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PPARα as target for pharmacological and nutritional agents affecting lipid metabolism

María del Carmen González, María Isabel Panadero, Emilio Herrera and Carlos Bocos
Facultad de Farmacia, Universidad San Pablo-CEU
Boadilla del Monte, Madrid, Spain

Abstract

Peroxisome proliferator-activated receptor alpha (PPARα) binds fatty acids, eicosanoids, or fibrates, and regulates transcription of specific genes. These genes are involved both in lipid and glucose metabolism as well as in cellular differentiation and inflammation. Thus, PPARα controls metabolic and inflammatory disturbances and therefore, its activation is expected to normalize lipid and glucose parameters and exert anti-inflammatory effects. In fact, PPARα

Correspondence/Reprint request: Dr. Carlos Bocos, Facultad de Farmacia, Universidad San Pablo-CEU Urbanización Montepríncipe, 28668 Boadilla del Monte, Madrid, Spain. E-mail: carbocos@ceu.es

agonists have already been demonstrated to benefit metabolic syndrome, type 2 diabetes and cardiovascular diseases. However, several findings have revealed either potential adverse effects or unefficient hypolipidemic action of PPARα activation, highlighting the need to search for pharmacological or nutritional solutions based on modulation of PPARα. This strategy could be either complementary to or replace the classical therapy. Thus, compounds that selectively and/or specifically modulate PPARα activity appear to be a promising alternative.

Abbreviations

ABC	:	ATP-binding cassette
ACBP	:	acyl-CoA binding protein
ACC	:	acetyl-CoA carboxilase
ACO	:	acyl-CoA oxidase
ACS	:	acyl-CoA synthetase
AF-1 or AF-2	:	ligand-independent transactivation function
aP2	:	adipocyte fatty acid-binding protein
apo	:	apolipoprotein
apo E-KO	:	apolipoprotein E-deficient
ARB	:	angiotensin receptor blocker
CD36	:	scavenger receptor
CDK	:	cyclin-dependent kinase
C/EBP	:	CAATT/enhancer binding protein
CBP	:	CREB binding protein
CETP	:	cholesteryl ester tranfer protein
CLA	:	conjugated linoleic acid
CLA-1	:	human receptor HDL
COUP-TF	:	chicken ovoalbumin upstream promoter transcription factor
CPT	:	carnitine palmitoyl transferase
CREB	:	cAMP response element-binding protien
CYP4A	:	cytochrome P450 superfamily
DBD	:	DNA-binding domain
DR (1-2)	:	direct repeat spaced by one or two nucleotides
ERE	:	estrogen response element
ERRα	:	estrogen receptor-related receptor alpha
ETYA	:	5,8,11,14 eicosatetraynoic acid
FAE	:	fatty acids with etanolamine
FABP	:	fatty acid binding protein
FAS	:	fatty acid synthase
FAT	:	fatty acid translocase

FATP	:	fatty acid transporter protein
FFA	:	free fatty acid
FXR	:	farnesol X receptor
GH	:	growth hormone
GLUT	:	glucose transporters
GR	:	glucocorticoid receptor
Gyk	:	glycerol kinase
HAT	:	histone acetyl transferase
HD or L-PBE	:	enoyl-CoA hydratase/3-hydroxyacyl-CoA deshydrogenase (L-bifunctional enzyme)
HDL	:	high density lipoprotein
HETEs	:	hydroxyeicosatetraenoic acids
HMG-CoA	:	hydroxymethylglutaryl-CoA
HNF-4	:	hepatocyte nuclear factor-4
HSD17β- or D-PBE	:	D-3 hydroxyacyl-CoA dehydratase / D3-hydroxyacyl-CoA dehydrogenase or D-bifunctional enzyme or 17-β-hydroxysteroide dehydrogenase
hsp	:	heat shock protein
JAK2/STAT5b	:	Janus kinase 2/signal transducer and activator of transcription 5b
LBD	:	ligand-binding domain
LDL	:	low density lipoprotein
LPL	:	lipoprotein lipase
LT	:	leukotriene
LXR	:	liver X receptor
MAPK	:	mitogen-activated protein kinase
MCAD	:	medium-chain acyl-CoA dehydrogenase
ME	:	malic enzyme
MCP-1	:	monocyte chemotactic protein 1
NcoR	:	nuclear co-repressor receptor
NF-κB	:	nuclear factor κB
NSAIDs	:	non-steroidal anti-inflammatory drugs
OEA	:	oleylethanolamide
p300	:	E1A associated factor
PBP	:	PPAR binding protein
PEPCK	:	phosphoenolpiruvate carboxy kinase
PGC-1	:	PPAR gamma coactivator-1
PKC	:	protein kinase C
PPAR	:	peroxisome proliferator-activated receptor
PPRE	:	peroxisome proliferator-response element
PUFA	:	polyunsaturated fatty acids
PRIP/RAP250	:	PPAR interacting protein

RABP	:	retinoic-acid binding protein
RAR	:	retinoid acid receptor
RXR	:	9-cis retinoid X receptor
RZR	:	retinoid Z receptor
SERM	:	selective estrogen receptor modulator
SCD-1	:	stearoyl-CoA desaturase 1
siRNA	:	small interfering RNA
SMRT	:	silencing mediator for retinoid and thyroid hormone receptor
Sp1	:	specific protein-1
SPPARM	:	selective PPAR modulator
SR-B1	:	murine scavenger receptor 1
SRC-1	:	steroid receptor coactivator 1
TAK1/TR4	:	TGF (beta)-activated kinase 1 (TAK1)
TBP	:	TATA box binding protein
TIO	:	3-ketoacyl-CoA thiolase
TNF	:	tumor necrosis factor
TR	:	thyroid receptor
TZD	:	thiazolidinedione
UCP	:	uncoupling protein
VLDL	:	very low density lipoprotein
VSMC	:	vascular smooth muscle cell

Introduction

Peroxisome proliferators-activated receptor (PPARs) are ligand-inducible transcription factors that belong to the nuclear hormone receptor superfamily, together with the receptor for thyroid hormone, retinoids, steroid hormones and vitamin D [1]. After binding to fatty acids, eicosanoids, or a mixed of structurally diverse compounds called peroxisome proliferators, PPARs regulate transcription of specific genes by interaction with specific peroxisome-proliferator-response elements (PPREs) on their promoter. PPARs are involved in the regulation of lipid and lipoprotein metabolism, glucose and aminoacid metabolism and control cellular differentiation, inflammatory responses, and cancer development. This diversity of actions suggests that PPARs may function as a key molecule implicated in diverse metabolic disorders, and increases the interest in the search for pharmacological and nutritional agents that target PPARs for the development of therapeutic solutions.

In this chapter we intend to review the activation mechanism of PPARα and to analyse those cases in which PPARα agonists do not attain the desirable effectiveness or even appear to produce adverse effects. Emergent

pharmaceutical and nutraceutical approaches that might be able to prevent or alleviate the negative effects of PPARα agonists will also be discussed.

General aspects of PPARα

Molecular aspects

In 1990, PPARs were first identified as receptors for fibrates, a class of hypolipidemic drugs used in humans since the late 1960s [2]. The name of PPARs was initially chosen because of their ability to induce the proliferation of peroxisome in rodent [3]. Since then, three related PPAR isotypes have been identified: PPARα (NR1C1), PPARβ (also called –δ, NR1C2), and PPARγ (NR1C3) [4].

PPARs are proteins of 49-56 kDa that contain multiple structural and functional domains. The amino terminal A/B domain contains a ligand-independent transactivation function (AF-1) [5] and conserved mitogen-activated protein kinase (MAPK) phosphorylation serine sites [6]. The C domain or DNA-binding domain (DBD) contains two zinc fingers, involved in heterodimerize with the 9-cis retinoid X receptor (RXR), in order to form a complex that is able to bind to the PPRE located in the promoter of PPAR target gene [7]. Functional PPREs are constituted by two copies of the core motif organized as a direct repeat spaced by one nucleotide (DR-1) [8] along with a 5′-extension. The D or hinge domain linking DBD and LBD allows for bending or conformational alteration of the protein. Through this D region, the receptor interacts with cofactors, it is involved in nuclear localization, and contains a protein kinase C (PKC) phosphorylation site [9]. Eventually, the carboxyl terminal E/F domain or ligand-binding domain (LBD) contains an hydrophobic pocket with two differences as compared to other nuclear receptors: a huge volume of the ligand-binding cavity, and an additional α-helix at the bottom of the pocket that participates in the entry of the ligand [10]. The E/F domain also contains a ligand-dependent transactivation function (AF-2) and includes interfaces involved in interaction with RXR, cofactors, and heat shock proteins [11].

Despite a common general structure of the LBD, binding of some ligands to PPARα, β and γ shows both species and isotype specificities. PPARs are activated by natural and synthetic compounds. Thus, fatty acids bind to all three PPAR isotypes [12], with PPARα exhibiting the highest affinity. In general, all isoforms present a higher selectivity to polyunsaturated fatty acids (PUFAs) ω-3 and ω-6 very long chain than to saturated or monounsaturated fatty acids [13]. The discovery that fatty acids can control the activity of transcription factors, such as PPARs, demonstrated that fatty acids are metabolic regulators [14], acting mainly in lipid metabolism. Eicosanoids, fatty acids derived from arachidonic acid via lipoxygenase pathway such as

hydroxyeicosatetraenoic acids (HETEs) [13] and leukotrienes (LTs), involved in inflammatory processes, are also ligands for PPARα.

PPARs are also activated by peroxisome proliferators, a large class of structurally diverse compounds that include hypolipidemic drugs, plasticizers, herbicides, and solvents. These hypolipidemic agents are fibrates such as clofibrate, fenofibrate, bezafibrate, gemfibrozil and, an experimental compound, Wy-14,643. Fibrates preferentially bind PPARα [3]. Various fatty acid or prostaglandin molecules have been proposed as natural PPARγ ligands, whereas the thiazolidinediones (TZDs), a class of antidiabetic drugs generally used as insulin sensitizers, selectively bind PPARγ [15]. This functional association between a key regulator of lipid metabolism and an antidiabetic drug emphasizes the link between lipid and glucose metabolism.

Finally, other synthetic compounds that bind to PPARα include the ETYA (5,8,11,14 eicosatetraynoic acid), an arachidonic acid analog [16], products from oxidized low density lipoproteins (LDL), and non-steroidal anti-inflammatory drugs (NSAIDs) which also appear to activate PPARγ [17]. Moreover, it seems that even RXR agonists, such as the natural ligand 9-cis retinoic acid or synthetic compounds (LG 1096 and LG 100268), can induce PPAR:RXR heterodimers and active PPARα target genes [18].

Mechanism of action

The ability of the PPAR to regulate gene expression involves complex interactions with ligands, other nuclear receptors and transcription factors, co-activators and co-repressors, binding to DNA, and participation of kinases.

Thus, PPARs form a functional transcriptional unit upon heterodimerization with retinoid X receptors (RXR) and activation by their ligands. PPARα:RXR heterodimer bind to PPRE located in the promoter region of target genes and regulate their transcription. Nevertheless, the ability of nuclear receptors to initiate or suppress the transcription process relies on their interaction with negatively or positively acting cofactors. These cofactors serve as a bridge between transcription factors and the basic transcription machinery, and more importantly, contain several enzymatic activities, controlling gene expression by specifically modifying chromatin and DNA structure. For instance, the coactivators cAMP response element-binding protein (CREB)-binding protein (CBP) and p300 can perform histone acetylation [19] and the two corepressors silencing mediator for retinoid and thyroid hormone receptor (SMRT) and nuclear co-repressor receptor (NCoR) present histone deacetylase activity [20]. Accordingly, in the absence of a ligand, PPAR:RXR heterodimers associate with corepressors which provokes a deacetylated state of histones and thus, gene transcription is prevented. On the contrary, ligand binding induces conformational change in the transactivation domain (AF-2) of the LBD of

PPAR, resulting in the release of corepressors and recruitment of coactivators [21]. Numerous coactivators including PBP (PPAR binding protein), PRIP/RAP250 (PPAR interacting protein), PGC-1 (PPAR gamma coactivator-1) or the steroid receptor coactivator 1 (SRC-1) have also been shown to interact with PPARs in vitro. Interaction of nuclear receptors with these coactivators results in the binding of the heterodimer to PPRE in the promoter, modification of the chromatine structure and either the activation or supression of the transcription of that target gene. This model implies that chromatine is flexible enough to allow looping (Figure 1).

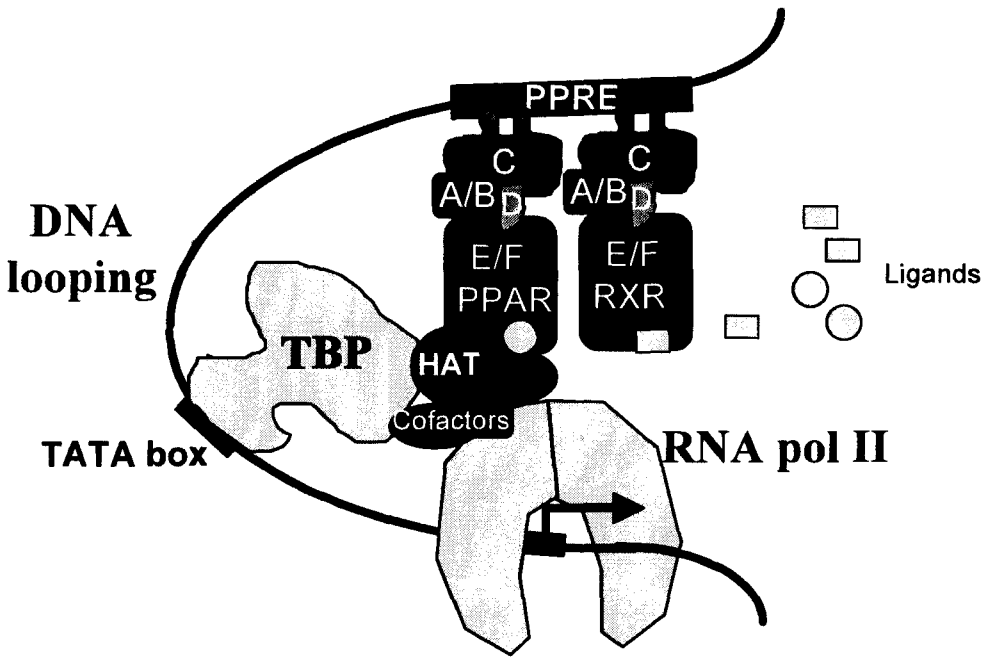

Figure 1. Mechanism of PPAR activation and function. PPARs regulate transcription of their target genes by heterodimerization with the 9-cis retinoic acid receptor (RXR). The PPAR/RXR heterodimer binds to PPRE located in the promoter of target genes, through the DBD of PPAR and RXR. Receptor activity is regulated by both phosphorylation of A/B domain and ligand-binding to LBD. The activated PPAR/RXR heterodimer associates with cofactors containing histone acetyl-transferase (HAT), modifying nucleosome structure, contacting general transcription factors and allowing gene transcription.

Physiologic aspects

Tissular expression of PPAR

The three PPAR subtypes are expressed in most tissues with levels that vary from one cell type to another. The expression patterns do not differ significantly between males and females [22]. High levels of PPARα are

detected in tissues with highly-activated fatty acid catabolism, such as brown fat, liver, kidney, heart, skeletal muscle, and duodenum [22]. In human liver and muscle, PPARα levels are generally lower than in rodent [23]. On the other hand, PPARγ is highly expressed in tissues characterized in lipid storage, such as adipose, and PPARβ is ubiquitously expressed.

PPAR target genes and functions related to lipid metabolism

PPARα mediates the control of liver fatty acid oxidation [4] and PPARγ regulates adipogenesis [24]. Therefore, the identification of PPAR target genes has been concentrated mainly on hepatocytes and adipocytes, indicating that PPARs exert a general regulatory effect on lipid homeostasis. In addition, liver is able to regulate the levels of non-esterified fatty acids, triacylglycerols, and ketone bodies, by modulating the relative rates of fatty acid uptake, esterification into triacylglycerols and oxidation, respectively [10]. PPARα target genes constitute a set of genes that participate in many if not all aspects of lipid catabolism. These include fatty acid transport across the cell membrane (fatty acid transport protein genes), intracellular binding (liver fatty acid binding protein gene), activation via the formation of acyl-CoA (long chain fatty acid acyl CoA synthase genes), catabolism by β-oxidation in peroxisomes and mitochondria, and catabolism by ω-oxidation in microsomes (acyl-CoA oxidase gene, CYP4A1 and CYP4A6 genes, medium-chain acyl-CoA dehydrogenase, and 3-hydroxy 3-methylglutaryl-CoA synthase genes) [25]. The role of PPARα in fatty acid oxidation is particularly important during fasting that results in an enhanced load of fatty acids in the liver, to be used as an energy source [26]. On the other hand, several PPARγ target genes have been identified including the genes encoding lipoprotein lipase (LPL), fatty acid transporter protein (FATP), adipocyte fatty acid-binding protein (aP2), acyl-CoA synthetase (ACS), phosphoenolpyruvate carboxy kinase (PEPCK) and malic enzyme (ME). This suggests that PPARγ controls lipid accumulation by regulating fatty acid uptake (LPL, FATP, aP2), NADPH production for lipogenesis (ME), glycerolgenesis (PEPCK), and fatty acid esterification (ACS) [10].

Several but not all of these genes that have been shown to be regulated by PPARa activators containing PPRE in their promoters (Table 1).

PPAR target genes and functions in glucose homeostasis

PPARs have also been demonstrated to have an important role in glucose homeostasis. Thus, PPARα agonists (fibrates and fatty acids) by up-regulating fatty acid oxidation and ketone body production, are able to spare glucose. Accordingly, PPARα null mice present hypoglycemia during starvation due to a reduced capacity for hepatic gluconeogenesis and a diminished capability to

oxidize fatty acids [26]. Moreover, PPARα null mice have been shown to be protected from high-fat diet-induced insulin resistance [27]. Furthermore, different fibrates tested in mice models of high-fat diet-induced or genetic insulin resistant rodents [28], lowered hyperinsulinemia and hyperglycemia markedly [29], by improving insulin action and glucose utilization. On the other hand, PPARγ agonists (thiazolidinediones) by enhancing lipid storage in adipose tissue and by increasing insulin-dependent glucose disposal and reducing hepatic glucose output, are able to regulate glucose utilization. Since increased levels of free fatty acids have been related to impaired insulin signalling in muscle and liver, the retention of fatty acids in adipose tissue is supposed to result in insulin sensitization. Thus, as commented above, PPARγ ligands are able to regulate genes controlling free fatty acid (FFA) release from adipocytes. For instance, they selectively induce the adipose tissue expression of genes involved both in fatty acid uptake, such as LPL, FATP and ACS [30], and in fatty acid accumulation, such as PEPCK, and glycerol kinase (GyK). On the other hand, TZDs are able to reverse the inhibition of insulin signalling produced by the tumor necrosis factor (TNF) alpha [31], and to correct the deficit in glucose transporters (GLUT) in fat and muscle tissues of diabetic animals [32]. They also regulate adipocyte-secreted hormones that have an impact on glucose homeostasis, such as adiponectin, a secreted adipocyte-specific protein with potential insulin-sensitizing activity, and leptin, resistin and other proteins mediating insulin resistance [33].

Regulation of lipoprotein metabolism by PPARα

Since the present chapter is dedicated to PPARα, we will now focus exclusively on the physiologic aspects of this PPAR subtype.

To summarize to this point, most of the effects of PPARα activators on lipoprotein metabolism have been mainly attributed to: 1) the induction of lipoprotein lipase (LPL) gene expression and the down-regulation of apo CIII gene, which enhances catabolism of triacylglycerols-rich lipoproteins [34]; 2) the increase in fatty acid uptake, and catabolism, and to the reduction of fatty acid synthesis leading to a decreased hepatic very low density lipoprotein (VLDL) secretion [35]; 3) the increase in LDL particle removal as a result of changes in plasma LDL composition and subsequent increase of LDL affinity for its receptor [36]; 4) the induced expression of the apo AI and apo AII (major constituents of HDL), and HDL receptors (murine scavenger receptor 1 (SR-B1) and its human homologue (CLA-I)) genes, which stimulate both HDL production and reverse cholesterol transport [37]. Apo AV, another component of lipoproteins regulated by PPARα agonists, has also been defined as a putative key molecule on triacylglycerol homeostasis [38] (Table 1).

Table 1. Main pathways of lipid metabolism and target genes regulated by PPAR. PPARα is involved in the regulation of fatty acid intra- and extra- cellular transport, fatty acid activation, lipogenesis, oxidation (mitochondrial, peroxisomal and microsomal), ketone body synthesis and thermogenesis. Apolipoprotein A-I (apo A-I); apolipoprotein A-II (apo A-II); apolipoprotein A-V (apo A-V); apolipoprotein C-III (apo C-III); lipoprotein lipase (LPL); murine scavenger receptor class B1 (SR-B1); fatty acid transport protein (FATP); fatty acid transporter (FAT/CD36); fatty acid binding protein (FABP); acyl-CoA binding protein (ACBP); acyl-CoA synthetase (ACS); malic enzyme (ME); acetyl-CoA carboxylase (ACC); fatty acid synthase (FAS); stearoyl-CoA desaturase 1 (SCD-1); carnitine palmitoyl transferase I (CPT-I); medium chain acyl-CoA dehydrogenase (MCAD); acyl-CoA oxidase (ACO); enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase or L-bifunctional enzyme (HD or L-PBE); β/3-ketoacyl-CoA thiolase (TIO); D-3-hydroxyacyl-CoA dehydratase/D3-hydroxyacyl-CoA dehydrogenase or D-bifunctional enzyme or 17-β hydroxysteroide dehydrogenase (17β-HSD or D-PBE); cytochrome P450 (CYP4A1)(CYP4A6); 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase); uncoupling protein-2 (UCP-2). Genes for which a functional PPRE has been identified are indicated with a plus sign.

Target genes	Gene function	PPRE
Extracellular transport		
Apo A-I	Blood transport of fatty acid	+
Apo A-II	Blood transport of fatty acid	+
Apo A-V	Blood transport of fatty acid	+
Apo C-III	Blood transport of fatty acid	-
LPL	Fatty acid release from lipoprotein-bound triacylglycerols	+
SR-B1	HDL receptor	+
Intracellular transport		
FATP	Fatty acid transport across cell membrane	+
FAT/CD 36	Fatty acid transport across cell membrane	+
FABP	Intracellular fatty acid binding	+
ACBP	Intracellular acyl-CoA binding	+
Fatty acid activation		
ACS	Fatty acid activation	+
Lipogenesis		
ME	NADPH production	+
ACC	Fatty acid synthesis	-
FAS	Fatty acid synthesis	-
SCD-1	Desaturation of acyl-CoA	+
Oxidation		
Mitochondrial		
CPT-I	Entry of acyl-CoA	+
MCAD	β-oxidation	+

Table 1. Continued

Peroxisomal		
ACO	β-oxidation	+
HD (L-PBE)	β-oxidation	+
TIO	β-oxidation	+
D-PBE (17-β-HSD IV)	β-oxidation	+
Microsomal		
CYP4A1	ω-oxidation	+
CYP4A6	ω-oxidation	+
Ketogenesis		
HMG-CoA synthase	Ketone body synthesis	+
Thermogenesis		
UCP-2	Thermogenesis	+

Regulation of intracellular lipid metabolism by PPARα

PPARα plays a key role in intracellular fatty acid metabolism by regulating genes codifying diverse crucial steps in lipid metabolism (Table 1). In fact, a high level of PPARα expression is observed in tissues with elevated fatty catabolism. First of all, PPARα modulates genes involved in fatty acid uptake. Intracellular fatty acid concentration is controlled, in part, by the activity of the fatty acid transport protein (FATP), and fatty acid translocase (FAT), also called CD36, which regulate the entry of fatty acids through the cell membrane, and by acyl-CoA synthetase (ACS) which traps fatty acids inside the cells by their conversion to ester derivates. Moreover, this enzyme catalyzes the first step of fatty acid metabolism by converting inactive fatty acids into active acyl-CoA derivates. PPARα activation mediates the up-regulation of FATP and ACS expressions in liver [30]. The implication of PPARα in fatty acid transport was further demonstrated by the lack of induction of FATP and FAT mRNA in liver by PPARα activator in PPARα-null mice [39].

Secondly, in the cytoplasm, free fatty acids bind to transport proteins, such as fatty acid binding protein (FABP) and acyl-CoA binding protein (ACBP). Hypolipidemic drugs are able to induce expression of FABP via PPARα [40]. Interestingly, fatty acid binding protein is a candidate to serve as a shuttle for PPARα ligands, since it binds fatty acids and hypolipidemic drugs and further, is found both in the cytosol and inside the nucleus [14].

Thirdly, PPARα regulates the expression of genes coding for enzymes implicated in the peroxisomal β-oxidation such as acyl-CoA oxidase (ACO), enoyl-CoA hydratase/3-hydroxyacyl-CoA deshydrogenase (bifunctional enzyme) and 3-ketoacyl-CoA thiolase [41]. The activities of these and other

enzymes, responsible for the metabolism of long-chain fatty acids, are stimulated in response to peroxisome proliferators [42], due to changes in the transcription rates of their genes. In fact, mutant animals lacking PPARα are resistant to the induction of the enzymes of the peroxisomal β-oxidation pathway by peroxisomal proliferators [43]. Nevertheless, fatty acid metabolism is mainly regulated by mitochondrial fatty acid oxidation and thus, PPARα has also been demonstrated to affect fatty acid import into mitochondria by up-regulating the expression of the carnitine palmitoyl transferase (CPT) I gene [44]. PPARα further regulates the mitochondrial β-oxidative spiral by modulation of the expression of the medium-chain acyl-CoA dehydrogenase (MCAD) gene [45]. One of the fates of the acetyl-CoA units produced at fatty acid β-oxidation in mitochondria is to be converted to ketone bodies, mainly acetoacetate and 3-hydroxybutirate. The mitochondrial hydroxymethylglutaryl-CoA synthase (mHMG-CoA synthase) is the main enzyme involved in ketone body formation and is directly controlled by PPARα [46]. On the other hand, PPARα appears to increase energy expenditure by inducing the expression of uncoupling proteins (UCPs) [47], such as UCP1, UCP2 and UCP3, which are mitochondrial transporters localized in the inner mitochondrial membrane that act to dissipate the proton gradient and increase thermogenesis while reducing the efficiency of ATP synthesis [48].

In accordance with this, PPARα-deficient mice display either a massive accumulation of lipids in liver when fed a high fat diet [26] or hypoglycaemia, hypoketonemia, and elevated plasma FA levels after fasting for 24 hours [26]. As already commented, through their effect on the expression of fatty acid transporters and oxidation genes, PPARα activators direct the fatty acid flux to the β-oxidation pathway and therefore diminish the fatty acid pool to be incorporated into triacylglycerol-rich lipoprotein. Moreover, PPARα maintains lipid homeostasis by controlling the fatty acid flux from peripheral tissues, such as adipose, to the liver. Interestingly, PPARα also plays a role in adipose tissue as a mediator of leptin-induced lipolysis [49].

Fourthly, the microsomal CYP4A enzymes are a distinct group of the cytochrome P450 superfamily which catalyze the ω-hydroxylation of fatty acids and eicosanoids, such as leukotriene LTB4, and are induced by fibrates and other peroxisome proliferators, through activation of PPAR. Thus, in this way, PPARα has an important role in the detoxification of xenobiotics [25], including some hypolipidemic drugs.

It has been shown that the lipogenic malic enzyme gene is up-regulated by peroxisome proliferators via PPARα [50], and its liver expression is lowered in PPARα null mice [51], suggesting that PPARα is also involved in fatty acid synthesis. However, such a role for PPARα appears complex since other important lipogenic genes, such as acetyl-CoA carboxilase (ACC) and fatty acid synthase

(FAS) [52], are down-regulated by polyunsaturated fatty acids (PUFAs) [52], and peroxisomal proliferators [53]. Furthermore, the enzyme stearoyl-CoA desaturase 1 (SCD1) which catalyzes Δ9 desaturation of saturated fatty acids, is up-regulated by fibrates but down-regulated by polyunsaturated fatty acids [54].

Regulation of PPARα expression

The regulation of PPARα expression has been clearly shown to respond to dexamethasone, a synthetic glucocorticoid, by significantly increasing the PPARα mRNA levels. This effect is mediated by the glucocorticoid receptor (GR). In fact, PPARα mRNA and protein levels change in parallel with the circadian rhythm of circulating glucocorticoids. Accordingly, stress or fasting, which induce an increase in the levels of plasma glucocorticoids, also result in enhanced synthesis of PPARα [55].

Contrary to this, conflicting results have been reported regarding the regulation of PPARα expression by either insulin [56,57] or fatty acids [57], although most of these studies were carried out in vitro. Nevertheless, in studies in vivo in rat during perinatal development, we found that PPARα mRNA and protein levels varied profoundly, reaching a high value during the suckling period and later declining in adult rats [58]. This difference was clearly related to the milk intake, rich in lipids [59]. Moreover, it was observed that the dietary fat was able to markedly increased hepatic PPARα expression, unless plasma insulin levels were high [59]. However, Suruga et al did not find any response of jejunal PPARα mRNA levels in adult rat to forced-feeding of a high fat diet [60]. In accordance with that, we also observed that the expression of the PPARα gene in adult rat seems to be less sensitive to nutritional changes than that found in neonates [61]. However, an induction of PPARα mRNA expression in adult rat hepatocytes by fatty acids [57] has also been reported, suggesting that positive effects of fatty acids on PPARα expression might be liver-specific [60].

On the other hand, exposure of primary culture of rat hepatocytes to growth hormone (GH) for several days decreases PPARα mRNA levels by 50% [62]. Finally, an up-regulation of PPARα gene expression by its own ligands, fibrates or free fatty acids, which would suggest an autoregulatory feedback loop of this nuclear receptor has been found both in rat hepatoma cell line [56] and in vivo studies in rat liver [63].

When PPAR fails (when PPARα activation and hypolipidemic effect are disconnected)

The use of the PPARα agonists, fibrates, as hypolipidemic agents for several decades has demonstrated their safety and efficacy for lipid lowering,

an important parameter in the prevention of cardiovascular diseases. PPARα also exerts pleiotropic anti-inflammatory and antiproliferative effects and prevents the proatherogenic effects of cholesterol accumulation in macrophages by stimulating cholesterol efflux. The ability of PPARα to reduce symptoms of metabolic syndrome (visceral obesity, insulin resistance, inflammation, and atherogenic dyslipidemia, that is, low HDL, high triacylglycerolemia, and small dense LDL) suggests that PPARα may have an optimum cardiovascular benefit in metabolic syndrome and/or other appearances of insulin resistance. However, several findings have revealed either potential adverse effects or unefficient hypolipidemic action of PPARα activation, underlining the need for further study [64].

Cases in rodents

Although there are reports showing that hepatic PPARα mRNA content and plasma triacylglycerols level are inversely related [65,66], in several studies carried out in the last two decades, fibrates were already described as failing, or not sufficiently correcting hypertriacylglycerolemia under certain conditions. Among these, a short treatment of the hypertriacylglycerolemic model of rats (Zucker obese rats) with fenofibrate, was able to reduce their plasma cholesterol but, raised triacylglycerol by 47% in comparison to untreated obese control rats [67]. In another study which compared the effects of fibrates in different groups of mice fed hyperlipidemic diets, it was observed that in animals receiving a standard diet, fenofibrate and gemfibrozil decreased the triacylglycerol level, whereas in animals receiving a hypercholesterolemic diet fenofibrate lowered plasma triacylglycerols and cholesterol but gemfibrozil did little to alter the influence of the hypercholesterolemic diet. A similar situation was found in animals fed sucrose (a hypertriacylglycerolemic diet), where the diminution in plasma triacylglycerol levels provoked by fenofibrate was not dose-dependent, and gemfibrozil was not effective on the plasma triacylglycerol level [68]. Moreover, in some cases the opposite effects of different fibrates in both normal and hyperlipidemic rats were found. Thus, in a study using bezafibrate and gemfibrozil, both drugs lowered plasma triacylglycerols to about the same extent in both chow-fed and hypertriacylglycerolemic rats. Gemfibrozil lowered LDL-cholesterol (LDL-C), but elevated HDL-cholesterol (HDL-C), whereas bezafibrate produced the opposite effects, that is, decreased HDL-C and tended to increase LDL-C. Furthermore, changes in liver triacylglycerol concentrations in hypertriacylglycerolemic rats produced by these drugs were opposite. Thus, although similar with regard to triacylglycerol lowering activity and mechanisms thereof, gemfibrozil and bezafibrate produced different effects in the lipoprotein metabolism of rats which may relate to potential differential

effects on atherogenesis [69]. Although gemfibrozil is structurally unique as compared with other fibrates [70], it is well known that different fibrates may have a somewhat different spectrum of effects [71].

In more recent studies, other cases where PPARα activation and hypotriacylglycerolemic effect are not connected have been found. Thus, another animal model showing the opposite effects of fibrates has been found in late pregnant rats where a hypertriacylglycerolemic condition is normally present in comparison to non-pregnant rats. Thus, although plasma triacylglycerols decreased during the first 2 days of fenofibrate treatment in pregnant rats, the effect disappeared on day 3, and plasma triacylglycerols were even enhanced at day 4. In virgin rats, fenofibrate decreased plasma triacylglycerols throughout the experiment. Such unexpected hypertriacylglycerolemia observed in pregnant rats after fenofibrate treatment may be the result of a rebound effect provoked by the catabolic condition of the mother [72], in which adipose tissue lipolytic activity is enhanced [73], and therefore an enhanced arrival of FFA to the liver might be supporting an augmented VLDL triacylglycerol production capability of the mother [74]. Surprisingly, it was found that, although fenofibrate was unable to reverse the typical hypertriacylglycerolemia of gestation, it efficiently exerted its expected molecular effects in pregnant liver (i.e. to induce fatty acid and lipoprotein catabolism, and to reduce TG-rich lipoprotein secretion) [63]. Related to that, a lack of sensitivity to PPAR-α activators has also been observed in virgin mice in comparison to lactating dams. Fibrates induced the UCP-3 mRNA in skeletal muscle during lactation. However, in virgin mice acute PPAR-α activation did not stimulate UCP-3 mRNA expression. A potential explanation for these findings might be in the levels of serum free fatty acid, higher in virgin rats than in lactating mothers [75].

Not quite so related but in the same sense, it was observed that in a model of myocardial ischemia-reperfusion injury in porcine heart, that one month of pretreatment with fenofibrate had no effect on myocardial substrate utilization, lipid accumulation, contractile function, or infarct size. Effects of treatment were absent even under conditions of increased FFA availability, that is, in a group receiving a high-fat diet. The expected protective effect of fenofibrate was absent despite a prominent expression of PPARα in porcine myocardium and an evident activation by fenofibrate in liver [76]. Accordingly, in another study, mice pretreated with a PPARα agonist for 8 weeks showed no differences compared to untreated mice in myocardial glucose or FFA oxidation or expression of PPARα or PPARα target genes in heart [77].

Further examples showing an unefficient action of PPAR activators were also found in mice genetically modified, such as obese diabetic db/db mice. In these mice rosiglitazone, Wy 14,643 or T0901317, normalized gene expression involved glucose homeostasis, lipid homeostasis and local gluco orticoid

activation. However, a large number of diabetes-associated gene alterations remained unaffected or were even aggravated by nuclear receptor agonist treatment. Thus, expression of many genes implicated in lipogenesis, peroxisomal and mitochondrial function was not improved by drug treatment. These findings indicated that a correction of causal diabetes-associated molecular alterations was achieved by the compounds only to a very limited extent [78]. On the other hand, the apolipoprotein E-deficient mice, a mouse model that mimics some features of human dyslipidemia and atherosclerosis, presented elevated triacylglycerol levels plus diminished HDL concentrations. However, fenofibrate treatment paradoxically increased total cholesterol and triacylglycerols and decreased HDL-cholesterol levels compared with controls. Thus, fenofibrate does not have a beneficial lipid-lowering activity in apo E-null mice [79]. Accordingly, atherosclerotic lesions in cibrofibrate-treated apoE-deficient mice were considerably augmented compared with untreated animals, and plasma cholesterol levels were also increased [80]. Surprisingly, atherosclerotic lesion areas at the aorta were less in PPARα-null mice on an apoE-/- background compared with the control apoE-/- mice, suggesting an atherogenic role of PPARα [81,82]. Related to this, in contrast to the several studies indicating a beneficial effect of PPARα activation on insulin sensitivity [28,83], PPARα null mice have also been shown to be protected from high-fat diet-induced insulin resistance [27]. Finally, in the human apoA-II transgenic mice, which share some similar phenotypic characteristics with (PPARα)-deficient mice, it was shown that a fibrate-induced PPAR alpha activation did not correct the combined hyperlipidemia. Thus, after two weeks of treatment with fenofibrate, human apoA-II transgenic mice presented paradoxically a remarkable increase in plasma triacylglycerols, mainly due to decreased VLDL catabolism and a partial impairment in PPAR alpha-signalling [84].

Cases in humans

Fibric acid derivatives are potentially well-suited to the treatment of dyslipidemia that is generally associated with type 2 diabetes mellitus and metabolic syndrome, as they are usually more effective than HMG-CoA reductase inhibitors for normalizing serum levels of HDL-cholesterol and triacylglycerols. Moreover, hypertriacylglycerolemia is a strong predictor of coronary heart disease [85]. However, fibrates have also been described as failing, or not sufficiently correcting hypertriacylglycerolemia under certain conditions [86], i.e., severe combined hyperlipidaemia [87], or in combined treatment with hormone replacement therapy in obese postmenopausal women [88]. Thus, many patients might not be receiving appropriate treatment for the dyslipidemia that commonly occurs in cardiovascular diseases, insulin resistance and/or the metabolic syndrome [63]. In this way, monotherapy with

fibrate is generally not capable of normalizing the lipid profile of severe combined hyperlipidemia. Indeed, fibrates are more active on hypertriacylglycerolemia while statins are more active on hypercholesterolemia, the two components of such hyperlipidemia [87]. For patients with diabetes who have hypertriacylglycerolemia, gemfibrozil has been shown to be effective; however, not all patients tolerate gemfibrozil therapy [86]. On the other hand, in one study overweight postmenopausal women were separated into two intervention groups: a lipid lowering fibrate group and a hormone replacement therapy group. The fibrate group was given gemfibrozil and the hormone group received estradiol. After 3 months, the fibrate group added the estradiol to their therapy and patients in the hormone group added gemfibrozil, for a further 6 months. Triacylglycerols were more markedly decreased in the group which began the fibrate treatment than the group with the hormone [88].

More evident was the case described in subjects affected by massive hypertriacylglycerolemia being hyporesponsive to either fibrates, or polyunsaturated fatty acids (PUFA), or fibrate-PUFA association. Curiously, coenzyme Q10 improved the efficacy of fenofibrate in massive hypertriacylglycerolemia patients not responding to fenofibrate alone [89]. Indeed, in cases of extreme hypertriacylglycerolemia, combination therapy with gemfibrozil and niacin may be required to reduce triacylglycerols to acceptable levels [86]. Paradoxically, when fibrates are given to patients with very high triacylglycerols levels but who respond to fibrate treatment, an increase in LDL cholesterol was observed [90]. On the other hand, in a study with hypertriacylglycerolemic subjects gemfibrozil, unlike other fibrates, was more potent in Type IIb compared to Type IV subjects [70].

Remarkably, a common observation in fibrate-treated patients is the considerable variation in induced lipid changes, indicating that polymorphisms in the PPAR-α gene may contribute to the different responses to fibrate treatment [91]. Hence, a significant genotype-dependent response to gemfibrozil treatment in HDL_2-cholesterol with the PPARα Leu162Val polymorphism was found. In fact, the missense PPARα Leu162Val mutation has been associated with abnormal serum lipid profiles and with different PPARα activation in vitro. Furthermore, in a study with fenofibrate for 3 years, patients were divided into high responders and low responders in their plasma triacylglycerol. In the high responders group, there was a prevalence of PPARα intron 7 GG homozygotes when compared to the low responders. Thus, these authors proposed that the best predictors of response to fenofibrate treatment were baseline triacylglycerol level and intron 7 genotype [92 and references therein]. Moreover, the relation between frequent genetic variations in gene encoding proteins involved in triacylglycerol-rich lipoprotein metabolism and the effectiveness of fenofibrate on lowering triacylglycerol

levels on hypertriacylglycerolemic subjects was also investigated. Thus, although fenofibrate induced a decrease in plasma triacylglycerol levels in all genotypes studied, mutation-specific differences were observed. The carriers of a lipoprotein lipase (LPL) Pro207Leu mutation presented residual hypertriacylglycerolemia after the treatment. Regarding the apolipoprotein (apo) E gene polymorphism, the apo E2 allele was associated with a better response to fenofibrate on all lipid parameters compared to apo E3 and E4 variants. The simultaneous presence of apo E2 and PPARα Leu162Val tended to improve fenofibrate response in LPL Pro207Leu heterozygote subjects [93]. In the same sense, it has also been related to the liver fatty acid binding protein (L-FABP) Thr94Ala missense mutation and the risk of exhibiting residual hypertriacylglycerolmia after a treatment with fenofibrate [94].

In some clinical trials with cardiovascular endpoints, fibrates have yielded either beneficial effects restricted to some types of patients or even negative outcomes. Thus, in the Helsinki Heart Study, cardiovascular disease risk reduction upon gemfibrozil treatment was most pronounced in obese patients with metabolic syndrome or diabetes and atherogenic dyslipidemia [95]. In the Bezafibrate Infarction Prevention trial, reduction in coronary events with bezafibrate was observed only in patients with elevated serum triacylglycerols concentrations. In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial, the significant beneficial effects of gemfibrozil were shown in diabetics or in nondiabetics with high insulin levels [64 and references therein]. Furthermore, in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, although fenofibrate lowered triacylglycerols as well as LDL cholesterol and hardly elevated HDL cholesterol, it did not reduce the number of total cardiovascular events. However, the interpretation of this study is complicated by the different and increasing use of statin throughout the study in both groups (placebo and fenofibrate) [96].

On the other hand, the factor that dominates in overweight-related metabolic syndrome is the permanent elevation of plasma free fatty acids (FFA) and the predominant utilization of lipids by the muscle, inducing a diminution of glucose uptake and insulin resistance. Therefore, the decreasing of plasma FFA and improving of insulin sensitization by PPAR agonists seems to be a logical and valuable goal for therapy [71]. However, only a few clinical trials report an improvement of glucose homeostasis after fibrate treatment [97,98]. Nevertheless, these beneficial effects of fibrates on glucose control may not be attributable alone to enhanced fatty acid oxidation related to PPARα activation, because some of these compounds also have modest PPARγ activity [99]. However, the FIELD study did not reveal any fenofibrate effect on glucose parameters in diabetic patients [100], and a recent report concluded that administration of fenofibrate for three months did not affect insulin sensitivity in obese subjects with type 2 diabetes [101]. Moreover, there

are data suggesting that PPAR activation in human do not correct insulin resistance induced by glucocorticoids and may even adversely affect blood pressure [102].

Interspecies differences

Rodents and man are very different in the way cellular processes are regulated by PPARα, so caution must be used in extrapolating data from the murine to the human situation. Furthermore, some authors suggest a more modest role of PPARα in humans than in rodents.

Induction of peroxisome proliferation

Human and rodent respond differentially to PPARα-induced peroxisome proliferation. Thus, in rodents, the peroxisomal ß-oxidation pathway is much more induced than the mitochondrial ß-oxidation pathway. On the contrary, in man, the mitochondrial ß-oxidation pathway is induced strongly, but the peroxisomal ß-oxidation is not. Hence, rodents are sensitive to activation by PPARα of peroxisome proliferation while humans are resistant. Furthermore, after chronic treatment with peroxisome proliferators, rodents present hepatocarcinoma, whereas epidemiological studies have not shown these adverse events in patients taking fibrates [103].

Hypolipidemic effect

There are evident differences between mouse and human lipid metabolism. For example, in mice HDL is the main transporter of cholesterol, whereas LDL is the principal carrier in humans. In addition, interspecies variations of the mode of regulation of metabolic genes may be considerable. Thus, the human apo-AI gene contains functional PPRE, whereas in rodents it is not functional due to a difference in three nucleotides between rodent and human PPRE. Activation of PPARα by fibrates induces human apo-AI expression, increasing HDL, whereas in rats fibrates suppress apo-AI expression and decrease HDL levels [103].

Anti-inflammatory properties

Related to the above explained different responses of peroxisomal ß-oxidation to peroxisome proliferators between human and rodents, PPARα activation by LTB_4 stimulates peroxisomal ß-oxidation of this leukotriene in rodent liver, providing a negative feedback control of inflammatory lipid stimuli [103]. Accordingly, PPARα knockout mice present a prolonged response to inflammation [104]. By PPARα activation, peroxisomal ß-oxidation is induced slightly or not at all in man, so no changes should be found in LTB_4 levels [103]. Some studies even suggest that PPARα activation

may be potentially proinflammatory and proatherogenic by stimulating the production of monocyte chemotactic protein 1 (MCP-1) in human endothelial cells [105].

Insulin-sensitizing effects

Animal studies have demonstrated a favourable fibrate effect on insulin sensitivity. However, results of human studies of the effects of fibrates on insulin sensitivity are less consistent. Some studies demonstrated that fibrates are able to improve insulin sensitivity in rodent models of insulin resistance [27,106]. On the contrary, PPARα null mice subjected to a high-fat diet or to aging are protected from developing insulin resistance [28] and PPARα overexpression in skeletal muscle provokes insulin resistance. A recent study indicates that in contrast to rodents no direct effects of fibrates on adipose tissue are present in humans. Further, fibrate treatment does not seem to perturb glucose homeostasis in humans since a study on overweight subjects failed to demonstrate any change in insulin sensitivity after 3-months administration of fenofibrate [101].

Effects on heart

Surprisingly, overexpression of PPARα in the heart turned out to be cardiotoxic [107]. The chronic stimulation of cardiac metabolism by PPARα activators resulted in cardiac hypertrophy and heart failure [91]. However, at present, there is no indication that fibrate treatment would increase chronic heart insufficiency in humans. This points to the above commented fact that PPARα activators exert species-specific activities and may induce peroxisome proliferation in mouse hearts, which could increase oxidative stress [64]. However, since fibrates have yielded mixed results in trials with cardiovascular endpoints, the question of whether activation of PPARα has detrimental effects in the hearts of patients deserves further study.

Factors modulating PPAR agonists response

There are several factors which may modulate PPARα activity, namely, its expression, its ligands (of both pharmacological and physiological origin), its coactivators and corepressors, and the posttranslational modifications of PPARα [64]. In its first level of control, the expression of PPARα can be modulated by a regulator (for example, glucocorticoids or insulin) [55,59] by the interaction of the corresponding nuclear receptor with the regulatory area of that gene. A second level of control is by acting on the nuclear translocation of the PPARα, via complexes with cytoplasmic proteins (for example, a heat-shock protein), or via cellular signalling that regulate the posttranscriptional modifications of PPAR. In a third level of action, the activation of PPARα in

the nucleus depends either on interaction with a ligand, or upon posttranslational modification (phosphorylation, acetylation, sumoylation or other modifications) [108]. Indeed, the transcriptional response is strongly influenced by the chemical structure of the ligand, the nature of the PPRE, the structure of the promoter, the levels of coactivators and corepressors, and physiological and pathological conditions [64].

Relative cellular content of PPARα

To date, there has been very little research into the regulation of PPARα expression. Studies have reported that the regulation of PPARα expression is carried out either by glucocorticoids [55], insulin or, even, by fatty acids and fibrates [59,63].

Interestingly, there has been speculation that relative amounts of PPARα might mediate different responses in gene expression. Thus, whereas high levels of PPARα would modulate both the lipid homeostasis gene expression (i.e. LPL, apo-AI) and genes associated with the peroxisome proliferation (i.e. ACO, bifunctional enzyme), low amounts of PPARα would only act on the regulation of the former [109]. Hence, in humans, the low amount of liver PPARα would be limiting and only the genes related to hypolipidemia may be induced upon exposure to ligand. On the contrary, in rodents, the high level of PPARα would be sufficient to activate both the genes associated with lipid metabolism and the genes related to peroxisome proliferation. A similar explanation would justify the different sensitivity of PPARα gene expression to nutritional changes found in liver of suckling rats (displaying a high PPARα level) and adult rats (having a low PPARα level) [61]. A profound reduction in the expression and activity of hepatic PPARα has also been shown in old rats, which become resistant to eliciting a hypotriacylglycerolemic response and to increasing the liver expression of PPARα target genes when treated with either gemfibrozil or bezafibrate [65].

Palmer et al. (1998) [23] suggested that low levels of PPARα expression in human liver may be responsible for human unresponsiveness to peroxisome proliferation. Moreover, it appears that part of the PPARα mRNA detected in the human samples may correspond either to a truncated form of PPARα with dominant negative activity [110] or a cDNA for PPARα isolated from human hepatocytes that do not encode a functional PPAR [23].

On the contrary, it has been observed in human hepatocytes that the lack of induction of ACO gene expression by PPAR activation was independent of the expression level of human PPARα [111]. Moreover, PPARα-null mice expressing human PPARα in their livers, functionally responded to Wy-14,643 and fenofibrate in a manner similar to wild-type mice, by controlling the expression of known target genes [112]. However, the mice having human

PPARα did not exhibit the hepatocarcinoma that was observed in mice having murine PPARα, despite that the expression levels of PPARα being comparable in both types of mice [112]. Therefore, since human and rat PPARα are not exactly identical in their DNA- and ligand-binding domains [113] and it has been reported that a single amino acid change in the ligand-binding domain of PPARα profoundly alters its transcriptional activity [114], the differences between species should be found in structural and functional differences between human and mouse PPARα rather than in the level of PPARα protein.

Nevertheless, the putative redundancy in the functions of PPARs α and δ as transcriptional regulators of fatty acid homeostasis should be kept in mind which suggests that the delta-subtype might compensate for deficiency of PPARα [115].

Translocation of PPARα

Like steroid receptors, the unbound form of PPARα is present in the cytosol, presumably, maintaining a cytoplasmic chaperone complex. After binding an agonist, the complex is dissociated and PPARα migrates to the nucleus. In fact, it has been shown that PPARs are able to bind both with heat shock protein (hsp) 72-kDa [116] and hsp90 [117].

The nature of ligand

The ligand-binding domain (LBD) of PPARα harbors a "large" ligand-binding pocket which allows it to accommodate mixed natural and synthetic ligands [64].

A wide variety of saturated and unsaturated fatty acids bind to PPARα, but with relatively low affinity and it is unclear whether at concentrations physiologically relevant. Therefore, it is difficult to establish their importance in vivo and the search for "the" endogenous physiologic PPARα ligand is still ongoing [64]. An alternative strategy has been to evaluate enzymatic pathways that could locally generate ligands. Thus, it was demonstrated that LPL releases fatty acids from triacylglycerol-rich lipoproteins that activate PPARα [118]. However, other lipases that were equally effective at generating free fatty acids were unable to activate PPARα, suggesting a selective regulation of that receptor [82]. In this same sense, it has been shown that de novo synthesized fatty acids regulate PPARα activity, whereas the fatty acids released from adipocytes are inactive. This observation would imply the existence of discrete physiological compartments. One possibility is that lipid binding proteins select the fatty acids to be shuttled to PPARα as commented below [119].

In a similar way to that shown for natural ligands, fibrates must be generally used at high doses (300-1200 mg/day), at least in humans, to achieve

efficacious lipid-lowering activity [120]. Interestingly, PPARα ligands also display distinct pharmacokinetic properties. Thus, fibrates appear to act preferentially in the liver and are low affinity ligands for PPARα, whereas high-affinity ligands such as Wy-14,643 and GW7647 are suspected of acting more efficiently in peripheral tissues [64,82]. These and other findings indicate tissue specificity of response to PPARα activation [76].

Interestingly, it has been suggested that alteration of gene transcription by fatty acids and fibrates is often disconnected. Thus, polyunsaturated fatty acids repress transcription of the Δ5- and Δ6-desaturases [121], whereas the same genes are induced by fibrates. Peroxisome proliferators, but not long chain fatty acids, induce carnitine palmitoyl transferase (CPT)-II in fetal rat hepatocytes [122]. In hepatocytes, lipoxygenase inhibitors impair activation of CPT-I gene expression by fibrates, but do not affect the induction by fatty acids [123]. These different responses and others indicate that not all of the fatty acid effects can be assigned to PPAR activation [124]. Hence, in conditions where both an enhanced arrival of free fatty acids to liver and treatment with fibrate would be coincident, an additional battery of genes might be affected, in comparison to those situations where either PPAR ligands would be present separately. That would be the case of late pregnant rats having an active lipolytic activity and treated with fenofibrate as compared to non-pregnant rats [74] and of animals receiving a high-fat diet along with fibrate [76]. Furthermore, if the action of fibrates on lipid metabolism is primarily mediated by their role as ligands for PPARα [13], an enhanced arrival of free fatty acids could decrease the availability of the former to their corresponding PPAR site, substantially reducing the capability of fibrates to activate PPARα and, consequently, its metabolic effects [14,74].

The role of fatty acid-binding protein (FABP) and other structurally related proteins which function as gateways for the transport of ligands, and participating in the interaction of fatty acids with PPARα cannot be ruled out [125]. Long-chain fatty acids can compete or displace peroxisome proliferators for binding to FABP [14]. Therefore a displacement of fibrates for binding to FABP by free fatty acids would be influenced by their intracellular concentration. Moreover, it has been suggested that impairing either the function of cytoplasmic retinoic-acid binding protein (RABP, which favours presentation of retinoic acid for enzymic formation of 9-*cis* retinoic acid, the ligand of RXRα) or the function of FABP might depress PPAR activity [14,76].

To add more complexity, the species differences in ligand activation of PPARα cannot be discarded. For example, fibrates are more effective than linoleic acid in promoting PPARα activation in cultured rat hepatoma cells,

whereas linoleic acid is more effective than fibrates in human hepatoma cells [126].

Cross-talk signalling

Several transcription factors such as RXRα, Sp1 [127], chicken ovoalbumin upstream promoter transcription factor (COUP-TF) [128], hepatocyte nuclear factor-4 (HNF-4) [129], thyroid receptor [130], LXRα [131], and TAK1/TR4 [132], as well as coactivator and integrator proteins [133], can modulate the transcriptional activity of PPARs. Thus, differences both in the expression of these factors and the interactions of one or several of these proteins upon addition of fibrates could modulate the capacity of PPAR to regulate the expression of target genes which might explain the interspecies differences found or between diverse type of cells [111,134,135]. Moreover, whereas fenofibrate behaves as a full agonist, gemfibrozil appears to act as a partial agonist and seems to be due to a differential recruitment of coactivators [136]. The binding of a partial agonist to PPAR might cause the receptor to interact with cofactors in a less efficacious manner than a full agonist [137]. Such distintive interaction between PPAR and its cofactor may transmit signals that result in a unique gene regulatory activity [120]. These observations would explain, for example, the differences found in the activity of fenofibrate and gemfibrozil on apoA-I expression [136].

Moreover, other receptors, such as the orphan receptor RZRα, can bind directly to PPRE and mimic or avoid the action of the heterodimer PPARα-RXRα [103]. PPARα interferes negatively with other nuclear signalling pathways such as the AP1 [138], the CAATT/enhancer binding protein (C/EBP) [139] and NF-κB pathways [140]. The subtype ß of PPAR has also been reported to be a physiological human antagonist of PPARα [141], and therefore, a higher expression of the PPARß isoform in human cells than in cells of rodent origin, could imply an impairment of PPARα activity in human cells [134]. In a similar way, PPAR function could be antagonised by dimerisation of the orphan LXRα with either PPARα or RXRα, leading to a complex that cannot bind to DNA responsive elements [103]. To further complicate the matter, certain fatty acids have also been shown to function both as PPARα agonists and as LXR antagonists. Moreover, fibrate esters display LXR agonist/antagonist activity that is dependent on the target gene context [142].

Several authors have also proposed a cross-talk between fibrates and estrogen. Thus, for example, mixed studies carried out either in wild-type, LDL. Receptor-deficient or ovariectomized mice have suggested the involvement of ovarian steroid hormones in the regulation of obesity and hypertriacylglycerolemia by fenofibrate [143,144]. Moreover, in late pregnant rats treated with fenofibrate, competition between fenofibrate and estradiol,

which levels during late pregnancy are extremely high [145], leading to lipid metabolism has been suggested [63,146]. Indeed, it appears that the gender of the animal affects how it responds to perturbations in PPARα expression, since gender-related differences in the phenotypes of PPARα null mice have been noted. Accordingly, studies using small interfering RNA (siRNA) to knockdown PPARα and studies with PPARα null mice and administration of an inhibitor of CPT-I [147] showed findings with sexual dimorphism [148]. In fact, it has been reported that heterodimers of PPAR and RXR can bind to estrogen response elements (ERE) in estradiol-responsive genes and negatively regulate expression [149]. Nevertheless, the possibility that peroxisome proliferators may also produce changes in serum estradiol levels cannot be discarded [146]. Eventually, the orphan nuclear receptor estrogen receptor-related receptor alpha (ERRα) activates PPARα gene expression via direct binding to the PPARα gene promoter, and so, many of the genes regulated by ERRα are known targets for PPARα [150].

Structure of the PPRE/promoter

It is well-known that gene regulation via hormone response elements is complex. Thus, the sequences DR1 and DR2 present in PPREs are able to bind PPAR/RXR heterodimer, but are also recognized by RXR homodimers, retinoic acid receptor (RAR)/RXR heterodimers, HNF4, and COUP-TF. The effectiveness of these interactions depends on the sequence of the regulatory element and the context of the promoter, suggesting cross-talk with other nuclear receptors that may generate different agonistic/antagonistic actions and influence metabolic control [38,64]. For example, the apo-CIII gene harbors one DR-1, but its promoter responds to PPAR only in non-hepatocyte cells [151] and therefore, fibrates appear to down-regulate apo-CIII gene expression through either of HNF4 or Rev-erb-α nuclear receptors [152]. Thus, PPARα activators would stimulate Rev-erb-α [153], which in turn inhibits apo-CIII [38]. Moreover, although the PEPCK gene promoter contains two DR1 sequences [154], it is responsive to fatty acids in adipocytes but not in hepatocytes [124]. On the other hand, several studies have indicated that PPAR/RXR complex up-regulate L-FABP expression, by competing with COUP-TFII for the DR1 sites in the proximal promoter of that gene. Thus, the relative cellular content of COUP-TFII (repressor) to PPAR/RXR (activator) determines the differential L-FABP expression found in different types of cells [155]. All these findings would explain why only three genes: apo-AV, apo-AII and CPT-I, have been firmly identified as a clear and direct target gene of PPARα activators [38].

On the other hand, a series of studies have demonstrated that the PPRE on human ACO promoter is non-responsive to PPARα whereas the rat PPRE is

highly responsive [156]. However, other studies indicated that in human cells, rat as well as human PPARα displayed no distinct responsiveness to peroxisome proliferators on a rat or human ACO PPRE [23,111]. Another study in human liver cells treated with fibrates indicates that the lack of peroxisome proliferation and the no-increase in the interaction of PPAR-RXR complexes with the PPRE in the ACO promoter are related. Accordingly, the characteristics of the response element, their specific sequence, spatial disposition, and extended sequence are determinants in the efficiency of PPAR to modulate the gene transcription [134 and references therein].

Phosphorylation and other posttranslational modifications

The phosphorylation-dephosphorylation process is an important regulation mechanism which is also valid for transcription factors. In fact, PPARα is a phosphoprotein. In the proximity of the DBD of PPARα, a N-terminal region called activating function–1 (AF-1) that may be phosphorylated is found [6]. Therefore, PPARα activity is dependent on its phosphorylated state [157]. It has been observed that the phosphorylation of PPARα may be enhanced by treatment with peroxisome proliferators like ciprofibrate, and by insulin [57,158]. Phosphorylation may inhibit gene transcriptional regulation by PPARα. Accordingly, cell treatment with phosphatase inhibitors decreased the levels of ciprofibrate-induced peroxisomal ACO mRNA [157]. Moreover, PKC is modified by peroxisome proliferators, increasing the phosphorylation level of some specific proteins [159]. Hence, PKC inhibition increases repression of the fibrinogen-β gene by PPARα by modulating its phosphorylation state [160]. On the other hand, PPARα activity has also been shown to be modulated either by Janus kinase 2/signal transducer and activator of transcription 5b (JAK2/STAT5b), a kinase complex regulated by growth hormone [161], or by MAPK [6]. Eventually, modification of the phosphorylation state of PPAR affects ligand affinity [103].

Pharmacological strategies

Metabolic syndrome and type 2 diabetes mellitus both increase the risk of cardiovascular disease and their prevalence is increasing worldwide. Thus patients need to mitigate or avoid these risk factors either through lifestyle or pharmacological approaches. Moreover, many of them are not receiving appropriate treatment for the individual components of metabolic syndrome which contributes to reducing the overall impact on cardiovascular and diabetic diseases [85,162]. Generally, fibrates, PPARα agonists, have been accepted as the most promising treatment [163]. However, as already commented, a number of studies in animals and humans have revealed potential adverse effects of PPARα action or failure of fibrates, highlighting

the need to search for alternative or complementary therapies. Furthermore, fibrates are weak agonists of PPARα, and high doses are required for effective treatment. While selective PPARα agonists with higher potency, as well as improved PPARα receptor specificity and possibly greater therapeutic benefit, are currently being developed [164], other alternative therapies are, or are not, demonstrating their usefulness [165,166].

PPARγ agonists

In both insulin-resistant diabetic rodent models and clinical research studies with type 2 diabetic patients, TZDs have clearly been demonstrated to be able to lower both fasting and postprandial glucose levels, as well as circulating insulin levels [167]. Treatment of patients with TZDs seems to have beneficial effects on most of the components of metabolic syndrome, providing pharmacological evidence that insulin resistance is the core abnormality in these patients and that the associated abnormalities are, in some way, mechanistically related to the impairment of insulin action [168]. Indeed, TZD treatment improves insulin sensitivity, reduces circulating triacylglycerol levels, modestly increases HDL levels, decreases blood pressure and diminishes plasminogen activator inhibitor-1 (PAI-1) levels [169]. On the other hand, differences in the lipid-lowering capability of TZDs have been shown, for instance, pioglitazone, but not rosiglitazone, lowers serum LDL and triacylglycerol levels in diabetic patients [170]. One possible explanation for these differences may be that pioglitazone has, albeit limited, PPARα activity [171].

RWJ-348260 is a PPARγ agonist with novel structural features compared to other PPARγ agonists reported previously. Thus, the TZD ring has been replaced with a carboxylic acid. In addition, the molecule also bears a fatty side chain [172]. RWJ-348260 has shown *in vitro* to selectively activate PPARγ receptor, and *in vivo* to efficaciously act as an insulin sensitizer in diabetic animals. Furthermore, in Zucker diabetic fatty rats, 8 weeks treatment with RWJ-348260 produced greater reductions in circulating triacylglycerol and cholesterol levels with less weight-gain than rosiglitazone [173]. Since RWJ-348260 produced a lower liver lipid accumulation compared to rosiglitazone in *db/db* mice, this PPARγ agonist appears to act with similar efficacy, but less side-effects, compared to rosiglizazone [173]. On the other hand, another molecule that binds to PPARγ is YM440, which induces an unique conformational change in the PPARγ protein and cofactors recruitment, different to those produced by TZDs [174]. Curiously, YM440 displays little effect on adipocyte differentiation and appears to act preferentially on the liver, there being a great difference with respect to other PPARγ agonists that improve both hepatic and peripheral resistance. Accordingly, chronic treatment

of Zucker diabetic fatty rats with YM440 improves glycogen metabolism and gluconeogenesis in liver [175]. In addition, this agent significantly decreased blood glucose to a level comparable to TZD-treated diabetic *db/db* mice, but without body weight gain [176].

PPARβ/δ agonists

Preclinical studies suggest that ligands of the less well-known receptor, PPARβ, may be beneficial in patients with dyslipidemia and insulin resistance, since PPARβ agonists have been shown to reduce triacylglycerols and LDL, and increase HDL levels in insulin-resistant animals [177,178]. Moreover, treatment of obese mice with a PPARβ agonist reduces obesity and insulin resistance via an effect on fatty acid oxidation in skeletal muscle. Furthermore, PPARβ also appears to have anti-inflammatory effects. Thus, a PPARβ agonist such as GW501516 has demonstrated similar effects as bezafibrate on circulating lipids and insulin resistance. Nevertheless, it has been suggested that the effects of PPARβ in the liver might be very similar to those of PPARα, at least, in the regulation of genes involved in lipid metabolism [179].

Dual PPARα/γ agonists and pan-agonists

The combined use of fibrates and insulin sensitizers results in a decrease in insulin resistance, with reduced blood glucose and triacylglycerol levels [180]. In addition, both PPARα and PPARγ selective activators have been shown to suppress vessel wall inflammatory activity and reduce atherosclerosis in experimental animal models through complementary mechanisms [181]. Therefore, an agent that simultaneously activates both PPARα and PPARγ is assumed to be useful for the treatment of these metabolism abnormalities [182]. Glitazars (a type of PPARα/PPARγ dual agonists) are new drugs in the treatment of diabetes as well as metabolic syndrome.

Muraglitazar (BMS-298585) binds with high affinity to both human PPARα and PPARγ. The *in vivo* pharmacological data in lean and *db/db* mice demonstrate that muraglitazar modulates the expression of PPAR target genes implicated in the regulation of glucose and lipid metabolic pathways in white adipose tissue and in liver, showing potent and efficacious antidiabetic and lipid-lowering actions [183]. Due to excellent findings in animals models, clinical trials in humans were initiated. A phase III clinical trial with type 2 diabetic patients compared treatment with muraglitazar versus pioglitazone, both in combination with metformin [184]. Muraglitazar plus metformin showed significantly greater improvement in glycemic control, reduction in plasma triacylglycerols and an increment in plasma HDL, than pioglitazone plus metformin. However, it was also found that death, nonfatal myocarcial infarction, or stroke occurred in a higher number of patients treated with

muraglitazar as compared to patients treated with pioglitazone or placebo. Furthermore, in clinical trials, tesaglitazar was shown to elevate blood creatinine levels in patients, indicating potential kidney toxicity. Therefore, these two promising compounds were discontinued in May 2006, and at present new molecules are being tested [185].

DRF 2519, an analogue of the TZDs, has shown dual activation of PPARα and γ. In an insulin-resistant *ob/ob* mouse model, DRF 2519 showed better alleviation of insulin resistance and dyslipidemia than rosiglitazone. In fatty Zucker rats, DRF 2519 showed greater reduction in plasma insulin, triacylglycerol and free fatty acid levels than rosiglitazone. In high-fat-fed Sprague Dawley rats, DRF 2519 better improved plasma lipid profiles than fenofibrate or rosiglitazone. These results indicate that DRF 2519 could be an interesting candidate in the management of metabolic disorders and associated complications [186]. Another novel PPARα/PPARγ dual agonist is LSN862, which shows improvements in glucose and lipid levels in rodent models of type 2 diabetes and dyslipidemia. LSN862 appears to display a high-affinity PPARγ partial agonism with relatively less but still significant PPARα agonist activity. In Zucker diabetic fatty rats, LSN862 showed higher glucose and triacylglycerol lowering activity and a greater increase in adiponectin levels than rosiglitazone. In addition, LSN862 in *db/db* mice demonstrated statistically better antidiabetic efficacy compared with rosiglitazone. In the humanized apo AI transgenic mouse, LSN862 and fenofibrate reduced, whereas rosiglitazone increased, VLDL levels. Moreover, LSN862 produced the maximal increase in HDL compared to fenofibrate or rosiglitazone [187]. These findings indicate that PPAR full agonist activity is not necessary to be an efficient insulin-sensitizing agent.

In addition, bezafibrate is a pan(α, β and γ)-PPAR activator with more than a quarter of a century of safe therapeutic use. Bezafibrate improves both insulin sensitivity and the blood lipid profile and reduces the risk of long-term cardiovascular complications in patients with features of metabolic syndrome [71]. Therefore, bezafibrate-based clinical studies support the use of pan-PPAR ligands as therapeutic agents against the metabolic syndrome. Nevertheless, since bezafibrate is a weak PPAR ligand, more powerful new pan-PPAR compounds should be sought to be used as a therapy for patients in which lipid and glucose metabolism disorders coexist [188].

Selective PPAR Modulators (SPPARMs)

The concept of Selective PPAR Modulators (SPPARMs) was suggested by analogy to Selective Estrogen Receptor Modulators (SERM), which proposes that each different ligand can have different agonist or antagonist properties depending on the cell context and the specific target gene in question. PPAR ligands with different chemical structures (SPPARMs) bind to LBD receptor,

inducing distinct conformational changes that lead to different binding affinities for the various cofactors and, thus, activate PPAR in distinct ways provoking differential gene expression and biological responses. Nevertheless, it should be kept in mind that, unlike classical steroid hormone receptors, PPAR receptors have a large ligand-binding pocket which is not fully filled with the ligand [189].

The best therapeutic strategy is to select a desired PPAR-mediated endpoint in one cell type without inducing an adverse PPAR-mediated effect in another [103]. A SPPARM with differential properties in the regulation of fat cell function is FK614. This compound behaves as a partial agonist in inducing the interaction of PPARγ with both transcriptional coactivators CBP and SRC-1, but as a full agonist with both PBP and PRIP, which are required for PPARγ-mediated adipogenesis. Therefore, in differentiating 3T3-L1 adipocytes, but not in mature adipocytes, FK614 induces aP2 mRNA expression and triacylglycerol accumulation, whereas TZDs produce the same effects at the two stages of adipocyte differentiation. Since FK614 behaves as SPPARM with stage-dependent selectivity, it may contribute to ameliorate insulin resistance without stimulating fat accumulation in adipocytes [190]. On the other hand, two angiotensin receptor blockers (ARBs), telmisartan and irbesartan, have also been proposed to function as SPPARMs. Telmisartan interacts directly with the PPARγ protein producing a distinct conformational change and selective cofactor binding as compared to a glitazone, resulting in differential gene expression profiles in adipocytes assessed by microarray analysis. Thus, the ARBs could be displaying the metabolic efficacy of PPARγ activation with low adverse effects (SPPARM activity) along with angiotensin receptor blocking activity [191].

Repression of PPAR

There is a theory that many synthetic PPARγ ligands are not pure PPARγ activators but, rather, partial agonists. Moreover, it has been proposed that for binding to PPAR these molecules might compete with endogenous ligands, which presumably act as pure agonists. Therefore, TZDs are able to increase insulin sensitization by displacement of endogenous PPARγ ligands that, by affecting key glucoregulatory genes, would impair the insulin action [168]. Thus, insulin sensitivity would be enhanced by reversing the repression with a TZD partial agonist. The same effect would be observed by reducing the level of the PPARγ receptor as occurs in heterozygous PPARγ+/– mice [168]. The concept of TZD as a partial agonist/antagonist has been supported when different TZDs have been shown to regulate both overlapping and different sets of genes, resulting in specific and common biological responses. Each TZD would display a different binding affinity, with different residence times

on the receptor, and therefore, slightly different ways of interacting with the binding pocket [192].

Since the most accepted action of TZD is to induce PPARγ activation, TZDs are generally considered as PPARγ agonists. On the contrary, the compound SR-202 has been identified as PPARγ antagonist. Curiously, both SR-202 and TZDs protect against high-fat diet-induced insulin resistance in animal models, suggesting that PPARγ antagonists in rodents might have effects similar to PPARγ agonists. However, in contrast to TZDs, SR-202 reduces adipogenesis and prevents obesity [193].

Contrary to this, a PPARα antagonist produces effects similar to those associated with the inhibition of PPARα, that is, disregulation of lipid and lipoprotein metabolism [194].

Rexinoids (RXR ligands)

RXR ligands (rexinoids) have been considered as a therapeutic approach to metabolic diseases. A heterodimer composed of RXR and a partner such as PPAR, can be activated by a RXR ligand, independently of the presence or absence of a ligand for the partner of the receptor. Moreover, RXR homodimers can selectively bind to functional PPREs and induce transactivation, both in the absence and in the presence of PPAR [195]. Therefore, rexinoid synthetic ligands such as targretin (LGD1069), can activate genes that have a PPRE in their promoter, in a independent PPAR-RXR way [196]. Thus, in mice, rexinoids have shown antidiabetic effects [197], but have also increased circulating triacylglycerol levels [198], and interfered with thyroid hormone receptor signalling, provoking a profound hypothyroidism [199].

Combined therapies

As discussed previously, there has been controversy about the appropriate hypolipidemic drug treatment in subjects with dyslipidemia. Statins are the drug of choice for patients who need to regulate the LDL-cholesterol, and fibrates for those who need to control HDL-cholesterol and/or triacylglycerol levels [200]. The simultaneous use of fibrates and statins could be suitable in patients who need to normalize all those lipid levels. However, a statin-fibrate combination, mainly gemfibrozil and to a lesser extent fenofibrate, may be associated with an increased risk of rhabdomyolysis [200,201,202].

Other lipid modifiying strategies use niacin, ezetimibe, bile acid sequestrants or cholesteryl ester transfer protein (CETP) inhibitors. Moreover, since fibrates, niacin, ezetimibe and statins regulate serum lipids by different mechanisms, combined therapy may produce benefits in patients with combined hyperlipidemia. Thus, combinations of some of these hypolipidemic

agents have been used effectively on dyslipidemic patients with low responsiveness to monotherapy [71].

Nicotinic acid (niacin), despite its modest effect in increasing serum glucose levels, has beneficial effects on all traditional blood lipid and lipoprotein fractions and produces a marked reduction in cardiovascular events. Niacin has even been combined with clofibrate in a molecule, etofibrate, that has exhibited its lipid-lowering effect on animals [35] and patients [203]. Niacin has also shown beneficial effects on FFA levels, possibly due to the inhibition of adipose tissue lipolysis [200,204].

Bile acid sequestrants (cholestyramine, colesevelam and colestipol) bind bile acids within the intestinal lumen thereby reducing the reabsorption of bile acids. This depletes the hepatic bile acid pool, thus stimulating an increase in bile acid synthesis from cholesterol, which lowers LDL cholesterol. Bile acid sequestrans are not absorbed by intestine and thus have no systemic drug-drug interactions, but may interfere with the absorption of some lipophilic vitamins and drugs [71,205].

Ezetimibe is a cholesterol absorption inhibitor that blocks the translocation of dietary and biliary cholesterol from the intestinal lumen into the enterocytes [206]. Ezetimibe reduces intestinal cholesterol absorption by binding to the ATP-binding cassette (ABC) transporters, which are proteins located in the brush border of enterocytes that promote efflux of cholesterol and plant sterols from enterocytes back into the lumen, limiting the amount of absorbed cholesterol [207].

Finally, the CETP inhibitors (JTT-705 and torcetrapib) have been shown to effectively increase HDL-cholesterol levels in humans, without significant side effects [208].

Other therapies

Alternatively, nuclear receptors have been suggested as putative targets for new therapeutic approaches, for instance, LXR, FXR and, presumably, orphan receptors [108]. Recent findings have shown that FXR regulates triacylglycerol levels and phospholipid transfer protein, apo AI, and apo AV, three key players in lipoprotein metabolism, which have been described as FXR target genes [209 and references therein].

On the other hand, some of the adverse effects of PPARα action are due to the compounds generated in oxidized fatty-acid metabolism [210], suggesting that antioxidant therapy coadministered with PPAR ligands, might alleviate or prevent the undesirable effects of PPAR agonists [103].

Finally, emerging therapies such as incretin mimetics, dipeptidyl peptidase IV inhibitors, protein tyrosine phosphatase 1B inhibitors, leptin receptor antagonists, endocannabinoid receptor blocking agents [200], anti-obesity drugs, angiotensin converting enzyme (ACE) inhibitors, and even aspirin and

dietary supplements might be proposed as potential future therapies (alone or in combination) [211]. The multiple risk factors present in many dyslipidemic patients makes using multifactorial pharmacological treatment recommendable [212].

Nutritional strategies

The most appropriate recommendation for dyslipidemic patients, moreover if obese, is a nutritionally balanced, moderately hypocaloric diet with a reduced intake of saturated fat and an increase in physical activity. A significant role in weight reduction has been reported with the use of natural hypoglycemic substances such as cinnamon extract, which can significantly reduce blood glucose levels and lipids, improving insulin sensitivity [213]. The control of appetite would also lead to better weight control. In fact, adipose tissue plays a crucial role in the regulation of food intake, because it secretes a number of endocrine and paracrine mediators, including leptin, adiponectin, resistin, and TNFα, which have been shown to influence appetite. Understanding the complex signalling system that underlies appetite control will probably offer new treatment approaches. Moreover, all three of the PPAR isotypes attenuate inflammatory responses [214], which is important, because inflammation is intimately connected to appetite, insulin resistance, obesity, and atherosclerosis [91].

As previously commented, the recognition of fatty acids as putative endogenous ligands for PPARs, prompted the identification of numerous natural compounds that can bind to one of the three PPAR subtypes, and stimulate its transcriptional activities in cells and its consequent biological activities. Since PPARα regulates fatty acid catabolism [215], PPARδ mediates macrophage VLDL signalling [216,217], and PPARγ controls adipocyte differentiation, lipid storage, and glucose homeostasis [218], all these effects have been employed to propose the use of natural PPAR agonists for the treatment of atherosclerosis [82,163], metabolic syndrome and other metabolic diseases [200].

Polyunsaturated fatty acids

Polyunsaturated fatty acids, especially those of the omega-3 class, are known to affect all the metabolic nuclear receptors that modulate triacylglycerol levels, LXR, FXR and HNF-4α, as well as the three PPARs [219]. The efficient hypotriacylglycerol effect of omega-3 fatty acids has been explained by their coordinated action on these 4 different nuclear receptors [219]. Although omega-3 fatty acids are more potent than omega-6 fatty acids as in vivo activators of PPARα, neither of these polyunsaturated fatty acids are particularly strong PPARα activators [220]. However, eicosanoids derived

from polyunsaturated fatty acid metabolism or oxidized fatty acids have greater affinity for PPARα than their precursor fatty acids and are more potent transcriptional activators of PPARα-dependent genes [220]. Different to PPARα, PPARγ has a preference for binding polyunsaturated fatty acids, but they are not particularly efficacious activators of PPARγ. However, intracellular conversion of polyunsaturated fatty acids to eicosanoids, through enhanced expression of 15-lipoxygenase, greatly increases PPARγ mediated gene activation [221].

Conjugated linoleic acid (CLA)

Conjugated linoleic acid (CLA) refers to a group of positional and geometric octadecadienoic acid isomers of linoleic acid that have conjugated bonds, i.e. it has two double bonds separated by a single bond. CLA is found naturally in animal tissues and food sources, including ruminant meats, poultry, eggs and dairy products. The two predominant isomers of CLA found in food and commercial preparations are cis-9,trans-11 CLA and trans-10,cis-12 CLA. Studies, mostly conducted in animals, have reported that CLA has beneficial effects on atherogenesis [222], diabetes [223], immune function [224] and body composition [225]. Although the action mechanism(s) of CLA are not clearly understood, it has been proposed that activation of PPARα and/or PPARγ may account, in part, for those effects of CLA reported in animal studies [226,227], although both CLA isomers have been shown to antagonize ligand-dependent activation of PPARγ [228].

Oleylethanolamide (OEA)

Amides of fatty acids with ethanolamine (FAE) are a family of natural active lipid mediators present in tissues and circulating blood [229] that participate in a variety of biological functions, including the regulation of feeding [230] and improvement of myocardial function in conditions of cardiomyopathy [231]. These compounds are produced naturally in mammalian cells through the concerted action of two enzymes: N-acyltransferase, which transfers a fatty acid from a donor phospholipid to the free amine in phosphatidylethanolamine, producing N-acyl-phosphatidylethanolamine, and phospholipase D, which converts N-acyl-phosphatidylethanolamine to the amide of the long-chain fatty acid with ethanolamine (FAE). A polyunsaturated member of this family, named anandamide (arachidonoylethanolamine) has been shown to increase food intake in rats by activating G protein-coupled cannabinoid receptors [232]. However, the monounsaturated FAE oleylethanolamide (OEA) decreases food intake and body weight gain and modulates lipid metabolism through a cannabinoid receptor-independent mechanism [229] that involves its high-affinity binding to and consequent activation of PPARα [230]. It has also

been shown in rats that OEA stimulates both adipose tissue lipolytic activity and hepatic fatty acid oxidation, the process being carried out through the activation of PPARα [233,234].

Isohumulones

Beer flavour is a complex balance of sweetness from sugars and bitter from the humulones in hop (Humulus lupulus) flowers. When wort (unfermented beer) is boiled, the humulone molecules rearrange by isomerization to form the more soluble isohumulones, which mostly comprise isohumulone, isocohumulone and isoadhumulone [235]. Although there are few studies concerning the biological activities of the isohumulones found in beer, it has been reported recently that isohumulones can activate PPARα and PPARγ [236]. Throughout its effect enhancing PPARγ expression, it has been proposed that isohumulones improve insulin resistance in diabetic mice [236], whereas throughout the effect enhancing PPARα expression, it has also been proposed that isohumulones raise plasma HDL-cholesterol levels and reduce liver cholesterol and triacylglycerols in C57BL/6N mice [237]. A similar mechanism could account for the effect of isohumulones in preventing diet-induced obesity in mice [238]. Taken together, these findings have given rise to the suggestion that isohumulones could prevent metabolic syndrome in humans, although such a proposal requires further study.

Vitamin E

Vitamin E is a generic term that refers to any of four tocopherols or tocotrienols (α, β, δ and γ) isoforms. The isoform found in highest concentration in plasma is the α-tocopherol, although γ-tocopherol is the most abundant in the diet [239].

One beneficial health effect has been reported by tocopherols which is mediated throughout their action modulating PPARγ expression or activity. Vitamin E deficiency increases the expression of the scavenger receptor CD36, and it has been shown that α-tocopherol down-regulates CD36 both mRNA and protein expression in oxidized LDL-stimulated THP-1 monocytes, and that it can prevent CD36 induction after stimulation with a specific agonist of PPARγ [240]. Besides, vitamin E prevents cholesterol induced atherosclerotic lesions and the induction of CD36 scavenger receptor mRNA expression in hypercholesterolemic rabbits [241]. Altogether these suggest an involvement of vitamin E effects at the level of the CD36 scavenger receptor in its protective role against atherosclerosis.

Aged mice have been shown to express reduced mRNA levels of PPARα and target genes, such as peroxisomal acyl-CoA oxidase. Reductions in PPARα gene expression might contribute to the prooxidant state observed in

aged animals, possibly due to a deficiency in the modulation of cellular redox state. Reciprocally, that prooxidant state may be a cause of the reduced expression of PPARα found in old rats [65]. Interestingly, supplementation of old mice with vitamin E caused elevations in PPARα and acyl-CoA oxidase transcripts to the levels seen in young animals [242], suggesting that balancing the cellular redox state may positively affect the transcriptional regulation of PPARα gene.

Tocopherols, and particularly γ-tocopherol, have also been shown to positively modulate the activity of PPARγ protein in human keratinocyte cell lines, enhancing the expression of its target gene involved in terminal keratinocyte differentiation, transglutaminase-1 [243].

Retinoids

Retinoid acid receptors (RAR) play a key role in hematopoiesis [244] and have also been reported to participate in several development processes, including embryonic, skeletal [245], myeloid [244] and nervous system, as well in wound healing and keratinisation [246]. More recently it has been shown that the expression level of PPARγ increases during monocyte/ macrophage development, and that its activity can be enhanced by retinoids [247]. This effect opens up the possibility that retinoid modulated PPARγ responsiveness could be utilized to boost the anti-atherogenic effects of PPARγ regulated gene expression.

In hematopoiesis, PPARγ expression is increased during differentiation of monocytes to macrophages and the activation of PPARγ/retinoid X receptor (RXR)-regulated genes induces macrophage differentiation [248]. PPARγ/RXR signalling has been shown to be involved in the induction of apoptosis in T lymphocytes [249] and myeloid leukemias. The combination of PPARγ ligands with a RXR agonist or a RAR agonist enhanced differentiation and growth-inhibitory effects in myeloid and lymphoid cells [250]. These findings suggest that by activating the transcriptional activity of target genes that control apoptosis and differentiation in leukaemias, the PPARγ ligation in combination with retinoids have a potential utility for the treatment of hematopoietic malignancies.

Both all-trans-retinoid acid and 9-cis-retinoic acid, which are ligands for RAR and RXR, inhibit vascular smooth muscle cell (VSMC) proliferation in vitro and neointima formation after mechanical injury of the vessel wall [251]. The mechanism by which retinoids inhibit VSMC proliferation seems to be throughout the activation of PPARγ, which heterodimerizes with RXR-α and has been shown to inhibit G1→S progression in VSMCs [252]. An antiproliferative activity by modulating G1→S cell cycle regulators has also been demonstrated by retinoids in human coronary artery smooth muscle cells

through inhibition of retinoblastoma protein (Rb) phosphorylation and elevation of cyclin-dependent kinase (CDK)-inhibitory protein p27Kip1 levels [253]. Thus, by modulating these cell cycle molecules in human coronary cells, natural retinoids may provide an appropriate treatment for proliferative vascular diseases.

Phytochemical therapies

Interestingly, the cholesterol-lowering effect and the beneficial antidiabetic action of soy intake might be related to the fact that soy isoflavones have been confirmed in being able to activate PPARs [254]. Furthermore, polyphenolic compounds such as resveratrol analogues have shown lipid and glucose lowering properties mediated by PPARα [255].

On the other hand, plant sterols and plant stanol esters inhibit intestinal cholesterol absortion, and are being added to some types of food and thus effectively reducing LDL-cholesterol [211].

Conclusions

Effective and well-tolerated drugs are urgently needed to control obesity, cardiovascular disease, type 2 diabetes mellitus and definitively, metabolic syndrome. Complete knowledge of how PPARs regulate metabolic pathways both intra and extracellularly, as well as additional experimental and clinical studies will be useful in the search for and development of novel molecules as therapy for those metabolic diseases. Moreover, evidence also suggests that there is a 'fibrate effect' mediating the favourable action of PPAR agonists in dyslipidemia beyond the favourable impact of these agents on lipid and lipoprotein metabolism. These changes affecting other areas are consistent with the pleiotropic effects of fibrates which are known to be related to their action mechanisms. These and other pleiotropic effects of PPAR agonists may be discovered and used to improve their effectiveness.

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