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Peroxisome Proliferator-Activated Receptor β/δ (PPAR β/δ) as a Potential Therapeutic Target for Dyslipidemia

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1. Introduction

Dyslipidemia is a powerful predictor of cardiovascular disease in patients at high risk (Turner et al., 1998), such as type 2 diabetic patients. Lowering of LDL-C is the prime target for treatment (2002), but even with intensification of statin therapy, a substantial residual cardiovascular risk remains (Barter et al., 2007; Miller et al., 2008; Fruchart et al., 2008; Shepherd et al., 2006). This may partly be due to atherogenic dyslipidemia. This term is commonly used to describe a condition of abnormally elevated plasma triglycerides and low high-density lipoprotein cholesterol (HDL-C), irrespective of the levels of LDL-C (Grundy, 1995). In addition to these key components, increased levels of small, dense LDL-C particles are also present, which in conjunction with the former components conform the also called "lipid triad" (Shepherd et al., 2005). Other abnormalities include accumulation in plasma of triglyceride-rich lipoproteins (TLRs), including chylomicron and very-low-density lipoprotein (VLDL) remnants. This is reflected by elevated plasma concentrations of non-HDL-C and apolipoprotein B-100 (apoB). Postprandially, there is also accumulation in plasma of TLRs and their remnants, as well as qualitative alterations in LDL and HDL particles. Thus, hypertriglyceridemia is associated with a wide spectrum of atherogenic lipoproteins not measured routinely (Taskinen, 2003). The presence of this lipid plasma profile with high triglyceride and low HDL-C levels have been shown to increase the risk of cardiovascular events independent of conventional risk factors (Bansal et al., 2007; Barter et al., 2007; deGoma et al., 2008). In fact, guidelines recommend modifying high triglyceride and low HDL-C as secondary therapeutic targets to provide additional vascular protection (2002). The presence of atherogenic dyslipidemia is seen in almost all patients with triglycerides > 2.2 mmol/l and HDL-C < 1.0 mmol/l, virtually all of whom have type 2 diabetes or abdominal obesity and insulin resistance (Taskinen, 2003). Most of these alterations are also characteristic of metabolic syndrome, which is defined as the clustering

of multiple metabolic abnormalities, including abdominal obesity, dyslipidemia (high serum triglycerides and low serum HDL-C levels), glucose intolerance and hypertension (Eckel et al., 2005; Grundy et al., 2005).

2. Hypertriglyceridemia is crucial in the pathogenesis of atherogenic dyslipidemia

It is now recognized that the atherogenic dyslipidemia is mainly initiated by the hepatic overproduction of the plasma lipoproteins carrying triglycerides, the VLDL, which induce a sequence of lipoprotein changes leading to atherogenic lipid abnormalities in type 2 diabetes mellitus and metabolic syndrome (Adiels et al., 2008). Under these pathological conditions, the presence of insulin resistance at the level of adipose tissue leads to enhanced lypolisis and reduced free fatty acid (FFA) uptake and esterification which results in increased flux into the liver of FFA, which are either oxidized or esterified for triglyceride production, leading to hepatic steatosis and oversecretion into plasma of larger triglyceride-rich VLDL particles (Chan & Watts, 2011). These particles compete with chylomicrons and its remnants for clearance pathways regulated by lipoprotein lipase, an endothelial-bound enzyme, and by hepatic receptors, thereby exacerbating postprandial dyslipidemia. In addition, insulin resistance increases hepatic secretion of apoC-III, which is attached to VLDL delaying the catabolism of TRLs by inhibiting lipoprotein lipase and binding of remnant TRLs to hepatic clearance receptors (Chan & Watts, 2011). Finally, expansion of the VLDL triglyceride pool leads to cholesterol depletion and triglyceride enrichment of LDL and HDL through cholesteryl ester transfer protein, which facilitates the movement of cholesterol esters to VLDL, intermediate-density lipoprotein (IDL) and LDL from cholesterol ester rich HDL, leading to the accumulation in plasma of small, dense LDLs and a reduction in HDLs (Taskinen, 2003).

Since residual risk remains even after achieving an optimal LDL-C concentration with statins (Barter et al., 2007), probably due to other risk factors, such as high triglycerides, low HDL-C levels, defective glucose metabolism and other non-lipid-related risk factors (Kannel, 1983; Castelli, 1992; Lorenzo et al., 2010; Cederberg et al., 2010), the development of new drugs aimed at improving risk reduction is necessary. Among the new drugs for the treatment of the risk factors leading to the residual risk, PPARβ/δ activators might have a promising future. Interestingly, PPARβ/δ agonists have been demonstrated to be effective raising HDL-C and lowering triglyceride concentrations (Kersten, 2008). In addition to their lipid-modifying properties, PPARβ/δ agonists improve insulin resistance, which may also confer protection against the development of dyslipidemia (Coll et al., 2010a). This review summarizes the effects of PPARβ/δ on dyslipidemia identified during the last few years.

3. The PPAR family

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 (Kersten et al., 2000). To be transcriptionally active, PPARs need to heterodimerize with the 9-*cis* retinoic acid receptor (RXR) (NR2B) (Figure 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 (Feige et al., 2006). 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.).

Thus, the transcriptional activity of PPARs is modulated by co-activators and co-repressors (Feige et al., 2006). One of the best described PPAR co-activators is PPARγ coactivator 1 α (PGC-1α). Silencing mediator for retinoic and thyroid hormone receptor (SMRT) and the nuclear receptor co-repressor (NCoR) are co-repressors that interact with the PPARs in the absence of ligands (Zamir et al., 1997). Receptor-interacting protein 140 (RIP140), an important metabolic regulator, is another ligand-dependent co-repressor which interacts with PPARs.

Finally, PPAR activity is also regulated at the post-transcriptional level by phosphorylation, ubiquitinylation, and sumoylation (for a detailed review see (Feige et al., 2006)).

However, the regulation of gene transcription by PPARs extends beyond their ability to trans-activate 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 (Daynes & Jones, 2002). Most of the anti-inflammatory effects of PPARs are probably explained by this mechanism (Kamei et al., 1996; Li et al., 2000). Thus, through this DNA-binding independent mechanism, PPARs suppress the activities of several transcription factors, 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). 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 suppress the activity of other transcription factors that use the same co-activators (Delerive et al., 1999; Delerive et al., 2002). In the second mechanism, activated PPAR-RXR heterodimers are believed to act through physical interaction with other transcription factors (for example AP-1, NF-κB, NFAT or STATs). This association prevents the transcription factor from binding to its response element and thereby inhibits its ability to induce gene transcription (Desreumaux et al., 2001). 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 (Johnson et al., 1997), preventing activation of downstream transcription factors.

The PPAR family consists of three members, PPARα (NR1C1 according to the unified nomenclature system for the nuclear receptor superfamily), PPARβ/δ (NR1C2) and PPARγ (NR1C3) (Auwerx et al., 1999). PPARα 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 (Braissant et al., 1996). PPARγ 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 γ isotype is the molecular target for the anti-diabetic drugs, thiazolidinediones. PPARβ/δ is ubiquitously expressed and, for this reason, was initially thought to be a "housekeeping gene" (Kliewer et al., 1994). However, studies with knockout mice (Barak et al., 2002; Peters et al., 2000; Tan et al., 2001) and the development of specific and high-affinity ligands for this receptor have shown that PPARβ/δ is a potential molecular target for prevention or treatment of several disorders. In this review we will highlight the role of PPARβ/δ in those metabolic processes with potential for treating dyslipidemia.

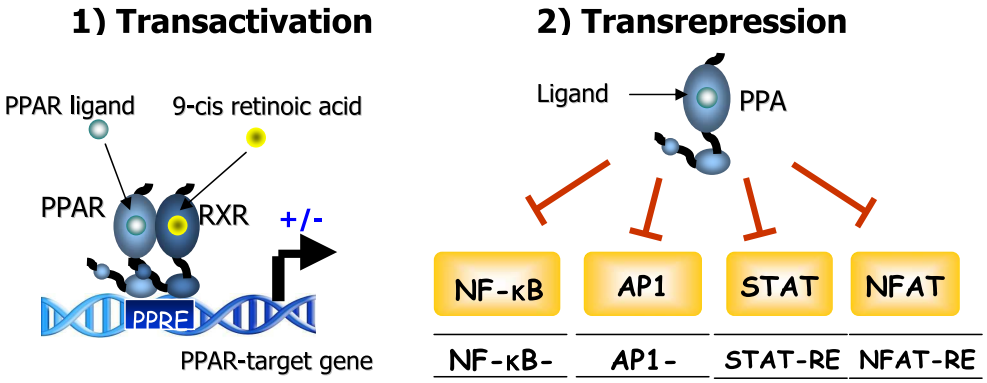

Fig. 1. Molecular mechanisms of Peroxisome Proliferator-Activated Receptors (PPARs). PPARs are ligand-activated transcription factors that regulate gene expression through two mechanisms: transactivation and transrepression. In transactivation PPAR-RXR heterodimers bind to DNA-specific sequences called 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 transrepression. Through this mechanism, PPARs inhibit the activity of several transcription factors such as NF-κB, which leads to anti-inflammatory effects. STAT denotes signal transducers and activators of transcription.

4. PPARβ/δ-specific features and ligands

The crystal structure of the ligand-binding domain of the PPARβ/δ isotype, which was first cloned in *Xenopus laevis* (Dreyer et al., 1992), revealed an exceptionally large pocket of approximately 1300 Å^3. This pocket is similar to that of PPARγ, but much larger than the pockets of other nuclear receptors (Takada et al., 2000; Xu et al., 1999), 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 PPARβ/δ in the low micromolar range (Xu et al., 1999; Forman et al., 1997; Yu et al., 1995; Krey et al., 1997). In addition, all-trans-retinoic acid (vitamin A) (Shaw et al., 2003) and fatty acids derived from VLDL (Chawla et al., 2003) can activate PPARβ/δFinally, the availability of three synthetic ligands (GW501516, GW0742 and L-165041) that activate PPARβ/δ at very low concentrations both in vivo and in vitro with high selectivity over other PPAR isotypes (Sznaidman et al., 2003) led to a huge increase in experimental studies on the role of PPARβ/δ in cellular processes. The EC_{50} for these compounds assessed with recombinant human PPARβ/δ were 1.0 nM for GW0742, 1.1 nM for GW501516 and 50 nM for L-165041 (Berger et al., 1999; Sznaidman et al., 2003).

5. Role of PPARβ/δ in lipoprotein metabolism

Treatment of the atherogenic dyslipidemia associated with type 2 diabetes mellitus and metabolic syndrome requires lowering triglycerides, increasing HDL-C and increasing the size of the LDL-C particle. Studies using the PPARβ/δ agonist GW501516 have demonstrated that this drug increased HDL-C (79%), and decreased triglycerides (56%), LDL-C (29%) and fasting insulin levels (48%) in obese rhesus monkeys, a model for human obesity and its associated metabolic disorders (Oliver, Jr. et al., 2001). A decrease in small dense LDL was also observed in treated animals (Oliver, Jr. et al., 2001). It has been suggested that the increase in HDL-C levels after PPARβ/δ 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 (Leibowitz et al., 2000; van, V et al., 2005). Apart from these beneficial effects of PPARβ/δ activation on HDL levels, treatment with this compound also increased HDL particle size in primates (Wallace et al., 2005), an effect which is thought to be protective against the progression of coronary artery disease in humans (Rosenson et al., 2002). In addition, PPARβ/δ 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 (van, V et al., 2005). However, additional studies are necessary to clearly demonstrate that the effects of these drugs are mediated through PPARβ/δ activation.

In obese and diabetic db/db mice, administration of a PPARβ/δ agonist modestly increased HDL particles, without affecting triglyceride levels (Leibowitz et al., 2000), 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 (Tanaka et al., 2003).

In mice, deletion of PPARβ/δ led to enhanced LDL and triglyceride levels (Akiyama et al., 2004). It has been proposed that the increase in triglycerides observed in these PPARβ/δ-null mice is caused by a combination of increased VLDL production and decreased plasma triglyceride clearance, as demonstrated by a decrease in postheparin LPL activity and increased hepatic expression of the LPL inhibitors Angptl3 and 4 (Akiyama et al., 2004). Recent findings obtained by our laboratory indicate that additional mechanisms can also contribute to the hypotriglyceridemic effect of PPARβ/δ (Barroso et al., 2011). Interestingly, the main factor influencing hepatic triglyceride secretion is fatty acid availability (Lewis, 1997). In liver, fatty acids are either incorporated into triglycerides or oxidized by

mitochondrial β-oxidation. An increase in fatty acid oxidation in liver would thus reduce the availability of fatty acids and subsequent hepatic triglyceride secretion. However, it was unknown whether the hypotriglyceridemic effect observed following PPARβ/δ activation involved increased hepatic fatty acid oxidation and the mechanisms implicated. The rate-limiting step for mitochondrial β-oxidation is the transport of fatty acid into mitochondria by liver carnitine palmitoyltransferase-1 (CPT1a). This fatty acid transporter is under the control of both PPARs and AMP-activated protein kinase (AMPK), which detects low ATP levels and in turn increases oxidative metabolism (Zhang et al., 2009) by reducing the levels of malonyl-CoA. Interestingly, PPARβ/δ activation can increase the activity of AMPK and the increase in fatty acid oxidation in human skeletal muscle cells following GW501516 treatment is dependent on both PPARβ/δ and AMPK (Kramer et al., 2007). It is worth noting that a recent discovered protein, lipin 1, plays an important role in hepatic fatty acid oxidation since it determines whether fatty acids are incorporated into triglycerides or undergo mitochondrial β-oxidation. In addition, the expression and compartmentalization of lipin 1 controls the secretion of hepatic triglycerides (Bou et al., 2009). Thus, in the cytoplasm, lipin 1 promotes triglyceride accumulation and phospholipid synthesis by functioning as an Mg^{2+}-dependent phosphatidate phosphatase (phosphatidic acid phosphatase-1, PAP-1). In contrast, in the nucleus lipin 1 acts as a transcriptional co-activator linked to fatty acid oxidation by regulating the induction of PGC-1α-PPARα-target genes (Finck et al., 2006). Lipin 1 induces PPARα gene expression and forms a complex with PPARα and PGC-1α leading to the induction of genes involved in fatty acid oxidation, including *Cpt1a* and *Mcad* (medium chain acyl-CoA dehydrogenase) (Finck et al., 2006).

When we examined the effects a high-fat diet (HFD) on hypertriglyceridemia and on the hepatic fatty acid oxidation pathway, we observed that exposure to HFD caused hypertriglyceridemia that was accompanied by reduced hepatic mRNA levels of PGC-1α and lipin 1, reduced hepatic phospho-AMPK levels and increased activity of extracellular-signal-regulated kinase 1/2 (ERK1/2) (Figure 2). Interestingly, drug treatment reduced hypertriglyceridemia, and restored hepatic phosphorylated levels of AMPK and ERK1/2. GW501516 treatment increased nuclear lipin 1 protein levels, leading to amplification of the PGC-1α-PPARα signaling system, as demonstrated by the increase in PPARα levels and PPARα-DNA binding activity and the increased expression of PPARα-target genes involved in fatty acid oxidation. These effects of GW501516 were accompanied by an increase in plasma β-hydroxybutyrate levels, demonstrating enhanced hepatic fatty acid oxidation.

The maintenance of AMPK phosphorylation following GW501516 treatment was accompanied by the recovery in the expression levels of *Lipin 1* and *Pgc-1*α and the increase in the mRNA levels of the *Vldl receptor* (Figure 2). Although we cannot rule out direct transcriptional activation of these genes by PPARβ/δ since it has been suggested that *Lipin 1*, the *Vldl receptor* (Sanderson et al., 2010) and *Pgc-1*α (Hondares et al., 2007) might be PPARβ/δ-target genes, most effects of GW501516 might be the result of the increase in AMPK phosphorylation (Kramer et al., 2007). In fact, it has been reported that this kinase upregulates the expression of *Lipin 1* (Higashida et al., 2008), the *Vldl receptor* (Zenimaru et al., 2008) and *Pgc-1*α (Lee et al., 2006b). The increase in AMPK phosphorylation following GW501516 treatment might involve several mechanisms. Since inhibitory crosstalk between ERK1/2 and AMPK has been reported (Du et al., 2008), the increase in phospho-AMPK levels could be the result of the inhibition by GW501516 of the phosphorylation of ERK1/2 induced by the HFD, which is in agreement with our previous study reporting that

GW501516 prevents LPS-induced ERK1/2 phosphorylation in adipocytes (Rodriguez-Calvo et al., 2008). It is important to note that a previous study found that obesity leads to increased hepatic ERK1/2 activity and that caloric restriction blunts this increase and improves insulin sensitivity (Zheng et al., 2009). In our study, the improvement in glucose tolerance caused by GW501516 was also accompanied by the reduction in phospho-ERK1/2 levels. An additional mechanism could involve SIRT1, since it has recently been reported that pharmacological PPARβ/δ activation increases the expression of SIRT1 (Okazaki et al., 2010), a deacetylase which regulates AMPK activity (Ruderman et al., 2010) through LKB1 acetylation (Lan et al., 2008), and might be essential to the regulatory loop involving PPARα, PGC-1α and Lipin 1 (Sugden et al., 2010). However, our findings made this possibility unlikely given that the increase in SIRT1 levels induced by GW501516 did not modify the acetylation status of LKB1. Interestingly, we showed that GW501516 increased the AMP/ATP ratio in liver, indicating that, in line with a previous study in skeletal muscle cells (Kramer et al., 2007), the underlying mechanism responsible for the increase in AMPK phosphorylation induced by this drug could be a modification of the cellular energy status. Previous studies have suggested that the reduction in ATP levels caused by GW501516 can be the result of a specific inhibition of one or more complexes of the respiratory chain, an effect on the ATP synthase system, or to mitochondrial uncoupling (Kramer et al., 2007). These potential changes would reduce the yield of ATP synthesis by the mitochondria, leading to AMPK activation.

In agreement with the reported regulation of PGC-1α (Canto et al., 2009; Jeninga et al., 2010; Lee et al., 2006a) and Lipin 1 (Higashida et al., 2008) by AMPK, exposure to the HFD reduced both *Pgc-1*α and *Lipin 1* expression. The reduction in Lipin 1 was likely to be the result of the decrease of PGC-1α, since it has been reported that genetic reduction of hepatic PGC-1α decreases the expression of *Lipin 1* (Estall et al., 2009). In addition, it has been shown that physiological stimuli that increase mitochondrial fatty acid oxidation induce *Pgc-1*α gene expression, which in turn activates the expression of *Lipin 1* (Finck et al., 2006). Interestingly, it has been reported that upregulation of *Lipin 1* in liver increases PPARα activity by two mechanisms: transcriptional activation of the *Ppara* gene and direct coactivation of PPARα in cooperation with PGC-1α (Finck et al., 2006). Thus, Lipin 1 is considered to be an inducible "booster" that amplifies pathways downstream PGC-1α-PPARα, mainly mitochondrial fatty acid oxidation (Finck et al., 2006). In agreement with this, GW501516 treatment prevented the reduction in PGC-1α, increased the nuclear protein levels of Lipin 1 and amplified the PGC-1αPPARα pathway, as demonstrated by the increase in the transcriptional activation of *Ppar*α and the increase in PPARα transcriptional activity. These effects subsequently enhanced hepatic fatty acid oxidation, as shown by the increase in β-hydroxybutyrate levels. The reduction in PGC-1α and Lipin 1 levels caused by the HFD and their restoration after GW501516 treatment observed in our study might also contribute to the changes of plasma triglyceride levels, since both proteins are involved in the control of hepatic triglyceride secretion and fatty acid oxidation (Zhang et al., 2004; Chen et al., 2008; Estall et al., 2009). Overall, these data implicated PGC-1α and Lipin 1 in the hypotriglyceridemic effect of PPARβ/δ and complemented the findings of a previous study reporting that elevated plasma triglyceride levels in PPARβ/δ-null mouse were related to a combination of increased VLDL production and decreased plasma triglyceride clearance (Akiyama et al., 2004).

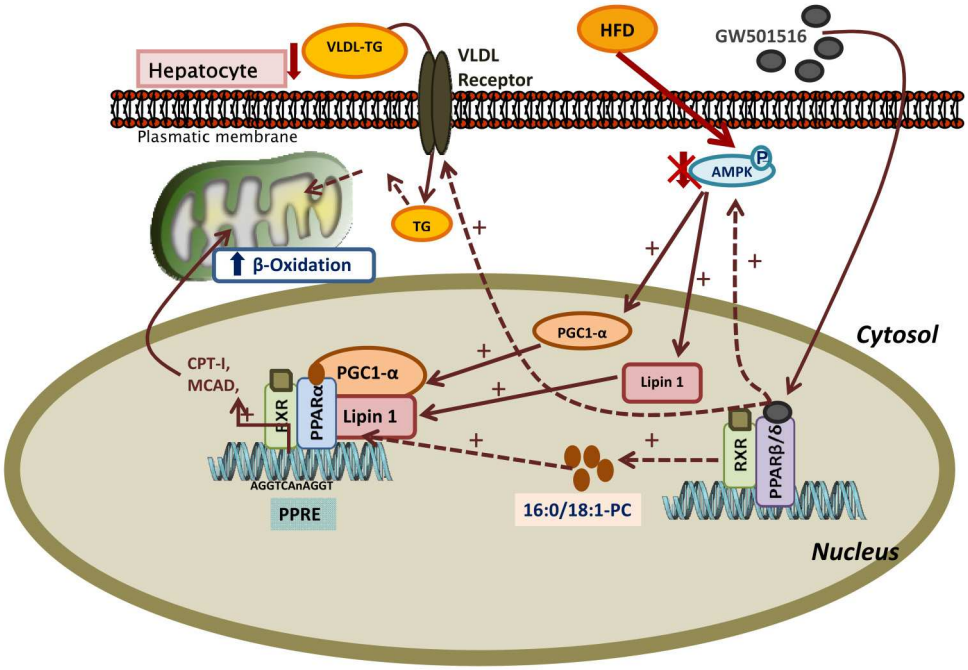

Fig. 2. A schematic of the potential effects of GW501516 (dashed lines) on liver metabolism is shown. Drug treatment with the PPARβ/δ agonist GW501516 prevents the reduction in phospho-AMPK levels and the subsequent increase in phospho-ERK1/2 levels caused by the HFD. In addition, GW501516 prevents the reduction in PGC-1α and increases Lipin 1 protein levels in the nucleus leading to amplification of the PPARα-PGC-1α pathway, which subsequently induces hepatic fatty acid oxidation. This pathway is additionally increased by GW501516 through the enhanced synthesis of the hepatic PPARα endogenous ligand 16:0/18:1-PC. As a result of the increase in this pathway the availability of fatty acids to be secreted as triglycerides might be compromised. The increase in the hepatic levels of the Vldl receptor can also contribute to reduce plasma triglyceride levels.

The data reported in our study also demonstrated that PPARβ/δ activation by GW501516 can amplify the PPARα pathway by an additional mechanism. Previous studies had demonstrated that hepatic fatty acid synthase (FAS) was necessary for the normal activation of PPARα target genes but did not identify the ligand involved in this process (Chakravarthy et al., 2005). Recently, this endogenous PPARα ligand was identified as 16:0/18:1-phosphatidilcholine (PC) (Chakravarthy et al., 2009). The synthesis of this ligand requires FAS activity, which yields palmitate (16:0), whereas 16:0/18:1-PC is generated through the enzymatic activity of CEPT1 (Chakravarthy et al., 2009). Subsequent binding of 16:0/18:1-PC to PPARα in the nucleus turns on PPARα-dependent genes and affects hepatic lipid metabolism. Interestingly, activation of PPARβ/δ by GW501516 induces FAS expression in liver as a result of increased glycolysis and the pentose phosphate shunt (Lee et al., 2006a). Our findings confirmed that GW501516 also increased *Cept1* expression and the levels of 16:0/18:1-PC, contributing to further amplification of the PPARα pathway.

The increase in fatty acid oxidation caused by GW501516 was apparently inconsistent with its lack of effects on hepatic triglyceride levels observed in our study. Several reasons may account for this. First, similar to the effects of GW501516, which restores Lipin 1 levels, hepatic *Lipin 1* overexpression leads to increased liver triglyceride content (Finck et al., 2006). This apparently conflicts with the effects of Lipin 1 on fatty acid oxidation, but it has been explained by hepatic triglyceride sequestration secondary to diminished triglyceride secretion, increased fatty acid uptake, or the PAP activity of Lipin 1 (Finck et al., 2006). Second, in our study we reported an additional possibility, the increase caused by GW501516 in the expression of the *Vldl receptor* in liver. The huge increase of this receptor observed in liver after GW501516 treatment might also reduce plasma triglyceride levels by increasing VLDL uptake by the liver. However, this can also lead to an increase in hepatic triglyceride content. Third, it has been reported that GW501516 improves hyperglycemia by increasing glucose flux through the pentose phosphate pathway and enhancing fatty acid synthesis in liver (Lee et al., 2006a). In that study, GW501516 increased liver triglyceride content but the authors reported that although this might raise concerns that long-term drug treatment might cause hepatic steatosis, they did not observe signs of fatty liver with treatment up to 6 months. In addition, long-term GW501516 treatment has been shown to reduce body weight and levels of circulating and liver triglycerides (Wang et al., 2004; Tanaka et al., 2003). In summary, our findings indicated that PPARβ/δ activation by GW501516 amplified the PPARα-PGC1-α pathway through the restoration of AMPK activity, contributing to the hypotriglyceridemic effect of this drug.

In humans, there are conflicting reports as to whether PPARβ/δ polymorphisms are associated with changes in plasma lipoproteins. Thus, while some studies found an association between a PPARβ/δ polymorphism and plasma lipids (Skogsberg et al., 2003), this was not confirmed in other studies (Gouni-Berthold et al., 2005). 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 PPARβ/δ -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 (Robitaille et al., 2007). The authors of this study concluded that the presence of the PPARβ/δ -87T>C polymorphism, which may result in enhanced PPARβ/δ 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 PPARβ/δ activation on lipoprotein metabolism are so promising that PPARβ/δ drugs are now in clinical trials for the treatment of human dyslipidemia.

6. Role of PPARβ/δ in insulin resistance

As stated above insulin resistance plays a crucial role in the development of hypertrigliceridemia, resulting in a sequence of lipoprotein changes leading to atherogenic dyslipidemia. Thus, those drugs, such as the PPARβ/δ ligands, which improve insulin resistance may also contribute to ameliorate the atherogenic dyslipidemia.

6.1 PPARβ/δ, inflammation and insulin resistance in adipose tissue

The expansion of adipose tissue, mainly in the form of visceral obesity, may contribute to enhanced inflammation in this tissue and insulin resistance through several processes. First, macrophages can infiltrate in adipose tissue, which contributes to the overproduction of

inflammatory cytokines, such as tumor necrosis factor α (TNF-α and interleukin 6 (IL-6) (Gustafson et al., 2009). Indeed, the infiltration of macrophages into adipose tissue correlates with the degree of insulin resistance (Mathieu et al., 2010). Second, as visceral fat (which is very sensitive to lipolytic stimuli) increases, so does the rate of lipolysis. This leads to increased free fatty acid (FFA) mobilization and elevated levels of circulating FFA. Several studies have consistently demonstrated that elevations of plasma FFA produce insulin resistance in diabetic patients and in nondiabetic subjects (Boden et al., 1991; Boden, 1997). Saturated FFA are potent activators of the Toll-like receptor-4 (TLR4) (Mathieu et al., 2006) and recent evidence suggests that inflammatory processes induced by obesity and a high-fat diet cause systemic insulin resistance via a mechanism involving this receptor (Shi et al., 2006). TLR-4 is expressed in virtually all human cells and binds a wide spectrum of exogenous and endogenous ligands, including bacterial lipopolysaccharide (LPS) (Akira et al., 2006). In the presence of LPS, the TLR4 complex (including CD-14 and an accessory protein, MD-2), recruits the adaptor protein, myeloid differentiation factor-88 (MyD88), which in turn recruits interleukin-1 receptor-associated kinase (IRAK). This leads to the activation of the pro-inflammatory transcription factor NF-κB (Shoelson et al., 2006) and the subsequent enhanced expression of several inflammatory mediators (including IL-6 and monocyte chemoattractant protein-1 [MCP-1]). These observations indicate that saturated FFA derived from adipocytes and from high-fat diets activate TLR and the inflammatory pathway in adipocytes and macrophages, which contribute to the synthesis and production of cytokines such as TNF-α (Nguyen et al., 2007). In addition, high-fat diets raise plasma LPS to a concentration that is high enough to increase body weight, fasting glycemia and inflammation (Cani et al., 2007). Furthermore, LPS receptor-deleted mice (CD14 mutants) are hypersensitive to insulin, and the development of insulin resistance, obesity and diabetes in this animal model is delayed in response to a high-fat diet (Cani et al., 2007). Experiments performed in our laboratory have demonstrated that the PPARβ/δ agonist GW501516 inhibits LPS-induced cytokine expression and secretion by preventing NF-κB activation in adipocytes (Rodriguez-Calvo et al., 2008). Of note, NF-κB activation by LPS requires mitogen-activated protein kinase (MAPK)–extracellular signal–related kinase (ERK)1/2 (MEK1/2) activation, since inhibition of this pathway reduces LPS-induced cytokine production in adipocytes (Chung et al., 2006). In agreement with this role of ERK1/2 in inflammation in adipocytes, the expression of pro-inflammatory cytokines in these cells drops when they are exposed to LPS in the presence of the MAPK pathway inhibitor U0126. Interestingly, in white adipose tissue from PPARβ/δ-null mice we observed increased ERK1/2 phosphorylation and NF-κB activity and higher expression of IL-6 compared with wild-type mice (Rodriguez-Calvo et al., 2008). Moreover, in the white adipose tissue of a genetic model of obesity and diabetes, the Zucker diabetic fatty (ZDF) rat, the reduction in the expression of PPARβ/δ correlated with an increase in ERK1/2 phosphorylation and NF-κB activity. These findings suggest that PPARβ/δ activation prevents LPS-induced NF-κB activation via ERK1/2, thereby reducing the production of pro-inflammatory cytokines involved in the development of insulin resistance.

In addition, it has been suggested that IL-6 is another of the mediators linking obesity-derived chronic inflammation with insulin resistance through activation of signal transducer and activator of transcription 3 (STAT3), with subsequent up-regulation of suppressor of cytokine signaling 3 (SOCS3). Recently we have demonstrated that the PPARβ/δ agonist GW501516 prevents both IL-6-dependent reduction in insulin-stimulated Akt phosphorylation and glucose uptake in adipocytes (Serrano-Marco et al., 2011). In addition,

this drug treatment abolished IL-6-induced SOCS3 expression in differentiated 3T3-L1 adipocytes. This effect was associated with the capacity of the drug to prevent IL-6-induced STAT3 phosphorylation on Tyr^{705} and Ser^{727} residues in vitro and in vivo. Moreover, GW501516 prevented IL-6-dependent induction of ERK1/2, a serine-threonine-protein kinase involved in serine STAT3 phosphorylation. Furthermore, in white adipose tissue from PPARβ/δ-null mice, STAT3 phosphorylation (Tyr^{705} and Ser^{727}), STAT3 DNA-binding activity and SOCS3 protein levels were higher than in wild-type mice. Several steps in STAT3 activation require its association with heat shock protein 90 (Hsp90), which was prevented by GW501516 as revealed in immunoprecipitation studies. Consistent with this finding, the STAT3-Hsp90 association was enhanced in white adipose tissue from PPARβ/δ-null mice compared to wild-type mice. Collectively, our findings indicate that PPARβ/δ activation prevents IL-6-induced STAT3 activation by inhibiting ERK1/2 and preventing the STAT3-Hsp90 association, an effect that may contribute to the prevention of cytokine-induced insulin resistance in adipocytes.

6.2 PPARβ/δ, inflammation and insulin resistance in skeletal muscle cells

FFAs may cause insulin resistance in skeletal muscle through several mechanisms, including effects on metabolism (Roden et al., 1996; Haber et al., 2003), signaling (Hirabara et al., 2007; Silveira et al., 2008) and mitochondrial function (Schrauwen et al., 2010; Hirabara et al., 2010). In addition, FFAs activate pro-inflammatory pathways, linking the development of this pathology to a chronic low-grade systemic inflammatory response (Wellen & Hotamisligil, 2005). In addition to FFA-induced inflammation through TLR, an additional pathway leads to FFA-mediated inflammation. This pathway involves intracellular accumulation of fatty acid derivatives. Once fatty acids are taken up by skeletal muscle cells they are either stored as fatty acid derivatives or undergo β-oxidation in the mitochondria. In the presence of high plasma FFA, fatty acid flux in skeletal muscle cells exceeds its oxidation, which leads to the accumulation of fatty acid derivatives, such as diacylglycerol (DAG), which can then activate a number of different serine kinases that negatively regulate insulin action. Thus, DAG is a potent allosteric activator of protein kinase Cθ (PKCθ), which is the most abundant PKC isoform in skeletal muscle (Griffin et al., 1999; Cortright et al., 2000; Itani et al., 2000). This PKC isoform inhibits the action of insulin by phosphorylating certain serine residues on insulin receptor substrate 1 (IRS1), including Ser^{307} in the rodent IRS-1 protein (reviewed in ref. (Gual et al., 2005)). This phosphorylation impairs insulin-receptor signaling through several distinct mechanisms (Hotamisligil et al., 1996). PKCθ also impairs insulin sensitivity by activating another serine kinase, IκB kinase β (IKKβ) (Perseghin et al., 2003). In addition to phosphorylating IRS-1 in Ser^{307}, IKKβ phosphorylates IκB. Thus, it activates the pro-inflammatory transcription factor NF-κB, which has been linked to fatty acid-induced impairment of insulin action in skeletal muscle in rodents (Kim et al., 2001; Yuan et al., 2001). Once activated, NF-κB regulates the expression of multiple inflammatory mediators, including IL-6. This cytokine correlates strongly with insulin resistance and type 2 diabetes (Pickup et al., 1997; Kern et al., 2001; Pradhan et al., 2001) and its plasma levels are 2-3 times higher in patients with obesity and type 2 diabetes than in lean control subjects (Kern et al., 2001).

Accumulation of fatty acid derivatives can be attenuated by mitochondrial β-oxidation. The rate-limiting step for β-oxidation of long-chain fatty acids is their transport into mitochondria via CPT-1. The activity of this enzyme is inhibited by malonyl-CoA, the

product of acetyl-CoA carboxylase, which, in turn, is inhibited by AMPK. This kinase is a metabolic sensor that detects low ATP levels and increases oxidative metabolism (Reznick & Shulman, 2006), by reducing the levels of malonyl-CoA. Interestingly, activation of fatty acid oxidation by overexpressing CPT-1 in cultured skeletal muscle cells (Sebastian et al., 2007) and in mouse skeletal muscle (Bruce et al., 2009) improves lipid-induced insulin resistance. Hence, this approach may provide a valid therapeutic strategy to prevent this pathology. Activation of PPARβ/δ by its ligands (including GW501516) enhances fatty acid catabolism in adipose tissue and skeletal muscle, thereby delaying weight gain (for a review see (Barish et al., 2006)). This increase in fatty acid oxidation in human skeletal muscle cells following PPARβ/δ activation by GW501516 is dependent on both PPARβ/δ and AMPK (Kramer et al., 2007). AMPK is activated by GW501516 by modulating the ATP:AMP ratio (Kramer et al., 2007). Despite these data, little information was available on whether the increase in fatty acid oxidation attained after PPARβ/δ activation prevented fatty acid-induced inflammation and insulin resistance in skeletal muscle cells. However, we have recently reported that the PPARβ/δ ligand GW501516 prevented palmitate-induced inflammation and insulin resistance in skeletal muscle cells (Coll et al., 2010b). Treatment with GW501516 enhanced the expression of two-well known PPARβ/δ-target genes involved in fatty acid oxidation, CPT-1 and pyruvate dehydrogenase kinase 4 (PDK-4), and increased the phosphorylation of AMPK. This prevented the reduction in fatty acid oxidation caused by palmitate exposure. In agreement with these changes, GW501516 treatment reversed the increase in DAG and PKCθ activation caused by palmitate. These effects were abolished in the presence of the CPT-1 inhibitor etomoxir, thereby implicating increased fatty acid oxidation in the changes. Consistent with these findings, PPARβ/δ activation by GW501516 blocked palmitate-induced NF-κB DNA-binding activity. Likewise, drug treatment inhibited the increase in IL-6 expression caused by palmitate in C2C12 myotubes and human skeletal muscle cells, as well as the protein secretion of this cytokine. Overall, these findings indicate that PPARβ/δ attenuates fatty acid-induced NF-κB activation and the subsequent development of insulin resistance in skeletal muscle cells by reducing DAG accumulation. Interestingly, it has been suggested that the hypotrigliceridemic effect of GW501516 in humans is dependent of the increase in CPT-1 expression observed in skeletal muscle (Riserus et al., 2008).

7. Conclusion

Reduction of LDL-C, the main target of hypolipidemic therapy, has been proved effective reducing morbidity and mortality associated to CVD. However, a high proportion of patients receiving statins, the most lipid-lowering family of drugs used, do not reach optimal LDL-C levels. In addition, even in those patients reaching the optimal LDL-C levels following statin treatment, a residual risk remains, probably due to the presence of other risk factors, including the presence of atherogic dyslipidemia (described by the presence of high triglycerides, low HDL-C levels, and the presence of small dense LDL particles), glucose metabolism alterations and additional non-lipid-related risk factors. Several studies have confirmed that PPARβ/δ plays an important role in the regulation of lipoprotein metabolism, leading to reductions in the levels of plasma triglycerides and LDL-C and increases in HDL-C in different animal models. Taken together, these effects attained following PPARβ/δ activation on lipoprotein metabolism are so promising that this nuclear

receptor has been considered a therapeutic target to prevent and treat dyslipidemia. However, as with any drug designed for human therapy, a great deal of research will be needed on the efficacy and safety of PPARβ/δ activators before they reach clinical use.

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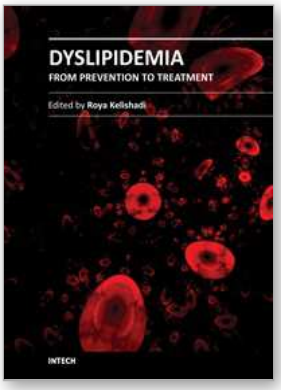

Dyslipidemia - From Prevention to Treatment
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Dyslipidemia has a complex pathophysiology consisting of various genetic, lifestyle, and environmental factors. It has many adverse health impacts, notably in the development of chronic non-communicable diseases. Significant ethnic differences exist due to the prevalence and types of lipid disorders. While elevated serum total- and LDL-cholesterol are the main concern in Western populations, in other countries hypertriglyceridemia and low HDL-cholesterol are more prevalent. The latter types of lipid disorders are considered as components of the metabolic syndrome. The escalating trend of obesity, as well as changes in lifestyle and environmental factors will make dyslipidemia a global medical and public health threat, not only for adults but for the pediatric age group as well. Several experimental and clinical studies are still being conducted regarding the underlying mechanisms and treatment of dyslipidemia. The current book is providing a general overview of dyslipidemia from diverse aspects of pathophysiology, ethnic differences, prevention, health hazards, and treatment.

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