dense LDL particle levels and increases plasma HDL-cholesterol. | [59,64, 65,67-70] |
| | | Protects endothelial function. | [76] |
| | | Induces microRNA-100 and decreases the uptake of VLDL in endothelial cells. | [77] |
| | | Prevents activation of NF-κB in the heart of mice fed an HFD. | [82] |
| | | Attenuates palmitate-induced ER stress in human cardiac cells through a mechanism that might involve the induction of autophagy. | [83] |
| GW0742 | ~ 1 nM | **Hepatocytes:** | |
| | | Prevents lipogenesis and SREBP-1 activation in hepatocytes via the induction of Insig-1. | [53] |
| | | **Cardiovascular system:** | |
| | | GW0742 (and GW501516) inhibit human macrophage foam cell formation and the inflammatory response induced by VLDL. | [62] |
| | | Regulates the expression of hepatic phospholipid transfer protein, | [67] |

| | | | |
|---|---|---|---|
| | | which contributes to maintenance of HDL levels. | |
| | | Protects endothelial function through several mechanisms and by up-regulating carnitine palmitoyltransferase-1 might prevent lipid-induced endothelial dysfunction. | [76,79] |
| | | **β-cells:** | |
| | | GW0742 (and GW501516) potentiate GLP-1 production by the small intestine. | [85] |
| L-165041 | 50 nM | **Cardiovascular system:** | |
| | | Protects endothelial cells. | [76] |
| MBX-802 | ~ 2 nM | **Cardiovascular system:** | |
| | | In dyslipidemic overweight patients, it reduces plasma triglycerides and NEFAs, and increases HDL-cholesterol. It improves insulin sensitivity and reduces the number of patients meeting diagnostic criteria for the metabolic syndrome, VLDL particle number and the presence of small LDL particles. | [60,71] |

BCL-6, B-cell lymphoma 6; DAG, diacylglycerol; $EC_{50}$, effective concentration 50 (assessed with human recombinant PPARβ/δ); ER, endoplasmic reticulum; GLP-1, glucagon-like peptide 1; Insig-1, insulin-induced gene; LDL, low-density lipoprotein; NEFA, non-esterified fatty acids; NF−κB, nuclear factor κB; PKCθ: protein kinase C θ; TLR4, Toll-like receptor 4; VLDL, very low-density lipoprotein.

## FIGURE LEGENDS

### Figure 1. Effects of PPARβ/δ activation on adipocytes and macrophages.

In macrophages, PPARβ/δ polarizes macrophages toward the alternatively activated, anti-inflammatory M2 phenotype, promoting insulin sensitivity in adipose tissue. In adipocytes, PPARβ/δ activation prevents LPS-induced NF-κB activation by inhibiting ERK1/2, thereby reducing the production of pro-inflammatory cytokines involved in the development of insulin resistance. Moreover, PPARβ/δ activation in adipocytes prevents IL-6-induced insulin resistance and induction of SOCS3 expression and STAT3 phosphorylation. Interaction of STAT3 with the chaperone heat shock protein 90 (Hsp90) contributes to many steps in STAT3 activation, and activated PPARβ/δ binds Hsp90 and prevents its interaction with STAT3, attenuating this pathway.

ERK1/2: extracellular signal–regulated kinase 1/2; IL: interleukin; IRS: insulin receptor substrate; JAK: Janus kinase; LPS: lipopolysaccharide; NF-κB: nuclear factor-κB; SOCS, suppressor of cytokine signaling; STATs: signal transducers and activators of transcription; TLR4, toll-like receptor 4; TRIF, Toll–IL-1 receptor domain-containing adaptor inducing interferon β.

**Figure 2. Effects of PPARβ/δ activation on skeletal muscle cells.**

PPARβ/δ activation increases the expression of genes involved in fatty acid oxidation (carnitine palmitoyltransferase 1, *Cpt-1;* pyruvate dehydrogenase kinase 4; *Pdk-4)* reducing the availability of fatty acids that can be accumulated in the form of fatty acid-derivatives, such as diacylglycerol (DAG). This effect reduces the activation of the DAG-PKCθ-NF-κB pathway that attenuates the insulin signaling pathway. Both PKCθ and IκKβ phosphorylate insulin receptor substrate (IRS) proteins on serine residues, attenuating insulin signaling and promoting insulin resistance. In addition, NF-κB activation increases the expression and secretion of pro-inflammatory cytokines that induce insulin resistance. PPARβ/δ activation also inhibits fatty acid-induced ER stress, which contributes to insulin resistance through several mechanisms, including the activation of the NF-κB pathway. The inhibition of ER stress following PPARβ/δ activation seems to involve the activation of AMPK and the subsequent inhibition of ERK1/2. Finally, PPARβ/δ provokes a switch in skeletal muscle fiber composition toward a higher oxidative capacity that contributes to prevent obesity and T2DM. The changes induced by PPARβ/δ activation are comparable to those observed in mice undergoing long-term exercise.

AMPK, AMP-activated protein kinase; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinases; IκK, IκB kinase; IRS, insulin receptor substrate; PKC, protein kinase C; NF-κB, nuclear factor-κB; SFA, saturated fatty acid; TLR4, toll-like receptor 4;

**Figure 3. Effects of PPARβ/δ activation on hepatocytes.**

When lipids are low, sterol regulatory element-binding protein (SREBP) precursors are transported by SCAP to the Golgi complex where they are proteolytically processed and migrate to the nucleus. Once in the nucleus, SREBP-1 binds to sterol-response elements in target genes encoding enzymes involved in lipogenesis. INSIG-1 bound to endoplasmic reticulum (ER) effectively is released from the SREBP precursor bound to SREBP cleavage-activating protein (SCAP). PPARβ/δ activation induces *Insig-1* gene expression, reducing the proteolytic processing of SREBP1 and ameliorating hepatic steatosis in animal models of obesity and diabetes. PPARβ/δ agonists activate AMPK, promoting fatty acid (FA) oxidation, thereby contributing to reduce plasma triglyceride (TG) levels. As a result of AMPK activation nuclear LIPIN 1 protein levels are also increased. This fact, together with the increase in the levels of the hepatic endogenous ligand for PPARα, 16:0/18:1-phosphatidilcholine, following PPARβ/δ activation, leads to the amplification of the PGC-1α-PPARα signaling system, which exacerbates mitochondrial fatty acid oxidation by upregulating the expression of genes such as carnitine palmitoyltransferase 1 (*Cpt-1*).

AMPK: AMP-activated protein kinase; ER, endoplasmic reticulum; FA: fatty acid; 16:0/18:1-PC; 16:0/18:1-phosphatidilcholine; SCAP, SREBP cleavage-activating protein; SREBP, sterol regulatory element-binding protein

**Box 1, FIGURE I:** Potential mechanisms linking obesity and insulin resistance.

Expansion of adipose tissue in obesity arises from increased adipocyte size [7]. Hypertrophic adipocytes contribute to metabolic dysregulation in obesity due to their pro-inflammatory, insulin resistant phenotype. Adipose tissue hypoxia contributes to deranged adipokine secretion and low-grade inflammation. Some adipokines secreted in excess by hypertrophic adipocytes such as monocyte chemoattractant protein 1 (MCP-1) are known chemoattractants for monocyte/macrophage infiltration. The majority of macrophages in obese adipose tissue aggregates in "crown-like structures" completely surrounding dead adipocytes and scavenging adipocyte debris. Besides increasing macrophage number, obesity induces a phenotypic switch of adipose tissue macrophages towards an M1-like pro-inflammatory profile; in turn, these activated macrophages abundantly secrete pro-inflammatory adipokines. Attenuation of insulin sensitivity increases the rate of lipolysis, with increasing levels of free fatty acids (FFAs). Under these conditions mitochondrial function is impaired, leading to increased levels of ROS, and the secretory machinery of the adipocyte gets overloaded, causing endoplasmic reticulum (ER) stress that reduces the release of insulin-sensitizing adipokines, such as adiponectin. All these processes result in altered adipokine secretion and chronic changes in FFA metabolism that result in storage of lipids in non-adipose tissue, leading to "lipotoxic" effects in these cells.

**Box 2, FIGURE I:**Structure and function of PPARβ/δ.

(A) The primary and tertiary structure of PPARβ/δ are shown. The ligand binding domain (LBD) contains transactivation, transrepression, and dimerization functions

(B) The modes of transactivation and transrepression by the PPARb/d /RXR heterodimer are shown. (C) The three different mechanisms of transrepression are shown. For details see box 2.

DBD, DNA-binding domain. PPRE, Peroxisome proliferator response element.

**BOX 1: Expansion of adipose tissue can promote the transition to a metabolically dysfunctional phenotype.**

As obesity develops, adipocytes undergo hypertrophy owing to increased triglyceride storage [7]. Hypertrophic adipocytes frequently become dysregulated, displaying cellular stress, such as ER stress that contributes to inflammation and insulin resistance in the adipose tissue. Additional pathways, such as the cascades involving toll-like receptors (TLRs) and other pro-inflammatory cytokine, including TNFα and IL-6, further reduce insulin sensitivity [19]. This prompts increased lipolysis and the subsequent release of FFAs in the circulation that exacerbates insulin resistance and causes a decrease in insulin-mediated glucose uptake. This is followed by changes in the secretion profile of adipokines that in turn negatively influence a number of key tissues. In addition, the increase in the circulating levels of FFAs leads to increased storage of lipids in ectopic tissues, leading to "lipotoxic" effects in liver, skeletal muscle, pancreas and heart, and contributing to insulin resistance in these tissues [8]. In liver, the increased availability of FFAs leads to a higher secretion of VLDL, facilitating the development of atherogenic dyslipidemia [61]. Reduced glucose uptake in the postprandial state contributes to systemic glucotoxic effects.

In lean subjects, adipose tissue $Th_2$ (T helper) T cells, Treg (regulatory T) cells, eosinophils and M2-like resident macrophages predominate. Treg cells secrete IL-10 and also stimulate IL-10 secretion from resident M2-like macrophages [94]. Eosinophils

secrete IL-4 and IL-13 and further contribute to an anti-inflammatory, insulin-sensitive phenotype. In obesity, immune cells are recruited and contribute to local inflammation. This process is initiated by the excessive secretion of known chemoattractants for monocyte/macrophage infiltration (like MCP-1) by hypertrophic adipocytes. Monocytes respond to these chemotactic signals, transmigrate into the adipose tissue and become polarized to the highly proinflammatory M1-like state. Once recruited, these M1-like macrophages secrete proinflammatory cytokines. Obesity also leads to a reduction in the content of eosinophils and a shift in the T cells population with a decrease in the content of Tregs and an increase in $CD4^+$ $Th_1$ and $CD8^+$ effector T cells, which secrete pro-inflammatory cytokines [94]. B cell numbers also increase and activate T cells, which potentiate M1-like macrophage polarization, inflammation and insulin resistance. The majority of macrophages in obese adipose tissue aggregate in "crown-like structures", completely surrounding dead adipocytes and scavenging adipocyte debris.

The vascular infrastructure generally does not keep pace with the rapid tissue expansion during obesity, leading to local hypoxia [95]. This process may be a very early event triggering the inflammatory state and ER stress may further amplify the inflammatory state. Reactive oxygen species formation due to mitochondrial dysfunction can also stimulate pro-inflammatory processes in obesity.

**Box 2. Structure and mechanisms of action of PPARβ/δ.**

PPARβ/δ is a member of the nuclear receptor (NR) superfamily of ligand-inducible transcription factors (TFs). The PPAR subfamily comprises three isoforms; PPARα (NR1C1), PPARβ/δ (NR1C2) and PPARγ (NR1C3) [12,13]. The receptor was initially cloned in *Xenopus laevis* and was named PPARβ. Subsequently, the human and

orthologues were cloned and named NUC-1 and PPARδ, respectively. Currently, we use the terminology PPARβ/δ [12].

PPARβ/δ has an N-terminal region (A/B domain) with a variable, ligand independent transactivation domain called Activation function 1 (AF-1), followed by a hinge (C domain) region, the DNA binding domain (C domain) and the E domain. The E domain contains the ligand-binding domain (LBD) and a ligand dependent transactivation region (AF-2) (**Figure I panel A**). The LBD mediates dimerization, transcriptional activation, and transcriptional repression functions. Ligand binding and activation of PPARβ/δ leads to heterodimerization with its obligate dimerization partner RXR, dissociation of co-repressor proteins, binding of the PPARβ/δ / RXR heterodimer to PPREs and transactivation (**Figure I panel B**). PPARβ/δ also regulates gene expression independently of binding to PPREs, and via crosstalk with other transcription factors (TFs), through a mechanism termed receptor-dependent transrepression. Most of the anti-inflammatory effects of PPARs are probably explained by this mechanism. Thus, through this DNA-binding independent mechanism, PPARs suppress the activities of several TFs, including nuclear factor κB (NF-κB), activator protein 1 (AP-1), signal transducers and activators of transcription (STATs) and nuclear factor of activated T cells (NFAT). Three main transrepression mechanisms exist by which ligand-activated PPARβ/δ-RXR complexes negatively regulate the activities of other TFs (**Figure I panel B)** [13]. In the first mechanism, transrepression 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 PPARβ/δ may suppress the activity of other TF by sequestering these co-activators. In the second mechanism, activated PPARβ/δ-RXR heterodimers physically interact with other TFs (for example AP-1, NF-

κB, NFAT or STATs), preventing their binding to their response elements and thereby inhibits their ability to induce gene transcription. The third transrepression mechanism relies on the ability of activated PPARβ/δ-RXR heterodimers to inhibit the phosphorylation and activation of certain members of the mitogen-activated protein kinase (MAPK) cascade, preventing activation of downstream TFs. Many of these proposed mechanisms were discovered in different cellular models, and thus cannot easily be brought into context with each other.

## Outstanding Questions

Given the ubiquitous expression of PPARβ/δ, can this expression pattern give rise to safety issues when treating diabetic patients chronically with PPARβ/δ activators?

Are the antidiabetic effects of PPARβ/δ ligands mediated primarily by the activation of AMPK and the subsequent reduction in ERK1/2 activity?

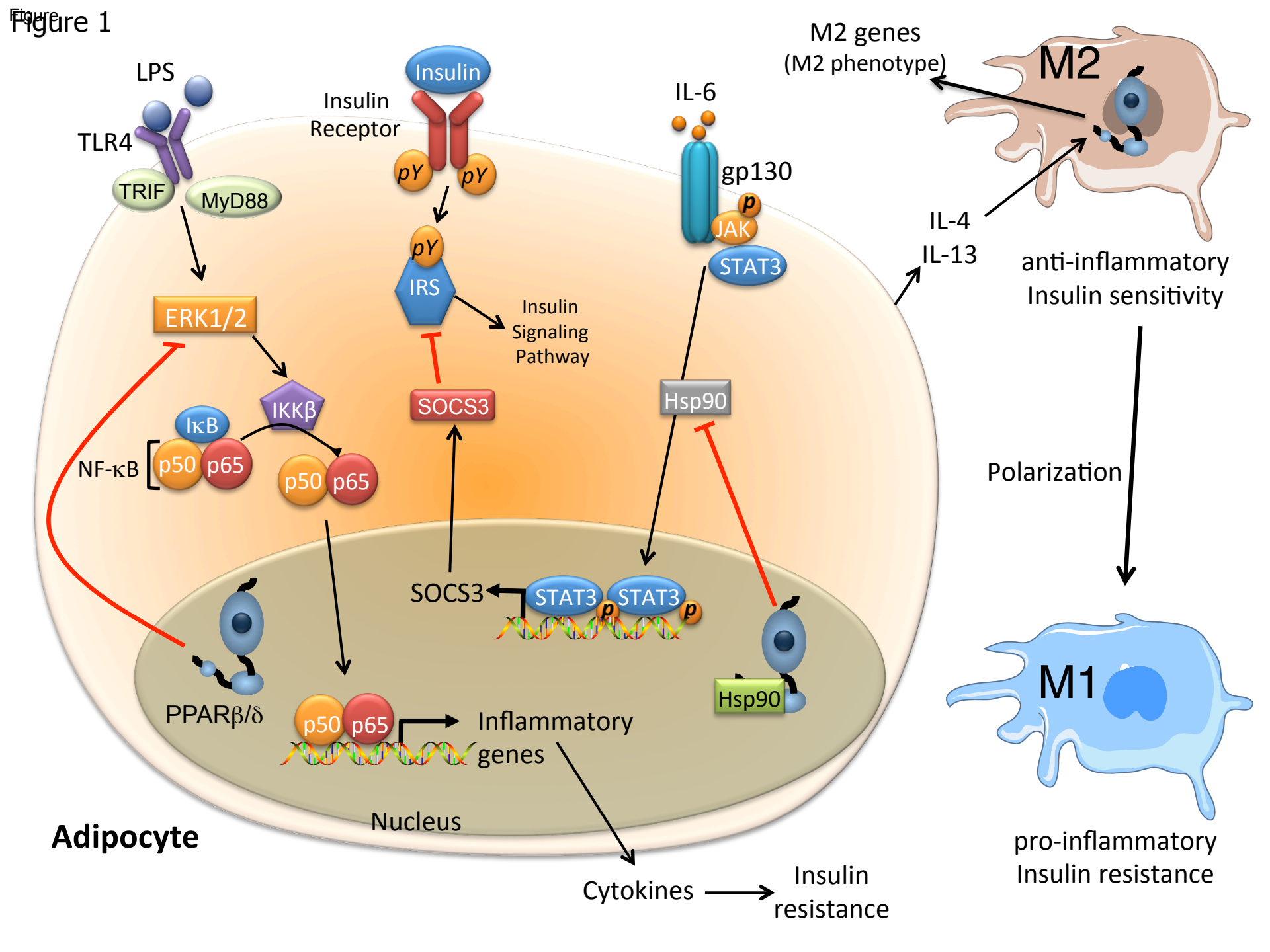
Can some of the more beneficial effects of certain drugs currently used in the treatment of T2DM, such as metformin, be attributed to the activation of PPARβ/δ-AMPK?

Can safety concerns regarding the role of PPARβ/δ ligands in carcinogenesis be fully ruled out in humans?

Are PPARβ/δ ligands capable of showing the same beneficial effects in diabetic patients as those observed in animal models of insulin resistance and T2DM?

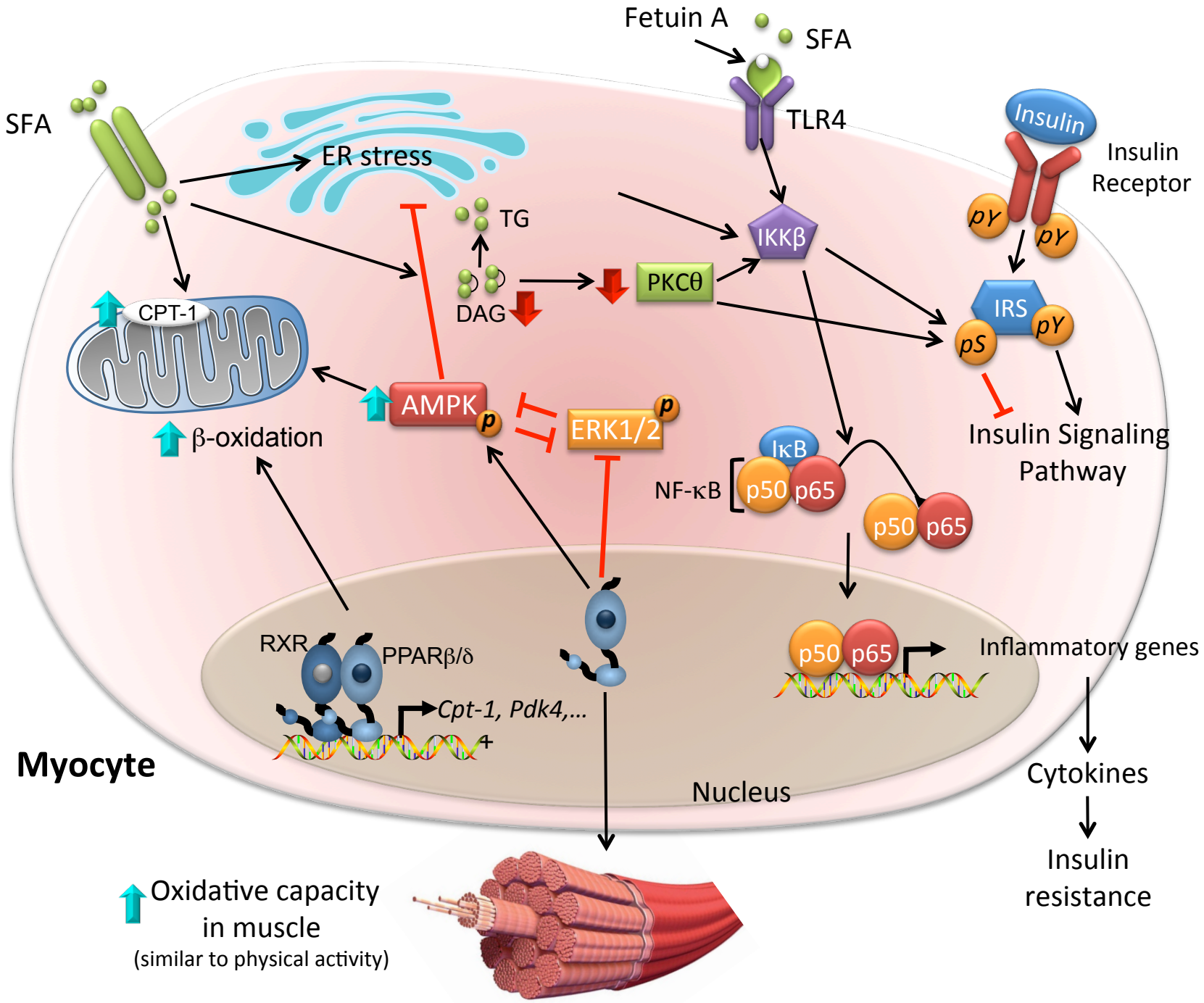
Are PPARβ/δ ligands future candidate drugs for the treatment of T2DM, dyslipidemia, NAFLD/NASH and CVD in humans?

Figure 1


LPS
TLR4
TRIF
MyD88
Insulin
Insulin Receptor
pY
pY
pY
IRS
Insulin Signaling Pathway
IL-6
gp130
p
JAK
STAT3
M2 genes
(M2 phenotype)
M2
IL-4
IL-13
anti-inflammatory
Insulin sensitivity
Polarization
ERK1/2
IKKβ
IκB
NF-κB
p50
p65
p50
p65
SOCS3
Hsp90
SOCS3
STAT3
p
STAT3
p
Hsp90
PPARβ/δ
p50
p65
Inflammatory genes
Nucleus
Adipocyte
M1
pro-inflammatory
Insulin resistance
Cytokines
Insulin resistance

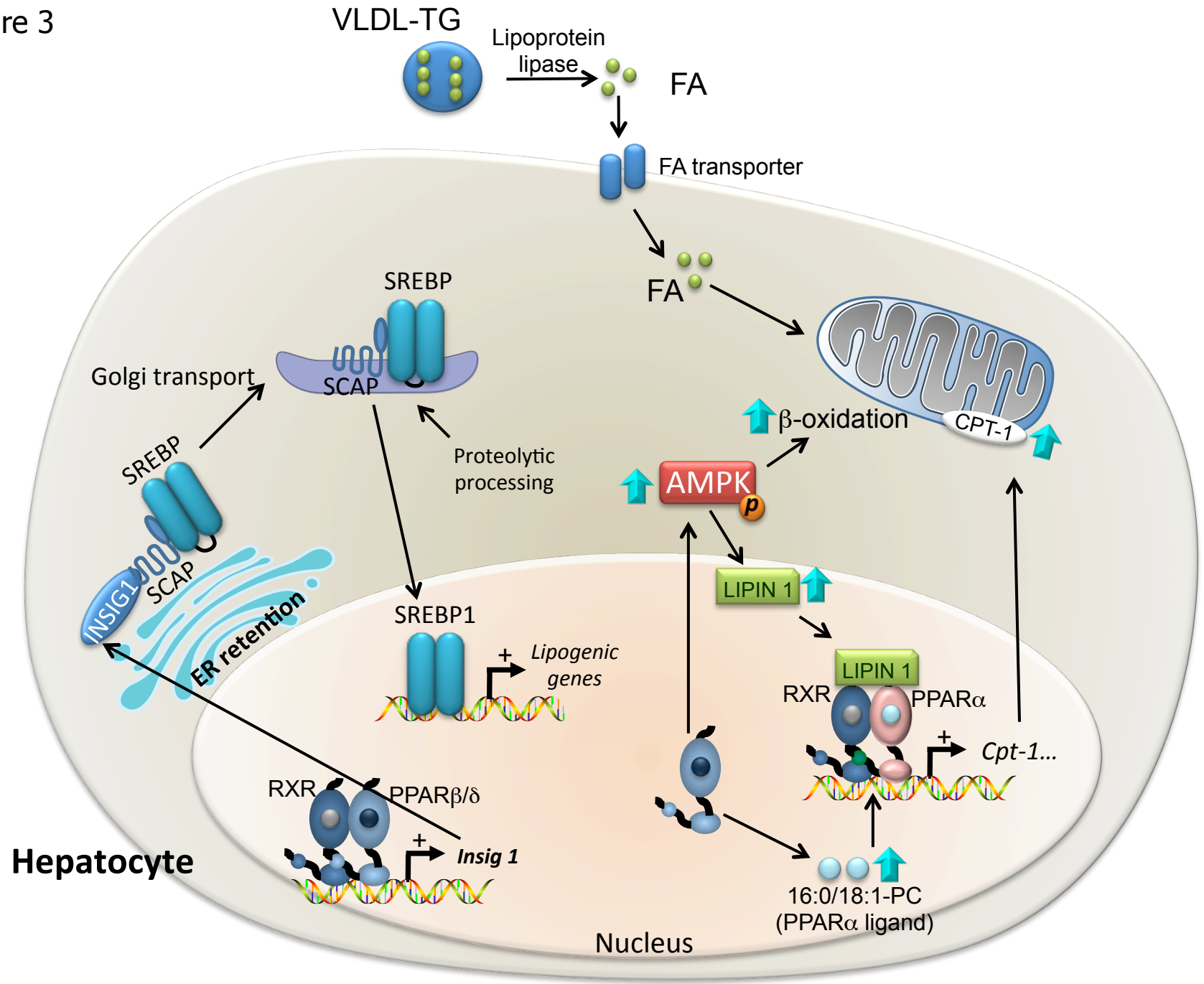
Figure

# Figure 2

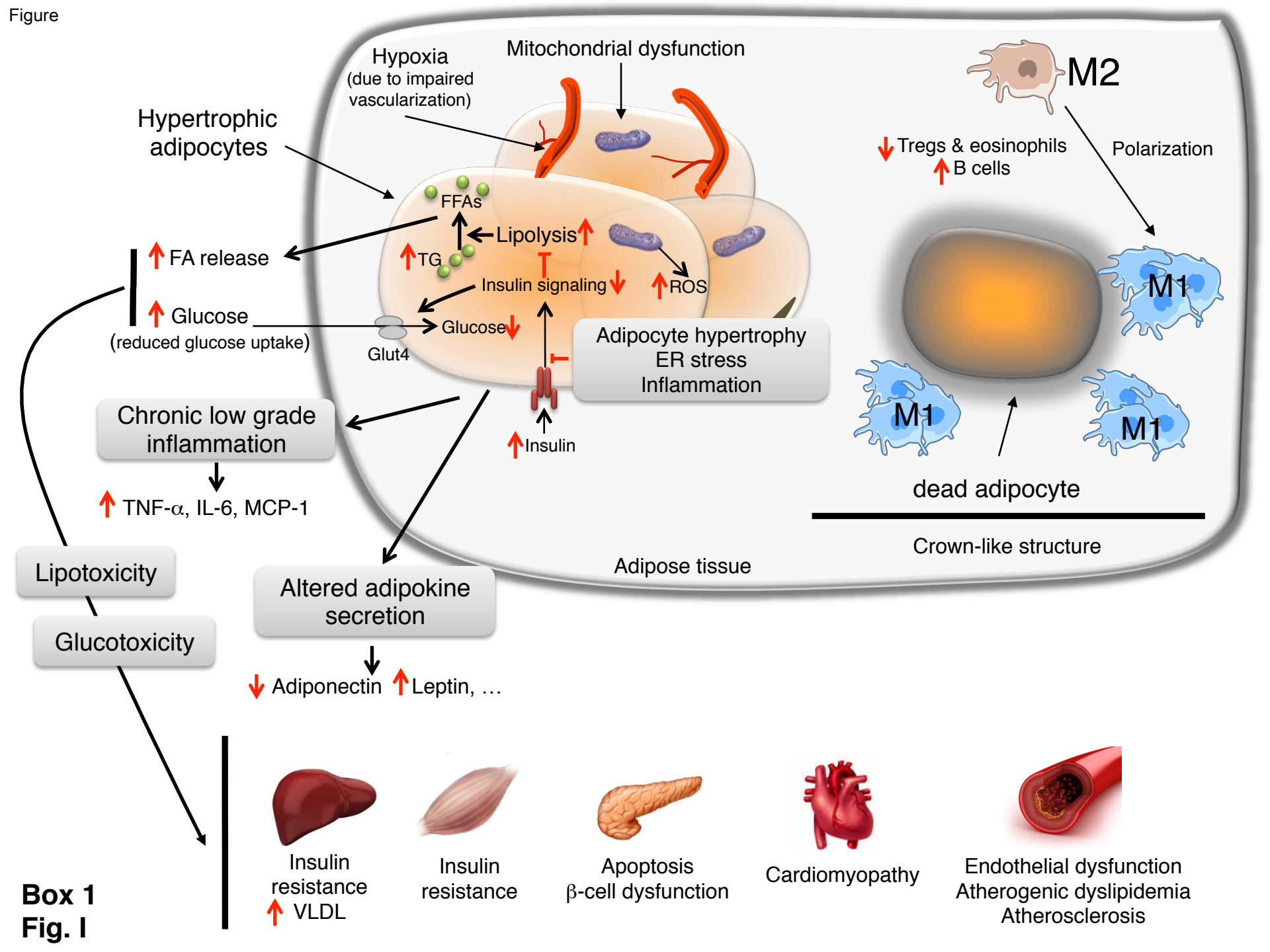


Figure

# Figure 3

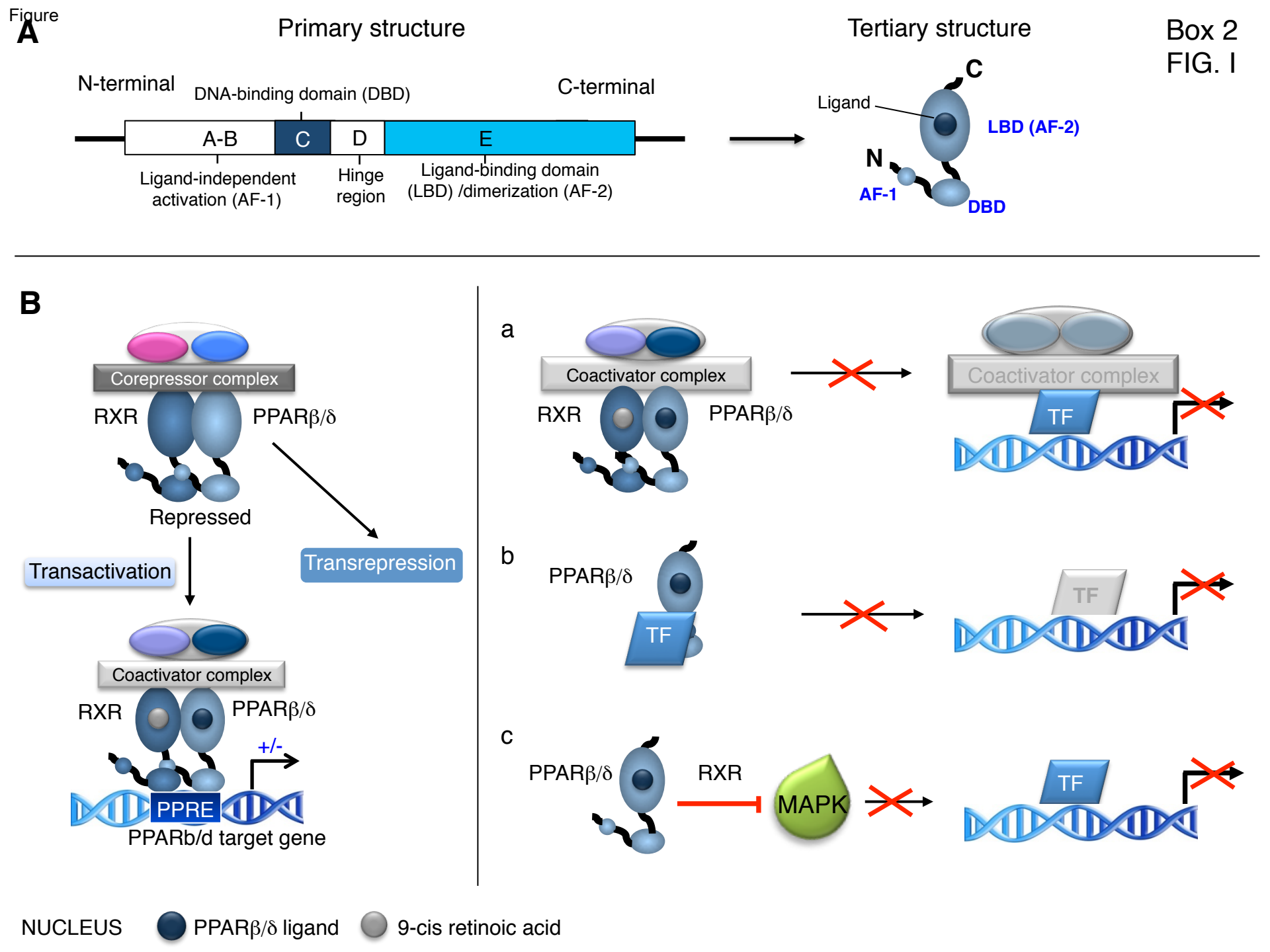


Figure




**Box 1**
**Fig. I**

Figure


A
Primary structure
Tertiary structure
Box 2
FIG. I
N-terminal
DNA-binding domain (DBD)
C-terminal
A-B
C
D
E
Ligand-independent activation (AF-1)
Hinge region
Ligand-binding domain (LBD) /dimerization (AF-2)
C
Ligand
LBD (AF-2)
N
AF-1
DBD
B
Corepressor complex
RXR
PPARβ/δ
Repressed
Transactivation
Transrepression
Coactivator complex
RXR
PPARβ/δ
+/-
PPRE
PPARb/d target gene
a
Coactivator complex
RXR
PPARβ/δ
Coactivator complex
TF
b
PPARβ/δ
TF
TF
c
PPARβ/δ
RXR
MAPK
TF
NUCLEUS
PPARβ/δ ligand
9-cis retinoic acid

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February 19th, 2016
**Dr. Iphigenia Tzameli,**
**Editor – Trends in Endocrinology and Metabolism**
**Revised Manuscript TEM-D-16-00020**

Dear Dr. Iphigenia Tzameli,

According to your instructions (see the mail sent the 8th of February), we submit a revised version of our Manuscript TEM-D-16-00020, now entitled "*Unravelling the effects of PPARβ/δ on insulin resistance and cardiovascular disease*".

First of all, I would like to thank the reviewers for their useful suggestions, following which we have made a number of modifications, as detailed below, which have allowed us to improve the manuscript. In addition, I have inserted all your suggestions, comments and explanations required in the new version of the manuscript, including amendments in the Title, Abstract, Main text, Boxes and Figures. The revised manuscript does not exceed 4000 words.

Therefore, I have addressed all the critiques and comments raised by the Reviewers and the Editor and, thus, I submit for your consideration the revised version of this manuscript in the hope that it will now be considered suitable for publication in Trends in Endocrinology and Metabolism.

I thank you in advance for your continued interest in our work.

Yours sincerely,

Manuel Vázquez-Carrera

**Comments for the Reviewer #1**

We would like to thank the Reviewer for his/her useful suggestions, which have allowed us to include changes to improve the manuscript. We are also very grateful for the comments made by this Reviewer ("*This review provides a fairly comprehensive description of prior publications that relate to PPARb/d and its potential utility in cardiometabolic diseases.*").

**. General comment**

***There is fairly strong clinical experience with two highly selective and potent delta agonists -GW501516 and MBX-8025- which were studied in patients with metabolic syndrome for periods of up to 12 and 8 weeks, respectively. The author needs to devote more effort to reviewing these data- they do not support a significant insulin sensitizing effect but to demonstrate clear and reproducible effects on lipid metabolism. There are also some suggestions of possible NASH benefits (hepatic fat decreased with GW501516) and possibly inflammation (CRP also decreased). I'd recommend less emphasis in the title and text on insulin resistance and inflammation per se – the author needs to achieve a more balanced review of connections between PPARd-inflammation – and insulin resistance with greater emphasis on the more clearly established changes in apolipoproteins / lipid metabolism.***

According to the suggestion of the reviewer I have changed the title of the manuscript to emphasize the cardiovascular effects of PPARβ/δ ligands. The title of the revised version of the manuscript is now "*Unravelling the effects of PPARβ/δ on insulin resistance and cardiovascular disease"*. In addition, I have expanded the effects of PPARβ/δ ligands on dyslipidemia and NAFLD/NASH without exceeding the limit of 4000 words. Moreover, I have included a new PPARβ/δ agonist, CER-002, which has completed Phase I for treatment of cardiovascular disease (http://www.cerenis.com/en/).

**Specific comments**

**Comment #1.** ***P. 4- Insulin resistance encompasses a lot more than a "defect in the ability of insulin to drive glucose into its target tissues" – patients are also resistant to other effects of insulin including suppression of hepatic glucose output and regulation of lipolysis.***

The reviewer is right. The sentence has been modified and in the revised version of the manuscript is presented as follows: "Insulin resistance, a hallmark of T2DM, encompasses defect in glucose uptake into insulin target tissues, suppression of hepatic glucose output and aberrant regulation of lipolysis."

**Comment #2.** ***P.5. It is not all clear that inflammation is a major driver of insulin resistance (in humans) – clinical experience with several anti-inflammatory mechanisms (eg ILb antibodies and anti-TNFa antibodies) has failed to confirm this hypothesis.***

The reviewer is right. In the case of TNF-α, several clinical studies have been performed to confirm its role in insulin resistance observed in preclinical studies. However, detailed analysis of these studies showed that they all suffered from short treatment durations (2 days to maximal 4weeks) and small sample sizes (7 to maximal 54 patients). Considering the genetic and metabolic heterogeneity of patients with type 2 diabetes, and the strong impact, among other factors, of life style changes on glucose parameters, these studies were underpowered. In contrast, other studies conducted in obese subjects or patients treated for additional conditions such as rheumatoid arthritis suggest that TNFα blockade may indeed alter insulin sensitivity or glycaemic parameters (Eur J Clin Invest 2004; 34: 641–642; Ann Rheum Dis 2005; 64: 765–766; Diabetes Care 2006; 29: 1712–1713; J Clin Endocrinol Metab 2011; 96: E146–150.). Therefore, additional clinical studies are needed to confirm whether TNFα antagonists might have a role in the treatment of diabetes.

Regarding IL-1β, a recent revision (Donath, M.Y. Nat Rev Drug Discov 2014; 13:465-76) concluded that IL-1β antagonists have the capacity to improve glucose metabolism in patients with diabetes after revising 8 clinical studies.

Although the improvement in HbA1c observed following anti-inflammatory treatments is low, it is likely that in the future the combination of various anti-inflammatory treatments will achieve greater efficacy (Diabetes Obes. Metab. 2013;15 Suppl 3:193-6).

Therefore, although several questions remain in the development of anti-inflammatory drugs for the treatment of type 2 diabetes, in my opinion, we cannot discard yet inflammation as a major driver of insulin resistance in humans.

**Comment #3.** ***P. 7 – Both Metabolex and Kalypsys are no longer in business. To my knowledge neither KD-3010 or MBX-8025 are in active development. Please, double check with ClinTrials.GOV. Please, note the Genfit molecule, GFT505 which is a dual PPAR$\alpha/\delta$ agonist – is in Ph2 for NASH.***

I have checked the ClinTrials.GOV web and I found no information about KD-3010, whereas CymaBay Therapeutics is evaluating the efficacy of MBX-8025 at present in primary biliary cirrhosis and in homozygous familial hypercholesterolemia. To avoid exceeding the limit of 4000 words of the manuscript I have decided not to mention whether the drugs are in active development. I have only mentioned that these drugs achieved clinical trials. In contrast, I have preferred to include the new PPARβ/δ agonist CER-002 that has completed Phase I clinical trial for treatment of cardiovascular disease.

In addition, in the new version of the manuscript I have included a paragraph about GFT505.

**Comment #4.** ***P. 10-11 – The text implies that mechanisms by which "lipotoxicity" leads to insulin resistance are largely based on inflammation. Most experts in this field believe that there is no evidence of overt inflammation in muscle (unlike adipose) despite increases in DAG and ceramides, etc. Whereas there is strong evidence that PPARd can induce fatty acid oxidation and augment mitochondrial function in muscle, there is scant evidence of an effect on "muscle inflammation" with PPARd nor much to link inflammation with the major effects on muscle metabolism and muscle function. Please address.***

There are conflicting findings about this subject. Some studies have found that inflammation, especially activation of the NF-κB pathway, contributes to lipid-induced insulin resistance in skeletal muscle [Diabetes 2002, 51:2005-11; Diabetes 2006, 55: 760-767; Diabetes 2008, 57: 2595-2602; Am J Physiol 2010, 299: E794-E801; Diabetes 2011, 60: 2810-2819; Diabetologia 2012, 55: 773-782]. However, I have softened the contribution of inflammation to insulin resistance in the skeletal muscle and in the beneficial effects of PPARβ/δ ligands in the revised version of the manuscript.

**Comment #5.** ***P. 13 Recent work in humans from Elizabeth Parks actually shows that patients with liver steatosis have increased fatty acid oxidation. Please also add increased fatty acid flux to the liver as a (the) major contributor to hepatic steatosis.***

In the revised version of the manuscript I have included this new contributor to hepatic steatosis (please, see reference 52).

**Comment #6.** ***The paper by Iwaisako et al. [PNAS 2012-E1369-76] is worth citing since it suggests an antifibrotic benefit of PPARd activation in NASH.***

Following your suggestion I have included this reference in the section dedicated to the effects of PPARβ/δ in hepatocytes (please, see Page 12, reference 15).

**Comments for the Reviewer #2**

We would like to thank the reviewer for his/her useful suggestions, which have allowed us to include changes to improve the manuscript. We are also very grateful for the comments made by this Reviewer ("*The manuscript by Vázquez-Carrera describes with high scientific accuracy the main preclinical and clinical information about the protective effects of PPARβ/δ activation in insulin resistance….).*

**Major comments**

**Comment #1.** ***The role of PPARβ/δ in high glucose induced insulin resistance in several tissues was not described.***

The reviewer is right. However, to avoid exceeding the limit of 4000 words of the manuscript, we have selected one reference showing the protective effects of PPARβ/δ under hyperglycemia conditions in endothelial cells: Quintela et al. (Br J Pharmacol. 2014, 171:3089-102.). Please, see page 15, reference 78.

**Comment #2.** ***The impairment of endothelium-dependent relaxant response to insulin should be mentioned as insulin resistance in vascular system.***

Following the suggestion of the reviewer I have included a comment mentioning this (please, see page 15, lines 336-339).

**Comment #3.** ***The impact of increased plasma HDL levels induced by PPARβ/δ activation in atherosclerosis and endothelial function should be described***

Following the suggestion of the reviewer, a reference has been included (please, see page 13, reference 66) in the revised version of the manuscript where the effects of HDL in atherosclerosis and endothelial function are described. Due to space limitations these effects cannot be described in the manuscript.

**Comment #4.** ***Vascular protective effects of PPARβ/δ activation, independently of the improvement of systemic lipid and glucose metabolism should be mentioned.***

The direct vascular protective effects of PPARβ/δ activation are mentioned in the following sentence: "*PPARβ/δ activation protects endothelial function in diabetes through several mechanisms; including increased levels of antioxidant genes, anti-inflammatory effects, regulation of angiogenesis and apoptosis inhibition [see reference 76 for review]*."

According to the instructions for authors of the journal, authors should *"Concentrate on the seminal references of the past 2–4 years (most references should be no more than 5 years old). Reviews can be cited to give the necessary background on the topic and refer to older data"*. Given that some of these effects were published before 2010 I cited a revision including the direct vascular protective effects of PPARβ/δ activation.

**Comment #5.** ***In outstanding questions include the mechanism involved on ERK1/2 inhibition by PPARβ/δ activation.***

Following the suggestion of the reviewer, this mechanism has been included in outstanding questions.

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January 22, 2016
Editor
Trends in Endocrinology and Metabolism

Dear Dr. Tzameli,

Here you will find enclosed the manuscript entitled “Unravelling the effects of PPARβ/δ on insulin resistance” to be submitted for publication in Trends in Endocrinology and Metabolism.

Sincerely yours,

Dr. Manuel Vázquez Carrera