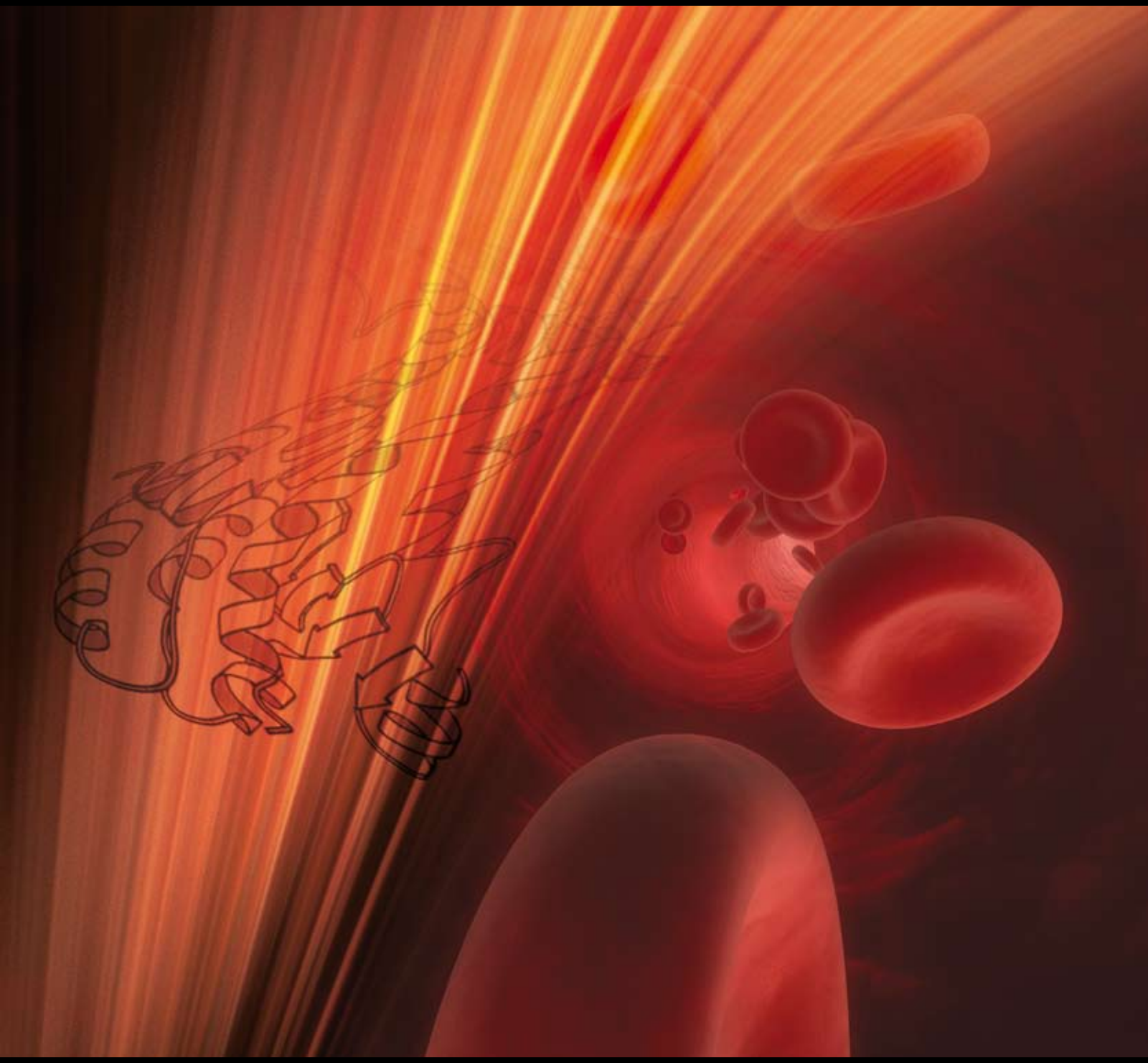
PPAR Research

PPARs and Obesity

Guest Editors: Francine M. Gregoire, Sander Kersten, and Wallace Harrington



PPARs and Obesity

PPAR Research

PPARs and Obesity

Guest Editors: Francine M. Gregoire,
Sander Kersten, and Wallace Harrington

Contents

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 78475, 1 page
doi:10.1155/2007/78475

Editorial
PPARS and Obesity

Francine M. Gregoire,[1] Sander Kersten,[2] and Wallace Harrington[3]

[1] *Department of Biology, Metabolex, Inc., 3876 Bay Center Place, Hayward, CA 94545, USA*
[2] *Division of Human Nutrition, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*
[3] *Department of Metabolic Diseases, GlaxoSmithKline Research, 5 Moore Drive, Research Triangle Park, NC 27709, USA*

Received 20 December 2006; Accepted 7 March 2007

Welcome to this special issue of PPAR Research dedicated to "PPARs and Obesity." Obesity and the interrelated disorders of the metabolic syndrome are a global health epidemic. To address this major problem, it is essential to understand the mechanisms regulating energy metabolism, and it has been known for years that PPARs play an important role in many facets of energy homeostasis. This is a very active and exciting field of research that, without a doubt, justifies a special issue. The genetic, molecular, and physiological aspects of PPARs as well as the metabolic effects of recently developed PPAR and RXR agonists are among the topics discussed. The issue begins with a review of key observations made in human subjects harboring genetic variations in PPARγ and a thorough overview of the metabolic effects of PPARs in genetically modified animal models. Over the last five years, the knowledge on PPAR delta biology has literally exploded and the potential therapeutic usefulness of this receptor in metabolic syndrome is now recognized. The interaction of PPARs with uncoupling proteins regulating energy expenditure is reviewed as are recent developments with RXR agonists. A closely related topic addresses the molecular and physiological functions of PPAR coactivators and corepressors in relationship to adipocyte energy metabolism. The selective PPAR modulator concept has attracted the attention of the field for over a decade; however the molecular bases underlying their differential mode of action have only begun to emerge recently. In addition to selective PPAR agonists, compounds that simultaneously activate two (dual agonists) or three (pan agonists) PPAR isoforms are in development. The potential advantages of these new combinations are discussed. The intriguing possibility that PPARs may mediate effects of caloric restriction on longevity is also considered. Finally, a growing body of evidence indicates that inflammation is a key feature of the obese state and that PPARs display strong anti-inflammatory properties. The evidence that PPARs may be interesting therapeutic targets to modulate obesity-induced inflammation is also reviewed. While these reviews just scratch the surface of PPAR/RXR interactions and regulation of energy balance, this special issue is packed with exciting, high quality reports from recognized experts in the field. We hope that the ideas presented here will generate further interest from the scientific community in this rapidly expanding area of research.

Francine M. Gregoire
Sander Kersten
Wallace Harrington

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 83593, 14 pages
doi:10.1155/2007/83593

Review Article

'Striking the Right Balance' in Targeting PPARγ in the Metabolic Syndrome: Novel Insights from Human Genetic Studies

Mark Gurnell

Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

Received 5 November 2006; Revised 13 December 2006; Accepted 13 December 2006

Recommended by Francine M. Gregoire

At a time when the twin epidemics of obesity and type 2 diabetes threaten to engulf even the most well-resourced Western healthcare systems, the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) has emerged as a *bona fide* therapeutic target for treating human metabolic disease. The novel insulin-sensitizing antidiabetic thiazolidinediones (TZDs, e.g., rosiglitazone, pioglitazone), which are licensed for use in the treatment of type 2 diabetes, are high-affinity PPARγ ligands, whose beneficial effects extend beyond improvement in glycaemic control to include amelioration of dyslipidaemia, lowering of blood pressure, and favourable modulation of macrophage lipid handling and inflammatory responses. However, a major drawback to the clinical use of exisiting TZDs is weight gain, reflecting both enhanced adipogenesis and fluid retention, neither of which is desirable in a population that is already overweight and prone to cardiovascular disease. Accordingly, the "search is on" to identify the next generation of PPARγ modulators that will promote maximal clinical benefit by targeting specific facets of the metabolic syndrome (glucose intolerance/diabetes, dyslipidaemia, and hypertension), while simultaneously avoiding undesirable side effects of PPARγ activation (e.g., weight gain). This paper outlines the important clinical and laboratory observations made in human subjects harboring genetic variations in PPARγ that support such a therapeutic strategy.

1. INTRODUCTION

The health of a nation has long been recognized to be a function of its wealth. Traditionally, countries with limited resources have struggled to eradicate diseases that are often considered a thing of the past in so-called "developed" or "industrialized" nations. However, in recent years it has become clear that wealth does not always equate with good health. Indeed, we now face the very real possibility that in the first half of this century, average life expectancy in industrialized countries such as the US and UK will plateau or decline, despite continuing economic growth and prosperity [1]. The obesity epidemic, which is currently sweeping through "Western civilization," is undoubtedly the single biggest factor behind this "unwanted reversal" [1]. Recent figures from the US reveal an alarming 75% increase in the prevalence of obesity over the past 25 years, such that a third of the population is now officially obese, that is to say, at least 20% heavier than their ideal weight [2]. Many Western European countries and Japan are not far behind. Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidaemia (particularly hypertriglyceridaemia and low high-density lipoprotein cholesterol (HDL-C)); this cluster of medical sequelae is often grouped together under the umbrella term "metabolic syndrome," and over the past decade the thresholds that must be met for the diagnosis of this entity have been progressively refined, culminating most recently in a consensus statement from the International Diabetes Federation (Table 1). Not surprisingly, subjects who meet the diagnostic criteria for this disorder are at significantly increased risk of atherosclerotic cardiovascular disease (reviewed in [3]).

So how can we arrest/reverse this apparently relentless march towards "metabolic meltdown"? The solution seems obvious: more effective obesity prevention and treatment. Limiting caloric intake and increasing energy expenditure to promote neutral (or in obese subjects negative) rather than positive energy balance is likely to yield enormous benefits at both the individual and population levels. Indeed, "lifestyle intervention" studies have already convincingly

Table 1: Diagnostic criteria for the human metabolic syndrome. WHO, World Health Organization; EGIR, European Group for the Study of Insulin Resistance; NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; IDF, International Diabetes Federation; T2DM, type 2 diabetes mellitus; IGT, impaired glucose tolerance; IR, insulin resistance; TG, triglycerides; HDL, high density lipoprotein cholesterol; BP, blood pressure; BMI, body mass index; WHR, waist hip ratio; WC, waist circumference; AER, albumin excretion rate; M, male; F, female.

WHO, 1999	EGIR, 1999	NCEP ATP III, 2001	IDF, 2005
T2DM or IGT or IR with ≥2 of the following	IR or hyperinsulinaemia, in nondiabetic subjects with ≥2 of the following	 ≥3 of the following	*Central obesity:* WC ≥ ethnicity specific cut-offs with ≥2 of the following
	Hyperglycaemia Fasting plasma glucose ≥ 6.1 mmol/L, but nondiabetic	*Hyperglycaemia* Fasting plasma glucose ≥ 6.1 mmol/L or treated with antidiabetic medication.	*Hyperglycaemia* Fasting plasma glucose ≥ 5.6 mmol/L or previously diagnosed T2DM
Dyslipidaemia TG >1.7 mmol/L and/or HDL <0.9 mmol/L (M) HDL <1.0 mmol/L (F)	*Dyslipidaemia* TG >2.0 mmol/L or HDL <1.0 mmol/L or treated for dyslipidaemia	*Hypertriglyceridaemia* TG ≥1.7 mmol/L	*Hypertriglyceridaemia* TG >1.7 mmol/L or treated for this lipid abnormality
		Low HDL cholesterol HDL <1.0 mmol/L (M) HDL <1.3 mmol/L (F)	*Reduced HDL cholesterol* HDL <1.03 mmol/L (M) HDL <1.29 mmol/L (F) or treated for this lipid abnormality
Hypertension BP ≥140/90 mmHg ± medication	*Hypertension* BP ≥140/90 mmHg or treated for hypertension	*Hypertension* BP ≥130/85 mmHg or treated for hypertension	*Hypertension* BP ≥130/85 mmHg or treated for hypertension
Obesity BMI ≥30 kg/m^2 or WHR >0.9 (M) WHR >0.85 (F)	*Central obesity* WC ≥94 cm (M) WC ≥80 cm (F)	*Central obesity* WC ≥102 cm (M) WC ≥88 cm (F)	*Central obesity* See above—core requirement for diagnosis of syndrome
Microalbuminuria Urinary AER >20 mcg/min			

demonstrated that the risk of developing complications such as T2DM can be significantly reduced using such an approach [4, 5]. Unfortunately however, while this is a laudable goal, most clinicians know only too well that in practice it is very difficult to achieve/sustain, and hence attention has turned towards seeking novel therapies that are capable of ameliorating/reversing weight gain, insulin resistance, and their unwanted sequelae. Understanding the genes that are involved in maintaining metabolic homeostasis in the face of differing nutritional and environmental stresses is essential to the rational development of these strategies.

In recent years, a group of transcription factors belonging to the nuclear receptor superfamily has emerged as key players in the regulation of mammalian metabolism. Peroxisome proliferator-activated receptor γ(PPARγ) is perhaps the best characterized of these so-called metabolic nuclear receptors, serving as it does to integrate the control of energy, glucose, and lipid homeostasis. The activity of PPARγ is governed by the binding of small lipophilic ligands, principally fatty acids, derived from nutrition or metabolism [6, 7], and activation of the receptor is a critical step in the pathway to adipocyte differentiation and fat cell maturation. Hence, it is easy to envisage how chronic exposure to high levels of dietary PPARγ ligands (provided in abundance in the Western diet) could promote the development of obesity, insulin resistance, and metabolic dysfunction, and why receptor modulation might offer a route to prevention/amelioration of these important cardiovascular risk factors. Indeed, drugs targeting PPARγ activity (thiazolidinediones (TZDs), e.g., rosiglitazone, pioglitazone) are already in widespread clinical use as effective antidiabetic agents, enhancing insulin sensitivity, elevating high-density lipoprotein cholesterol (HDL-C) levels, and lowering blood pressure [8]. Importantly other studies have begun to examine whether these agents actually lower cardiovascular event rates [9], and if they are capable of reducing the risk of progression to overt T2DM in those with existing impaired glucose regulation [10].

Paradoxically however, TZDs actually promote weight gain rather than weight loss. A significant part of this increase can be attributed to enhanced adipogenesis, consistent with TZDs acting as high-affinity agonists for PPARγ [11–13]. In addition, fluid retention and expansion of the extracellular compartment (possibly through altered renal sodium handling [14]) may contribute to weight gain in some patients, especially those with preexisting cardiac impairment [15]. Together, these observations raise an important question: is it possible to develop more selective PPARγ modulators, with even greater potential to improve metabolic dysfunction, yet at the same time with reduced propensity to cause weight gain and fluid retention? Clearly, the answer to this question

is dependent on the basic biology of PPARγ and whether it proves possible to regulate receptor function in a tissue- and a target-gene-specific manner.

This paper summarizes the important contributions that human genetic studies have made to our understanding of the role of PPARγ in the regulation of mammalian metabolic homeostasis, emphasizing the potential benefits and limitations that we can expect from more targeted approaches to modulating receptor function, and thus ensuring that in an era marked by an increasing prevalence of obesity, diabetes and cardiovascular disease, PPARγ remains more of "a help" than "a hindrance."

2. PPARγ-STRUCTURE, FUNCTION, AND LIGAND REGULATION

The human nuclear receptor superfamily comprises 48 ligand-inducible transcription factors that respond to a variety of stimuli including steroid and thyroid hormones, vitamins, lipid metabolites, and xenobiotics. PPARγ is the third member of a subdivision within the superfamily that also includes PPARα and PPARδ [25, 26]. Together, the PPARs function as key transcriptional regulators that govern metabolic homeostasis by serving as lipid sensors, responding to dietary fatty acids and their derivatives. However, each has a distinct pattern of tissue expression, and consistent with this, specific roles in the regulation of energy metabolism (reviewed in [25, 26]). The importance of these receptors in physiology and disease is evidenced by the fact that PPARα and PPARγ are the molecular targets for the lipid-lowering fibrate class of drugs and TZDs, respectively, while PPARδ ligands are currently being developed in anticipation that they will offer a novel approach to tackling obesity and metabolic dysfunction through effects on energy expenditure, HDL-C metabolism, and macrophage inflammatory responses (reviewed in [26]).

Differential promoter usage, coupled with alternate splicing of the *PPARG* gene, generates two protein isoforms: PPARγ2, expressed from a single γ2 promoter, contains an additional 28 N-terminal amino acids and is nearly adipose-specific; PPARγ1, whose expression can be regulated by multiple (γ1, γ3, γ4) promoters, is more ubiquitously distributed [27–29]. Like other nuclear receptors, PPARγ exhibits a modular structure consisting of distinct functional domains: the N-terminal A/B domain harbors a ligand-independent transcriptional activation function (AF1), which is stronger for the γ2 than γ1 isoform; the central DNA-binding domain, containing two zinc finger motifs, facilitates interaction with specific binding sites (PPAR response elements (PPREs)) in target gene promoters; the larger C-terminal domain mediates ligand-binding, heterodimerization with the retinoid X receptor (RXR), and contains a powerful ligand-dependent activation (AF2) function (Figure 1(a)).

Initially, PPARγ was considered to be a constitutively active receptor, recruiting transcriptional coactivators (e.g., steroid receptor coactivator-1 (SRC-1)) to classical target genes (e.g., adipocyte protein 2 (aP2)) even in the absence of ligand. More recently however, Guan et al. have shown that the unliganded PPARγ/RXR heterodimer can actively silence a subset of genes (e.g., adipocyte glycerol kinase (GyK)), in a manner analogous to that seen with the thyroid hormone (TR) and retinoic acid (RAR) receptors [24] (Figure 1(b)). Transcriptional silencing is mediated through recruitment of a multiprotein corepressor complex, containing either NCoR (nuclear receptor corepressor) or SMRT (silencing mediator of retinoic acid and thyroid receptors), together with histone-modifying enzymes (e.g., histone deacetylase 3 (HDAC 3)), which condense chromatin structure, thus impeding gene transcription. In contrast, binding of cognate or exogenous ligand(s) induces a conformational change in the heterodimer such that it now dissociates from any bound corepressor proteins and instead recruits a coactivator complex, containing histone acetyltransferases (e.g., CREB-binding protein (CBP)), which relaxes the chromatin structure so as to permit greater levels of gene transcription (Figure 1(c)).

A variety of putative endogenous activators has been described for PPARγ, including fatty acids, eicosanoids, and derivatives of oxidized low-density lipoproteins [30]. The prostaglandin J2 derivative 15-deoxy-$\Delta^{12,14}$-PGJ2 is also capable of activating PPARγ *in vitro*, although it is doubtful as to whether it exists at sufficient concentrations *in vivo* to serve as a physiological ligand. Recently, Tzameli et al. have reported the existence of an as yet undefined ligand(s) that is produced transiently during adipocyte differentiation [31].

3. PPARγ-A KEY THERAPEUTIC TARGET IN THE HUMAN METABOLIC SYNDROME

Patients with the metabolic syndrome typically require a "cocktail of drugs" to treat the individual components of the disorder and its associated atherosclerotic complications (e.g., oral hypoglycaemic agents, insulin, statins, fibrates, antihypertensives, aspirin, etc.). Unfortunately, many of these drugs confer little benefit in terms of correcting the underlying metabolic disturbance, and indeed some even exacerbate the situation, for example, insulin-induced weight gain. Not surprisingly then, compliance with these complex treatment regimens is often poor.

In contrast, drugs that target PPARγ appear, at least in theory, to offer an attractive and perhaps more logical approach to treating the metabolic syndrome, by virtue of their ability to ameliorate insulin resistance and other facets of the condition [8]. Set against this however is the well-documented increase in body weight that is observed with currently available TZDs [8]. It is these observations that have led scientists and clinicians alike to ask whether it is possible to retain/enhance the metabolic benefits of PPARγ activation, yet at the same time minimize undesirable side effects. The following sections outline the human genetic evidence that supports such a strategy, with specific reference to each of the key components of the metabolic syndrome.

3.1. PPARγ and adipogenesis

In vitro studies suggest that PPARγ is the ultimate effector of adipogenesis in a transcriptional cascade that also involves

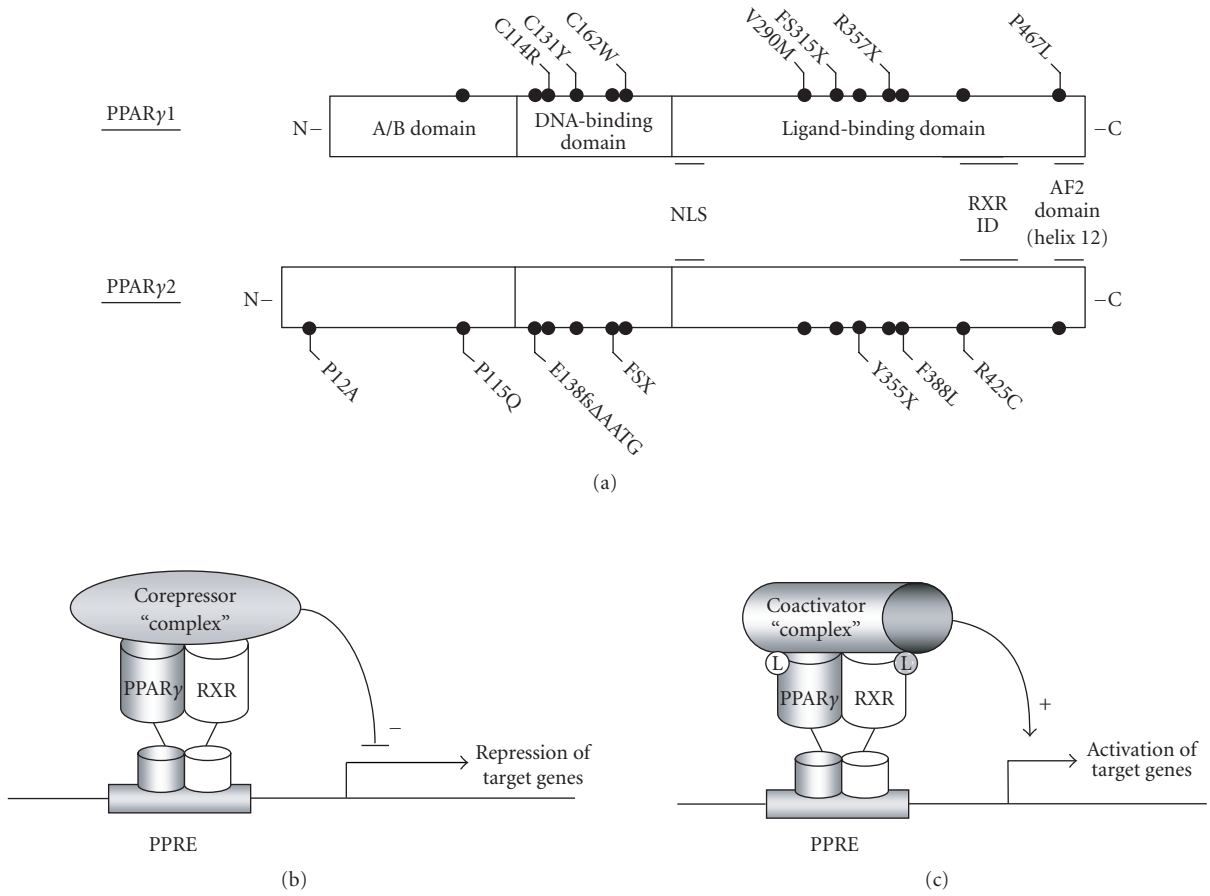


Figure 1: Structure function of PPARγ. (a) Schematic representation of the three principal domains of PPARγ, denoting the positions of several of the natural genetic variants that have been identified in the human receptor. Note that mutations and polymorphisms have been depicted based on the nomenclature (γ1 or γ2) used in the primary publication [16–23]. FSX denotes the mutation (A^{553}ΔAAAiT)fs185(stop186); FS315X denotes the mutation (A^{935}ΔC)fs312(stop315). (b) In the absence of exogenous ligand, PPARγ recruits a corepressor complex to a subset of target genes (e.g., adipocyte glycerol kinase), thereby repressing basal transcription [24]. (c) Addition of ligand induces a conformational change in the receptor, which promotes corepressor release and coactivator recruitment. For other target genes (e.g., aP2), the receptor appears to be constitutively active even in the absence of exogenous ligand [24]. NLS denotes nuclear localization signal; RXR denotes retinoid X receptor; ID denotes interaction domain; AF2 denotes activation function 2; PPRE denotes PPAR response element.

members of the C/EBP transcription factor family [32]. Modulation of PPARγ expression and/or action in rodent cell lines has conclusively shown that the receptor is both essential and, in the presence of PPARγ agonists, is sufficient for adipogenesis [33]. Consonant with this, PPARγ knockout mice fail to develop adipose tissue [34–36], while their heterozygous counterparts have reduced fat depots [36]. Studies in human tissues point to a similar critical role for PPARγ in the regulation of adipogenesis. Exposure of cultured primary human preadipocytes to PPARγ activators (e.g., TZDs) induces their differentiation [32], while both chemical and biological receptor antagonists efficiently block this process [37].

It comes as no surprise then to learn that human subjects treated with synthetic PPARγ agonists (e.g., rosiglitazone, pioglitazone) gain weight through enhanced adipogenesis [8]. Despite this, metabolic function in the majority of TZD recipients improves. This apparent TZD paradox undoubtedly reflects the ability of these agents to modify adipocyte function and free fatty acid storage in a favorable manner that promotes insulin sensitization; however, it may also be dependent, at least in part, on PPARγ activation mediating depot-specific rather than global changes in adipogenesis. For example, it is notable that the increase in fat mass observed in type 2 diabetics treated with TZDs is not uniformly distributed, with a tendency to accumulate subcutaneous (e.g., limb/gluteal) fat, whereas visceral adipose tissue volume is reduced or unchanged (reviewed in detail in [38]). Consistent with this, preadipocytes isolated from subcutaneous abdominal adipose tissue have been shown in some (although not all) studies to differentiate more readily in response to TZDs than cells from visceral depots taken from the same subjects [39].

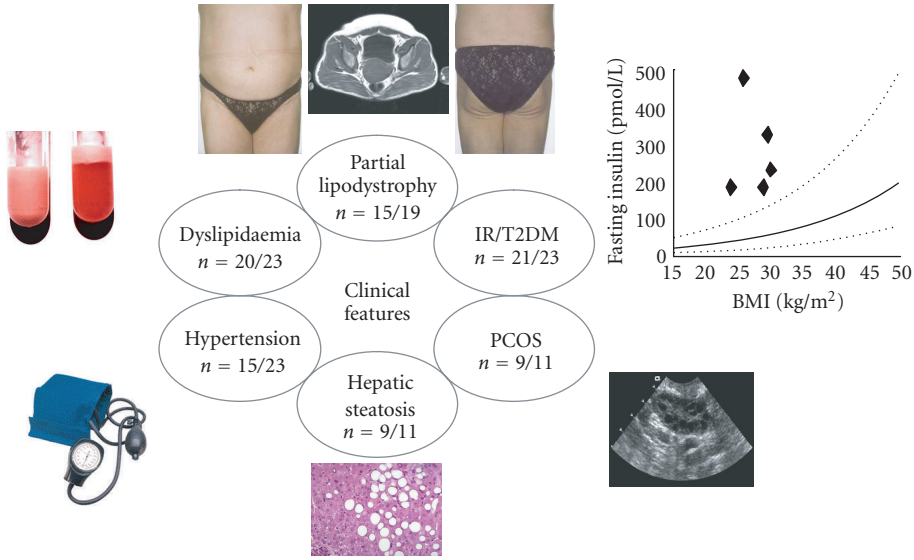


Figure 2: Clinical features exhibited by adult subjects harboring loss-of-function mutations in human PPARγ. For each parameter shown, the numerator denotes the reported number of affected individuals, and the denominator denotes the number of subjects for whom relevant information is available.

3.1.1. *Gain- and loss-of-function mutations*

With PPARγ agonists promoting adipogenesis, it would seem reasonable to speculate that gain-of-function PPARγ mutations should increase body fat mass. Ristow et al. have provided support for this hypothesis, with the identification of four morbidly obese (BMI 37.9 to 47.2 kg/m^2) German subjects, all of whom harbored a gain-of-function mutation (Pro115Gln PPARγ2) within the N-terminal domain of the receptor [40]. The transcriptional activity of PPARγ is subject to modification through phosphorylation of a serine residue at codon 114 [41, 42], and mutation of the adjacent proline was shown to interfere with this process, resulting in a receptor with constitutive transcriptional activity and enhanced adipogenic potential [40]. Subsequently however, a fifth subject, with only a mildly elevated BMI (28.5 kg/m^2), was found to carry the same amino acid substitution, which is in marked contrast to the findings of the original study [43]. Thus, for now the significance of this particular genetic variant remains unclear, and further mutation carriers must be identified to confirm whether Pro115Gln does indeed predispose to obesity and, if so, whether there is a depot-specific pattern to the accretion of adipose tissue.

In contrast, there is now a compelling body of data from the study of human subjects with loss-of-function mutations in PPARγ to confirm a pivotal role for this receptor in human adipogenesis. To date, twelve different heterozygous mutations (missense, nonsense, and frameshift) have been identified within the DNA- (DBD) and ligand-binding (LBD) domains of the receptor (Figure 1(a)) [16–23], with functional studies, where available, confirming that the mutant receptors are transcriptionally impaired. In keeping with their dominant mode of inheritance, several of the mutants have also been shown to be capable of inhibiting the activity of their wild-type counterpart in a dominant negative manner, reflecting either aberrant corepressor recruitment to DNA-bound mutant receptors [16, 44], or transcriptional interference through coactivator sequestration by DNA-binding deficient mutants [23]. In contrast, other mutants appear to lack dominant negative activity, with the clinical phenotype purported to be a consequence of haploinsufficiency [18, 20–22]. In keeping with the latter, Al-Shali et al. have recently identified a kindred harboring a novel heterozygous A > G mutation at position −14 within intron B of *PPARG* (upstream of exon 1), which reduces promoter activity of the PPARγ4 isoform [45]. This mutation cosegregated with a phenotype of partial lipodystrophy and metabolic dysfunction similar to that observed in subjects harboring loss-of-function mutations within the DBD or LBD [45].

Together, these reports describe more than twenty adult subjects, the majority of whom exhibit a stereotyped pattern of partial lipodystrophy, in which subcutaneous fat is diminished in the limbs and gluteal region, while being preserved/increased in the subcutaneous and visceral abdominal depots (Figure 2) [16–23]. Some phenotypic differences have been observed with facial and neck adipose tissues, which were reported to be increased in individuals from two kindreds, but normal or reduced in most other cases [16–23]. These findings are again strongly suggestive of a depot-specific role for PPARγ in human adipogenesis, and complement the observations made in diabetic subjects receiving TZD treatment. Clearly one challenge is to understand why visceral adipose tissue appears relatively refractory to PPARγ regulation despite expressing comparable levels of receptor to its subcutaneous counterpart. Studies of fat biopsies from different depots in PPARγ mutation carriers might offer a unique route to addressing this important question.

Interestingly, a transgenic knockin mouse model based on the human Pro467Leu mutation (Pro465Leu) has recently been reported by two independent groups [46, 47]. Heterozygous *Pparg*$^{P465L/+}$ mice have normal total adipose tissue weight, but exhibit reduced intra-abdominal fat mass and increased extra-abdominal subcutaneous fat compared to wild-type (WT) animals, that is, altered body fat distribution, but in a manner which is quite distinct from that observed in human subjects. In addition, unlike their human counterparts, the *Pparg*$^{P465L/+}$ mice were also insulin-sensitive. These findings initially raised concerns as to the suitability of using rodent models to explore the consequences of loss-of-function mutations in human PPARγ. Importantly however, in the model of Gray et al., expression of the P465L mutant on a hyperphagic ob/ob background grossly exacerbated the insulin resistance and metabolic disturbances associated with leptin deficiency, despite reducing whole body adiposity and adipocyte size [47]. Thus, in the mouse coexistence of the P465L PPARγ mutation and the leptin-deficient state creates a mismatch between adipose tissue expandability and energy availability, thereby unmasking the deleterious effects of PPARγ mutations on carbohydrate metabolism and recapitulating the clinical phenotype observed in human subjects.

3.1.2. Polymorphisms

The most prevalent human PPARγ genetic variant reported to date is the Pro12Ala polymorphism, substituting alanine for proline at codon 12 in the unique PPARγ2 amino-terminal domain [48]. The allelic frequency of the Ala variant differs quite markedly depending on the study population, ranging from 1% to 23% [49]. In functional assays, Ala12-PPARγ exhibits reduced binding to DNA and modest impairment in target gene transactivation in both the absence and presence of PPARγ agonists [48]. An association with lower BMI in the primary study appeared to suggest a corresponding genotype-phenotype correlation, and led to the hypothesis that improved insulin sensitivity might be accounted for entirely by changes in adiposity [48]. However, numerous subsequent cross-sectional studies have yielded conflicting results, demonstrating either no difference [50] or a modestly greater BMI [51] in carriers of the Ala allele. In an attempt to resolve this issue, Masud and Ye completed a meta-analysis using data from 30 independent studies with a total of 19 136 subjects [52]. They concluded that in the samples with a mean BMI value $\geq 27\,kg/m^2$, Ala12 allele carriers had a significantly higher BMI than noncarriers, whereas no difference was detected in the samples with a BMI value $<27\,kg/m^2$. A further analysis using data from publications in which BMI for the three genotype groups (i.e., Pro/Pro, Pro/Ala and Ala/Ala) were presented separately revealed that the Ala12 homozygotes had significantly higher BMI than heterozygotes and Pro12 homozygotes [52].

Importantly, the effects of the Ala allele have recently been shown to be subject to modification by other genetic and environmental factors, and indeed this may in part explain the apparently discordant results of the studies reported hitherto. For example, variations in dietary polyunsaturated fat versus saturated fat intake appear to influence BMI in carriers of the Ala variant [53]. In the Quebec Family Study, carriers of the Pro12 allele had lower BMI, waist circumference and fat mass (both subcutaneous and visceral) at baseline, but responded to an increase in dietary fat with a gradual increase in BMI and waist circumference, an effect which was not observed in their Ala counterparts [54]. Together, these and other studies support the notion of gene-nutrient interaction at the PPARγ locus.

3.2. PPARγ and insulin sensitivity

3.2.1. Genetic evidence for a link

Several lines of evidence point to a link between the level of PPARγ transcriptional activity and insulin sensitivity: (1) the *in vitro* binding affinities of TZD and non-TZD PPARγ ligands correlate closely with their *in vivo* potencies as insulin sensitizers [11, 55]; (2) RXR ligands, which can activate the PPARγ-RXR heterodimer, also exhibit insulin-sensitizing effects in rodents [56]; (3) mice exhibiting enhanced PPARγ activity, due to a mutation at serine 112 (serine 114 in human PPARγ2), which results in a constitutively more active receptor (through inhibition of phosphorylation), are protected from obesity-associated insulin resistance [57]; (4) mice lacking PPARγ in fat, muscle, or liver are predisposed to developing insulin resistance [58–61].

Importantly, studies of human PPARγ genetic variants have provided independent validation of the pharmacological and animal data. For example, severe insulin resistance (with or without overt T2DM) has proved to be a remarkably consistent finding in subjects with loss-of-function PPARγ mutations, being evident even in early childhood in affected individuals (Figure 2) [16–23]. Equally impressive has been the finding that of more than 40 different reported associations of genetic variation and population risk to T2DM, Pro12Ala has emerged as the most widely reproduced [62]. The Ala allele is protective against the risk of developing T2DM, and it has been estimated that the global prevalence of T2DM would be ∼25% lower simply by virtue of everybody carrying one or more copies of the Ala allele [49, 62, 63], implying that PPARγ is perhaps the single most important "diabetogene" identified to date.

In light of the findings with Pro12Ala, several groups have sought to determine whether other single-nucleotide polymorphisms (SNPs) within PPARγ might also influence T2DM risk at a population level. In a study of ∼4000 Asian subjects, a link with a second polymorphism C1431T (for which the presence of a T allele conferred a reduced diabetes risk when compared with CC homozygotes (OR = 0.73, $P = .011$)) has been reported [64]. Other workers have taken analysis of this genetic variant further, establishing it to be in tight allelic disequilibrium with the Ala12 variant in a separate study population (70% of all Ala carriers also carried the C1431T polymorphism) [65]. Having genotyped individuals from three separate cohorts (1997 subjects with T2DM, 2444 nondiabetic children, and 1061 middle-aged

controls—all from a similar area in Tayside, Scotland) for the *PPARG* Pro12Ala and C1431T polymorphisms, they concluded that the Ala12 variant was underrepresented in the T2DM population when compared with similarly aged nondiabetic adults (OR = 0.74, $P = .0006$). The 1431T variant was also underrepresented in the T2DM versus adult population. Intriguingly however, when the Ala12 variant was on a haplotype not bearing the 1431T variant, it conferred greater protection (OR = 0.66, $P = .003$); in contrast, when it was present in haplotypes containing the 1431T variant (70% of Ala12 carriers), this protection was absent (OR = 0.99, $P = .94$). Further studies are awaited with interest.

Thus, it is clear that the relationship between PPARγ activity and insulin sensitivity in humans is complex, with evidence for a gene dosage effect, which is subject to modification by other genetic and environmental factors.

3.2.2. Mechanisms of action

Adipose tissue

Given its high level of expression in adipose tissue and its pivotal role in adipogenesis, it is likely that receptor activation in adipocytes contributes significantly to the clinical efficacy of PPARγ ligands in ameliorating insulin resistance. Consistent with this, mice lacking adipose tissue have been shown to be refractory to the antidiabetic effects of TZDs [66], while adipose-specific deletion of PPARγ (which is associated with progressive lipodystrophy) predisposes mice to hepatic steatosis, and high-fat feeding-induced skeletal muscle insulin resistance [58]. In addition, because PPARγ2 is virtually exclusively expressed in fat cells, any metabolic effects of the Pro12Ala polymorphism, including those on glucose homeostasis, are likely to be secondary to alterations in adipose tissue metabolism. Several mechanisms have been advanced to explain how modulating PPARγ activity in fat benefits whole-body insulin sensitivity.

(i) Regulation of free fatty acid flux in adipocytes

Circulating levels of free fatty acids (FFAs) are a major determinant of insulin sensitivity [38]. Several studies have shown that the antidiabetic efficacy of TZDs correlates with their ability to lower circulating FFA levels [38]. Murine and cellular studies indicate that PPARγ activation in adipose tissue may exert coordinated effects on FFA flux (promoting uptake/trapping, while simultaneously impairing release), through the regulation of a panel of genes involved in FFA metabolism: adipocyte lipoprotein lipase (LPL) expression is upregulated in response to TZD treatment, thereby potentially enhancing release of FFAs from circulating lipoproteins [67]; simultaneous upregulation of FFA transporters such as CD36 and FATP (fatty acid transport protein) on the adipocyte surface facilitates their uptake [68]; TZDs may also reduce FFA efflux from adipocytes through enhanced expression of genes that promote their storage in the form of triglycerides (e.g., glycerol kinase directs the synthesis of glycerol-3-phosphate directly from glycerol; phosphoenolpyruvate carboxykinase permits the utilization of pyruvate to form the glycerol backbone for triglyceride synthesis) [69, 70]. If similar effects on FFA uptake and trapping are observed in human adipocytes, then treatment with TZDs and other PPARγ activators is likely to promote the safe storage of FFAs in adipose tissue, and prevent "ectopic" deposition in other sites such as liver and skeletal muscle, where they are capable of inducing "lipotoxicity." Observations in human subjects with genetic variations in PPARγ are consistent with this hypothesis. For example, it appears that even the existing residual adipose tissue depots in individuals with loss-of-function mutations in PPARγ are dysfunctional, resulting in exposure of skeletal muscle and liver to unregulated fatty acid fluxes, with consequent impairment of insulin action at these sites [19]. In addition, there is evidence that the Pro12Ala polymorphism facilitates insulin-mediated suppression of lipolysis, hence decreasing FFA release [49]. It is worth noting however that others have failed to detect any relationship between circulating FFA levels and Pro12Ala status [71].

(ii) Modulation of adipokine release

In addition to regulating circulating FFA levels, adipocytes also serve as a rich source of signalling molecules (e.g., leptin, adiponectin, tumour necrosis factor-α (TNFα), and resistin), many of which have far-reaching metabolic effects in other tissues. Collectively these adipocyte-derived hormones are referred to as adipokines, and several have been identified as targets for transcriptional regulation by PPARγ. In general, TZDs and other PPARγ agonists enhance the expression of adipokines that facilitate insulin action while simultaneously suppressing those which are antagonistic, thereby altering the profile of adipocyte gene expression in a manner that promotes insulin sensitization. For example, activation of PPARγ inhibits the expression of TNFα, resistin, and retinol-binding protein 4 (RBP4), all of which are associated with insulin resistance [72–74]. In contrast, adiponectin gene expression is increased following TZD treatment, thereby promoting fatty acid oxidation and insulin sensitivity in muscle and liver [75]. Circulating adiponectin levels have been shown to correlate closely with insulin sensitivity, and inversely with fat mass (especially visceral adiposity) [76], suggesting that this adipokine may represent a critical link between PPARγ activation and insulin sensitization [75, 76]. Consonant with this, circulating adiponectin levels have been shown to be dramatically reduced in individuals harboring loss-of-function PPARγ mutations when compared with healthy controls [77, 78]. In contrast, to date, no definitive correlation between the Pro12Ala polymorphism and adipokine release has been established, with existing studies providing conflicting results.

(iii) Promotion of glucose uptake into adipocytes

There is evidence to suggest that PPARγ is also capable of directly modulating the insulin signal transduction pathway in adipose tissue. The GLUT4 (insulin-dependent) transporter

is a key modulator of glucose disposal in both muscle and fat. Binding of insulin to its tyrosine kinase receptor engages a cascade of intracellular phosphorylation events, including activation of phosphatidylinositol-3-OH kinase (PI(3)K) and other downstream kinases, which promote trafficking of GLUT4 containing vesicles to the plasma membrane. A second pathway, which involves a distinct group of signalling molecules including the c-Cbl protooncogene product and CAP (c-Cbl-associated protein), acts in concert to augment this process. Several groups have shown that PPARγ activation in adipose tissue can influence insulin signalling at various points in these pathways, for example, through upregulation of insulin receptor substrates-1 and -2 (IRS-1, IRS-2) [79, 80], the p85 subunit of PI(3)K [81], and CAP [82, 83]—all of which might be predicted to enhance GLUT4 activity. Increased glucose uptake into adipocytes contributes to whole-body glucose disposal, and provides important substrate for triglyceride synthesis.

(iv) Regulation of adipocyte 11β-hydroxysteroid dehydrogenase type 1 activity

Prolonged exposure to hypercortisolaemia, as occurs in subjects with Cushing's syndrome, is associated with many features of the metabolic syndrome (visceral obesity, glucose intolerance, hypertension, and dyslipidaemia). While circulating cortisol levels in ordinary obese non-Cushingoid individuals are normal (if not slightly reduced), there is evidence to suggest that local regeneration of cortisol within adipose tissue could contribute to the development of insulin resistance in the setting of visceral obesity [84]. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) directs the production of active cortisol from inactive cortisone in liver and fat, thereby facilitating cortisol-induced adipocyte differentiation. In keeping with this, adipose-specific overexpression of 11β-HSD1 in transgenic mice induced a phenotype of insulin resistance and central obesity [85]. PPARγ ligands have been shown to downregulate adipocyte 11β-HSD1 expression and activity [86], and the subsequent modulation of glucocorticoid-induced gene expression may conceivably contribute to their insulin sensitizing actions. Studies of 11β-HSD1 activity in adipose tissue from subjects with loss-of-function mutations in PPARγ should provide a unique opportunity to examine the role of PPARγ in regulating human 11β-HSD1 function.

Skeletal muscle and liver

Maintenance of normal glucose homeostasis is critically dependent on retention of insulin sensitivity in key target tissues including liver and skeletal muscle. In addition to the beneficial effects of lowering circulating FFA levels and inducing a more favorable adipokine milieu to promote insulin sensitivity, there is some evidence to suggest that PPARγ activation at both of these sites might directly influence glucose and lipid homeostasis. For example, TZDs have been reported to facilitate insulin-stimulated glucose uptake in cultured human skeletal muscle cells, by enhancing insulin-stimulated PI(3)K activity and GLUT4 translocation [87, 88]. Thus, while skeletal muscle expresses relatively low levels of PPARγ protein when compared with adipose tissue, its dominant role in insulin-mediated glucose disposal suggests that PPARγ activation at this site may contribute significantly to the glucose lowering effect of TZD treatment. Unfortunately, to date attempts to resolve this issue using animal models of muscle-specific PPARγ deletion have proved unsuccessful with two separate groups reporting conflicting findings [59, 60]. Similarly, it remains to be seen whether activation of PPARγ in human liver benefits or impairs metabolic function, with further studies needed to clarify its role in the regulation of hepatic gluconeogenesis and susceptibility to hepatic steatosis.

3.3. PPARγ and lipid homeostasis

As might be predicted for a group of drugs that improve insulin sensitivity, TZDs raise HDL cholesterol in the majority of treated diabetics (typically by 5%–10%) [8]. Intriguingly however, their effects on hypertriglyceridaemia have been somewhat more variable, with reductions in triglyceride levels observed more often with pioglitazone than rosiglitazone. One hypothesis that has been advanced to explain this apparent discrepancy is that pioglitazone may also be acting as a partial PPARα agonist (akin to a fibrate), while at the doses used in clinical practice rosiglitazone retains pure γ-agonist activity [89]. However, data on mechanisms underlying the effects of TZDs on lipids in humans is limited and, moreover, caution needs to be exercised when attempting to extrapolate from animal studies, given the significant species-specific differences that exist in lipoprotein metabolism.

To date, virtually all subjects with loss-of-function mutations in PPARγ have exhibited hypertriglyceridaemia and/or low HDL levels, with relatively unremarkable LDL cholesterols [16–23] (Figure 2). It remains unclear however, as to whether these abnormalities are simply a "metabolic consequence" of severe insulin resistance *per se*, or whether they indicate an additive and independent effect of dysfunctional PPARγ signalling in relation to lipoprotein metabolism. Further studies of the reverse cholesterol transport pathway in monocyte-derived macrophages from these subjects may help to address this important issue.

Although there is an extensive body of data concerning the potential effects of the Pro12Ala polymorphism on glycaemic control, there are relatively few studies focusing on its consequences for lipid homeostasis. Moreover, given the potential confounding effect of insulin resistance, cohort selection (particularly with respect to diabetic status and/or BMI) is critical when trying to identify a specific independent link. Accepting these limitations, there is some evidence to suggest that the Ala allele may confer benefits for HDL metabolism. For example, in the original study of Deeb et al., higher HDL cholesterol (and lower triglyceride) levels were observed among elderly subjects with the Ala/Ala genotype compared with Pro/Ala and Pro/Pro genotypes [48]. A similar association has been described in over 4000 Singapore Asians whose genotype was analyzed as a dichotomous

variable (i.e., presence or absence of the Ala variant), and in whom Ala allele carriers had significantly higher HDL cholesterol compared with Pro/Pro homozygotes [64]. However, other groups have reported conflicting findings, with some detecting an association of lower HDL cholesterol levels with the presence of the Ala allele [50].

3.4. PPARγ and blood pressure regulation

Hypertension has been reported in a significant proportion of subjects harboring PPARγ mutations [16–23]. While this is not unexpected, given the well-recognized associations of insulin resistance and T2DM with hypertension, it is noteworthy that in some cases the hypertension has been of an unusually early onset and severity [16, 19, 23]. Indeed, on occasion it has been the dominant clinical feature, manifesting even in the absence of diabetes and its associated microvascular complications. In contrast, TZD therapy is associated with a modest reduction in blood pressure in a variety of clinical settings, including nondiabetic hypertensive subjects [89]. Taken together, these findings suggest possible additional effects on blood pressure regulation, which are independent of insulin sensitivity, and indeed several lines of evidence suggest that PPARγ may directly regulate vascular tone, for example, through blockade of calcium channel activity in smooth muscle, inhibition of release of endothelin-1, and enhancement of C-type natriuretic peptide release [89].

While no studies of vascular tone or endothelial function have yet been reported in human subjects with PPARγ mutations, mice heterozygous for the equivalent Pro465Leu mutation were found to be hypertensive in the absence of insulin resistance [46]. The hypertension in *Pparg*$^{P465L/+}$ mice was associated with increased expression of RAS components in various adipose depots—angiotensinogen (AGT) and angiotensin II receptor subtype 1 (AT1R) in inguinal and gonadal fat, respectively [46]. Interestingly, transgenic mice expressing AGT in adipose tissue have higher BP and increased fat mass [90]. Thus, it is conceivable that modulation of RAS activity in adipose tissue contributes to the decrease in blood pressure, which is seen with TZDs and other PPARγ agonists.

Data relating to differences in blood pressure and Pro12Ala status have proved less informative, with studies again reporting conflicting findings [91, 92], which are likely to reflect other genetic and environmental influences that are at work in the different study populations.

3.5. PPARγ and atherosclerosis

Collectively, the individual components of the metabolic syndrome conspire to dramatically increase the risk of cardiovascular disease [93]. PPARγ activation with exogenous ligands such as the TZDs would be predicted to confer significant benefits in this setting, through the amelioration of insulin resistance, dyslipidaemia, and possibly hypertension, albeit at a potential cost of mild weight gain (as a consequence of enhanced adipogenesis and fluid retention). Indeed retrospective human studies have indirectly suggested an atheroprotective effect of TZDs [94], and more recently a prospective trial demonstrated that pioglitazone protected patients with T2DM, albeit modestly, from cardiovascular events [9].

It was therefore surprising and of potential therapeutic concern when Tontonoz et al. reported that PPARγ activation in a premacrophage cell line induced expression of CD36 (also known as FAT—fatty acid translocase), a cellular scavenger receptor for atherogenic low-density lipoprotein (LDL) [95]. Enhanced CD36 expression might be predicted to increase intracellular accumulation of oxidized LDL cholesterol, which could then be catabolized to generate PPARγ ligands (e.g., 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE) capable of further receptor activation, thereby creating a vicious feedforward cycle of increasing lipid uptake, and ultimately driving conversion of the macrophage into an atherogenic foam cell [95, 96]. The finding that PPARγ is expressed at relatively high levels in human atherosclerotic plaques further served to fuel concerns [97].

However, almost coincident with these observations, several groups reported that PPARγ ligands could reduce the release of inflammatory cytokines (e.g., TNF-α and IL-6) from macrophages, an effect that might be predicted to be antiatherogenic [98, 99]. Several anti-inflammatory mechanisms have been proposed, including inhibition of NF-kB, AP1, and STAT signalling by PPARγ [100].

Subsequent studies have further redressed the balance, with the demonstration that PPARγ ligands exert an opposing effect on SR-A, a second LDL scavenger receptor, downregulating its expression in mouse macrophages [101]. In addition, the nuclear receptor LXRα (liver X receptor α), which enhances expression of ABCA1 (ATP-binding cassette transporter A1), a protein which mediates cellular cholesterol efflux [102], has also been shown to be a PPARγ target gene in human and mouse macrophages [103, 104]. Taken together, these data suggest a broader spectrum of PPARγ effects within the macrophage with the overall balance favouring cholesterol efflux and an antiatherogenic effect.

At first glance, the finding that only six subjects from a cohort of more than 20 affected PPARγ mutation carriers [18, 23] have documented atheromatous coronary disease might seem surprisingly modest, especially when one considers the severity of insulin resistance, dyslipidaemia, and hypertension found in this group, coupled with the potentially deleterious consequences of dysfunctional PPARγ signalling inside mutant macrophages. However, it is important to note that four of the six affected subjects are/were relatively young females in whom atheromatous coronary disease in the general population is a relatively uncommon occurrence. Accordingly, given that many of the remaining mutation carriers are still relatively young (<50 years), with a predominance of females, it would seem premature to exclude the possibility of accelerated vascular disease in this high-risk group.

There is also an emerging body of epidemiological evidence to suggest an association between the naturally occurring PPARγ polymorphisms and arterial intima media thickness (IMT), and thus indirectly, cardiovascular risk. A

study of 154 Japanese T2DM patients found those carrying the Ala12 allele to have a significantly lower carotid IMT than their Pro/Pro counterparts, despite no observed differences in gender, age, fasting blood glucose, lipid profile, or HbA1c [105]. However, differences in BMI and the degree of insulin resistance between the two groups were not reported. Yan et al. used IMT as a secondary outcome measure to investigate the prevalence of the C161T PPARγ polymorphism within 4 different Chinese cohorts; 248 subjects with insulin resistance syndrome (IRS), 163 with essential hypertension, 115 with T2DM and 121 normal controls. They observed that the CC genotype (prevalence 75%) was significantly associated with increased IMT compared to CT and TT genotypes (prevalence 22% and 4%, resp.) within 248 "metabolic syndrome" patients [106]. However interestingly, the prevalence of neither the Pro12Ala nor C161T polymorphism within PPARγ was overrepresented in a large Caucasian cohort (1170 individuals) with angiographically proven coronary heart disease [50], and it is clear that further large-scale studies are needed.

4. SELECTIVE PPARγ MODULATION

The ability of TZDs such as rosiglitazone and pioglitazone to enhance insulin sensitivity makes them attractive agents for use in the treatment of T2DM and the metabolic syndrome. Unfortunately however, the initial excitement that followed the introduction of TZDs into clinical practice has been tempered by the realization that for many patients, they afford only modest benefits in terms of glycaemic control—typically lowering glycosylated haemoglobin levels by 1.0%–1.5%—at a cost of weight gain and, in some instances, fluid retention/peripheral oedema [8]. Nevertheless, they represent a "step in the right direction" and have served to emphasize the potential benefits and limitations of modulating PPARγ function in human subjects.

For those seeking to develop the next generation of PPARγ ligands, two (related) key questions must be answered: (1) how much PPARγ activation is desirable, (2) is it possible to separate the receptor's adipogenic actions from those mediating improved insulin sensitivity, that is, to develop selective receptor modulators (so-called SPPARMs) that are capable of regulating glucose and lipid metabolism without promoting adipogenesis. Taking this a step further, if such agents favourably altered receptor function at other sites, for example, within macrophages and the vasculature, then it is conceivable that we might have access to a class of drugs which is almost tailor-made for treating the metabolic syndrome. Precedent for such an approach is provided by raloxifene, a selective oestrogen receptor (ER) modulator (SERM), which is an ER antagonist in breast and endometrium, but an agonist in bone. Examination of the properties of PPARγ in adipocytes suggests that it may be possible to selectively modulate its function in an analogous manner. For example, inside mature adipocytes, certain PPARγ target genes, for example, GyK, require exogenous ligand for activation, while others, for example, aP2, are activated even in the absence of synthetic ligand [24]. The concept of differential modulation of PPARγ activity is also supported by the work of Li and Lazar who have demonstrated that a form of this protein rendered constitutively active by fusion to the powerful VP16 transactivation domain could switch on the adipogenic gene program, yet it was unable to transrepress other PPAR target genes such as that encoding resistin [107].

Promisingly, several groups have independently identified PPARγ ligands with partial agonist activity and only mild/modest effects on adipogenesis, yet with retention of insulin sensitizing properties. MCC-555 is one such compound, whose ability to stimulate PPARγ is highly context-specific [108]. FMOC-L-Leucine, a chemically distinct receptor ligand, whose gene-specific effects appear to reflect differential coactivator recruitment, has been shown to improve insulin sensitivity, yet exert relatively weak adipogenic effects in rodent diabetic models [109]. Similarly, YM440, an analog of the oxadiazolidinediones, improved glycaemic control, but did not alter body fat weight in diabetic db/db mice [110].

The discovery of such compounds has prompted widespread screening of libraries of both structurally related and chemically distinct molecules with the subsequent identification of an array of potential SPPARMs: PAT5a, an unsaturated TZD with partial agonist activity, is a potent antidiabetic agent with only weak adipogenic activity [111]; similar properties have been reported for the novel non-TZD-selective PPARγ modulators nTZDpa [112] and KR-62980 [113]; a panel of N-benzyl-indole-selective PPARγ modulators, with partial agonist activity *in vitro*, exhibited potent glucose-lowering activity in db/db mice, but attenuated increases in heart weight and brown adipose tissue when compared with full agonists [114]. Interestingly, the message that seems to be emerging from these and other similar studies is that 'activation in moderation' is the way forward for PPARγ, thus confirming the adage that you can indeed have 'too much of a good thing.'

5. CONCLUSIONS

In just over a decade, PPARγ has evolved from modest beginnings as a simple regulator of adipogenesis to become a key therapeutic target in the fight against the 21st century epidemics of obesity, insulin resistance, and the metabolic syndrome. While pharmacological and animal studies have yielded important information regarding the role of this receptor in the regulation of energy, glucose, and lipid homeostasis, there is little doubt that defining the metabolic consequences associated with polymorphisms and mutations in the human PPARγ gene has contributed significantly to our understanding of the biology of this receptor. Given the significant species-specific differences that exist in metabolism, particularly in relation to lipid homeostasis, it is critical that we continue to identify and study these human experiments of nature, in order to complement the impressive pharmacological and functional genomic approaches that are currently being used to facilitate the development of more superior ligands with enhanced

therapeutic impact. Given the apparent inexorable rise in the prevalence of obesity, insulin resistance, and T2DM, the need for such novel therapies could not be more urgent.

ACKNOWLEDGMENTS

The author would like to acknowledge the support of the Wellcome Trust, and the clinicians and colleagues who have referred cases for study and contributed to the laboratory and physiological studies reported herein.

REFERENCES

[1] S. J. Olshansky, D. J. Passaro, R. C. Hershow, et al., "A potential decline in life expectancy in the United States in the 21st century," *New England Journal of Medicine*, vol. 352, no. 11, pp. 1138–1145, 2005.

[2] C. L. Ogden, M. D. Carroll, L. R. Curtin, M. A. McDowell, C. J. Tabak, and K. M. Flegal, "Prevalence of overweight and obesity in the United States, 1999–2004," *Journal of the American Medical Association*, vol. 295, no. 13, pp. 1549–1555, 2006.

[3] R. H. Eckel, S. M. Grundy, and P. Z. Zimmet, "The metabolic syndrome," *The Lancet*, vol. 365, no. 9468, pp. 1415–1428, 2005.

[4] J. Tuomilehto, J. Lindström, J. G. Eriksson, et al., "Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance," *New England Journal of Medicine*, vol. 344, no. 18, pp. 1343–1350, 2001.

[5] W. C. Knowler, E. Barrett-Connor, S. E. Fowler, et al., "Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin," *New England Journal of Medicine*, vol. 346, no. 6, pp. 393–403, 2002.

[6] J. Auwerx, "PPARγ, the ultimate thrifty gene," *Diabetologia*, vol. 42, no. 9, pp. 1033–1049, 1999.

[7] B. M. Forman, J. Chen, and R. M. Evans, "The peroxisome proliferator-activated receptors: ligands and activators," *Annals of the New York Academy of Sciences*, vol. 804, pp. 266–275, 1996.

[8] H. Yki-Järvinen, "Thiazolidinediones," *New England Journal of Medicine*, vol. 351, no. 11, pp. 1106–1118, 2004.

[9] J. A. Dormandy, B. Charbonnel, D. J. Eckland, et al., "Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive study (PROspective pioglitAzone clinical trial in macro vascular events): a randomised controlled trial," *The Lancet*, vol. 366, no. 9493, pp. 1279–1289, 2005.

[10] DREAM trial investigators, "Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial," *The Lancet*, vol. 368, no. 9541, pp. 1096–1105, 2006.

[11] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[12] V. Fonseca, "Effect of thiazolidinediones on body weight in patients with diabetes mellitus," *American Journal of Medicine*, vol. 115, no. 8, supplement 1, pp. 42–48, 2003.

[13] T. M. Larsen, S. Toubro, and A. Astrup, "PPARγ agonists in the treatment of type II diabetes: is increased fatness commensurate with long-term efficacy?" *International Journal of Obesity*, vol. 27, no. 2, pp. 147–161, 2003.

[14] Y. Guan, C. Hao, D. R. Cha, et al., "Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption," *Nature Medicine*, vol. 11, no. 8, pp. 861–866, 2005.

[15] S. Mudaliar, A. R. Chang, and R. R. Henry, "Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications," *Endocrine Practice*, vol. 9, no. 5, pp. 406–416, 2003.

[16] I. Barroso, M. Gurnell, V. E. F. Crowley, et al., "Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension," *Nature*, vol. 402, no. 6764, pp. 880–883, 1999.

[17] A. K. Agarwal and A. Garg, "A novel heterozygous mutation in peroxisome proliferator-activated receptor-γ gene in a patient with familial partial lipodystrophy," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 1, pp. 408–411, 2002.

[18] R. A. Hegele, H. Cao, C. Frankowski, S. T. Mathews, and T. Leff, "PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy," *Diabetes*, vol. 51, no. 12, pp. 3586–3590, 2002.

[19] D. B. Savage, G. D. Tan, C. L. Acerini, et al., "Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 52, no. 4, pp. 910–917, 2003.

[20] D. B. Savage, M. Agostini, I. Barroso, et al., "Digenic inheritance of severe insulin resistance in a human pedigree," *Nature Genetics*, vol. 31, no. 4, pp. 379–384, 2002.

[21] G. A. Francis, G. Li, R. Casey, et al., "Peroxisomal proliferator activated receptor-γ deficiency in a Canadian kindred with familial partial lipodystrophy type 3 (FPLD3)," *BMC Medical Genetics*, vol. 7, p. 3, 2006.

[22] R. A. Hegele, E. Ur, T. P. Ransom, and H. Cao, "A frameshift mutation in peroxisome-proliferator-activated receptor-γ in familial partial lipodystrophy subtype 3 (FPLD3; MIM 604367)," *Clinical Genetics*, vol. 70, no. 4, pp. 360–362, 2006.

[23] M. Agostini, E. Schoenmakers, C. Mitchell, et al., "Non-DNA binding, dominant-negative, human PPARγ mutations cause lipodystrophic insulin resistance," *Cell Metabolism*, vol. 4, no. 4, pp. 303–311, 2006.

[24] H.-P. Guan, T. Ishizuka, P. C. Chui, M. Lehrke, and M. A. Lazar, "Corepressors selectively control the transcriptional activity of PPARγ in adipocytes," *Genes and Development*, vol. 19, no. 4, pp. 453–461, 2005.

[25] R. M. Evans, G. D. Barish, and Y.-X. Wang, "PPARs and the complex journey to obesity," *Nature Medicine*, vol. 10, no. 4, pp. 355–361, 2004.

[26] G. D. Barish, V. A. Narkar, and R. M. Evans, "PPARδ: a dagger in the heart of the metabolic syndrome," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 590–597, 2006.

[27] L. Fajas, D. Auboeuf, E. Raspé, et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[28] L. Fajas, J.-C. Fruchart, and J. Auwerx, "PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[29] H. Sundvold and S. Lien, "Identification of a novel peroxisome proliferator-activated receptor (PPAR) γ promoter in man and transactivation by the nuclear receptor RORα1," *Biochemical and Biophysical Research Communications*, vol. 287, no. 2, pp. 383–390, 2001.

[30] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[31] I. Tzameli, H. Fang, M. Ollero, et al., "Regulated production of a peroxisome proliferator-activated receptor-γ ligand during an early phase of adipocyte differentiation in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 279, no. 34, pp. 36093–36102, 2004.

[32] E. D. Rosen, C.-H. Hsu, X. Wang, et al., "C/EBPα induces adipogenesis through PPARγ: a unified pathway," *Genes and Development*, vol. 16, no. 1, pp. 22–26, 2002.

[33] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[34] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[35] E. D. Rosen, P. Sarraf, A. E. Troy, et al., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[36] N. Kubota, Y. Terauchi, H. Miki, et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[37] M. Gurnell, J. M. Wentworth, M. Agostini, et al., "A dominant-negative peroxisome proliferator-activated receptor γ (PPARγ) mutant is a constitutive repressor and inhibits PPARγ-mediated adipogenesis," *Journal of Biological Chemistry*, vol. 275, no. 8, pp. 5754–5759, 2000.

[38] H. Bays, L. Mandarino, and R. A. DeFronzo, "Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 2, pp. 463–478, 2004.

[39] M. Adams, C. T. Montague, J. B. Prins, et al., "Activators of peroxisome proliferator-activated receptor γ have depot-specific effects on human preadipocyte differentiation," *Journal of Clinical Investigation*, vol. 100, no. 12, pp. 3149–3153, 1997.

[40] M. Ristow, D. Müller-Wieland, A. Pfeiffer, W. Krone, and C. R. Kahn, "Obesity associated with a mutation in a genetic regulator of adipocyte differentiation," *New England Journal of Medicine*, vol. 339, no. 14, pp. 953–959, 1998.

[41] E. Hu, J. B. Kim, P. Sarraf, and B. M. Spiegelman, "Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ," *Science*, vol. 274, no. 5295, pp. 2100–2103, 1996.

[42] M. Adams, M. J. Reginato, D. Shao, M. A. Lazar, and V. K. Chatterjee, "Transcriptional activation by peroxisome proliferator-activated receptor γ is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site," *Journal of Biological Chemistry*, vol. 272, no. 8, pp. 5128–5132, 1997.

[43] M. Blüher and R. Paschke, "Analysis of the relationship between PPAR-γ 2 gene variants and severe insulin resistance in obese patients with impaired glucose tolerance," *Experimental and Clinical Endocrinology and Diabetes*, vol. 111, no. 2, pp. 85–90, 2003.

[44] M. Agostini, M. Gurnell, D. B. Savage, et al., "Tyrosine agonists reverse the molecular defects associated with dominant-negative mutations in human peroxisome proliferator-activated receptor γ," *Endocrinology*, vol. 145, no. 4, pp. 1527–1538, 2004.

[45] K. Al-Shali, H. Cao, N. Knoers, A. R. Hermus, C. J. Tack, and R. A. Hegele, "A single-base mutation in the peroxisome proliferator-activated receptor γ4 promoter associated with altered in vitro expression and partial lipodystrophy," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 11, pp. 5655–5660, 2004.

[46] Y.-S. Tsai, H.-J. Kim, N. Takahashi, et al., "Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARγ," *Journal of Clinical Investigation*, vol. 114, no. 2, pp. 240–249, 2004.

[47] S. L. Gray, E. D. Nora, J. Grosse, et al., "Leptin deficiency unmasks the deleterious effects of impaired peroxisome proliferator-activated receptor γ function (P465L PPARγ) in mice," *Diabetes*, vol. 55, no. 10, pp. 2669–2677, 2006.

[48] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[49] M. Stumvoll and H. Häring, "The peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism," *Diabetes*, vol. 51, no. 8, pp. 2341–2347, 2002.

[50] M. M. Swarbrick, C. M. L. Chapman, B. M. McQuillan, J. Hung, P. L. Thompson, and J. P. Beilby, "A Pro12Ala polymorphism in the human peroxisome proliferator-activated receptor-γ2 is associated with combined hyperlipidaemia in obesity," *European Journal of Endocrinology*, vol. 144, no. 3, pp. 277–282, 2001.

[51] B. A. Beamer, C.-J. Yen, R. E. Andersen, et al., "Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor-γ2 gene with obesity in two Caucasian populations," *Diabetes*, vol. 47, no. 11, pp. 1806–1808, 1998.

[52] S. Masud and S. Ye, "Effect of the peroxisome proliferates activated receptor-γ gene Pro12Ala variant on body mass index: a meta-analysis," *Journal of Medical Genetics*, vol. 40, no. 10, pp. 773–780, 2003.

[53] J. Luan, P. O. Browne, A.-H. Harding, et al., "Evidence for gene-nutrient interaction at the PPARγ locus," *Diabetes*, vol. 50, no. 3, pp. 686–689, 2001.

[54] J. Robitaille, J.-P. Després, L. Pérusse, and M.-C. Vohl, "The PPAR-γ P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Québec family study," *Clinical Genetics*, vol. 63, no. 2, pp. 109–116, 2003.

[55] T. M. Willson, J. E. Cobb, D. J. Cowan, et al., "The structure—activity relationship between peroxisome proliferator-activated receptor γ agonism and the antihyperglycemic activity of thiazolidinediones," *Journal of Medicinal Chemistry*, vol. 39, no. 3, pp. 665–668, 1996.

[56] R. Mukherjee, P. J. A. Davies, D. L. Crombie, et al., "Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists," *Nature*, vol. 386, no. 6623, pp. 407–410, 1997.

[57] S. M. Rangwala, B. Rhoades, J. S. Shapiro, et al., "Genetic modulation of PPARγ phosphorylation regulates insulin sensitivity," *Developmental Cell*, vol. 5, no. 4, pp. 657–663, 2003.

[58] W. He, Y. Barak, A. Hevener, et al., "Adipose-specific peroxisome proliferator-activated receptor γ knockout causes

insulin resistance in fat and liver but not in muscle," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15712–15717, 2003.

[59] A. L. Hevener, W. He, Y. Barak, et al., "Muscle-specific Pparg deletion causes insulin resistance," *Nature Medicine*, vol. 9, no. 12, pp. 1491–1497, 2003.

[60] A. W. Norris, L. Chen, S. J. Fisher, et al., "Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones," *Journal of Clinical Investigation*, vol. 112, no. 4, pp. 608–618, 2003.

[61] K. Matsusue, M. Haluzik, G. Lambert, et al., "Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes," *Journal of Clinical Investigation*, vol. 111, no. 5, pp. 737–747, 2003.

[62] J. C. Florez, J. Hirschhorn, and D. Altshuler, "The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits," *Annual Review of Genomics and Human Genetics*, vol. 4, pp. 257–291, 2003.

[63] A. Memisoglu, F. B. Hu, S. E. Hankinson, et al., "Prospective study of the association between the proline to alanine codon 12 polymorphism in the PPARγ gene and type 2 diabetes," *Diabetes Care*, vol. 26, no. 10, pp. 2915–2917, 2003.

[64] E. S. Tai, D. Corella, M. Deurenberg-Yap, et al., "Differential effects of the C1431T and Pro12Ala PPARγ gene variants on plasma lipids and diabetes risk in an Asian population," *Journal of Lipid Research*, vol. 45, no. 4, pp. 674–685, 2004.

[65] A. S. F. Doney, B. Fischer, J. E. Cecil, et al., "Association of the Pro12Ala and C1431T variants of PPARG and their haplotypes with susceptibility to type 2 diabetes," *Diabetologia*, vol. 47, no. 3, pp. 555–558, 2004.

[66] L. Chao, B. Marcus-Samuels, M. M. Mason, et al., "Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones," *Journal of Clinical Investigation*, vol. 106, no. 10, pp. 1221–1228, 2000.

[67] K. Schoonjans, J. Peinado-Onsurbe, A.-M. Lefebvre, et al., "PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene," *EMBO Journal*, vol. 15, no. 19, pp. 5336–5348, 1996.

[68] B. I. Frohnert, T. Y. Hui, and D. A. Bernlohr, "Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene," *Journal of Biological Chemistry*, vol. 274, no. 7, pp. 3970–3977, 1999.

[69] H.-P. Guan, Y. Li, M. V. Jensen, C. B. Newgard, C. M. Steppan, and M. A. Lazar, "A futile metabolic cycle activated in adipocytes by antidiabetic agents," *Nature Medicine*, vol. 8, no. 10, pp. 1122–1128, 2002.

[70] J. Tordjman, G. Chauvet, J. Quette, E. G. Beale, C. Forest, and B. Antoine, "Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells," *Journal of Biological Chemistry*, vol. 278, no. 21, pp. 18785–18790, 2003.

[71] O. Vaccaro, F. P. Mancini, G. Ruffa, et al., "Fasting plasma free fatty acid concentrations and Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPAR) γ2 gene in healthy individuals," *Clinical Endocrinology*, vol. 57, no. 4, pp. 481–486, 2002.

[72] P. Peraldi, M. Xu, and B. M. Spiegelman, "Thiazolidinediones block tumor necrosis factor-α-induced inhibition of insulin signaling," *Journal of Clinical Investigation*, vol. 100, no. 7, pp. 1863–1869, 1997.

[73] C. M. Steppan, S. T. Bailey, S. Bhat, et al., "The hormone resistin links obesity to diabetes," *Nature*, vol. 409, no. 6818, pp. 307–312, 2001.

[74] Q. Yang, T. E. Graham, N. Mody, et al., "Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes," *Nature*, vol. 436, no. 7049, pp. 356–362, 2005.

[75] N. Maeda, M. Takahashi, T. Funahashi, et al., "PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein," *Diabetes*, vol. 50, no. 9, pp. 2094–2099, 2001.

[76] R. S. Ahima and J. S. Flier, "Adipose tissue as an endocrine organ," *Trends in Endocrinology and Metabolism*, vol. 11, no. 8, pp. 327–332, 2000.

[77] T. P. Combs, J. A. Wagner, J. Berger, et al., "Induction of adipocyte complement-related protein of 30 kilodaltons by PPARγ agonists: a potential mechanism of insulin sensitization," *Endocrinology*, vol. 143, no. 3, pp. 998–1007, 2002.

[78] R. K. Semple, M. A. Soos, J. Luan, et al., "Elevated plasma adiponectin in humans with genetically defective insulin receptors," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 8, pp. 3219–3223, 2006.

[79] M. Iwata, T. Haruta, I. Usui, et al., "Pioglitazone ameliorates tumor necrosis factor-α-induced insulin resistance by a mechanism independent of adipogenic activity of peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 50, no. 5, pp. 1083–1092, 2001.

[80] U. Smith, S. Gogg, A. Johansson, T. Olausson, V. Rotter, and B. Svalstedt, "Thiazolidinediones (PPARγ agonists) but not PPARα agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes," *FASEB Journal*, vol. 15, no. 1, pp. 215–220, 2001.

[81] J. Rieusset, C. Chambrier, K. Bouzakri, et al., "The expression of the p85α subunit of phosphatidylinositol 3-kinase is induced by activation of the peroxisome proliferator-activated receptor γ in human adipocytes," *Diabetologia*, vol. 44, no. 5, pp. 544–554, 2001.

[82] V. Ribon, J. H. Johnson, H. S. Camp, and A. R. Saltiel, "Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor γ activation stimulates expression of the CAP gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 25, pp. 14751–14756, 1998.

[83] C. A. Baumann, N. Chokshi, A. R. Saltiel, and V. Ribon, "Cloning and characterization of a functional peroxisome proliferator activator receptor-γ-responsive element in the promoter of the CAP gene," *Journal of Biological Chemistry*, vol. 275, no. 13, pp. 9131–9135, 2000.

[84] I. J. Bujalska, S. Kumar, and P. M. Stewart, "Does central obesity reflect "Cushing's disease of the omentum"?" *The Lancet*, vol. 349, no. 9060, pp. 1210–1213, 1997.

[85] H. Masuzaki, J. Paterson, H. Shinyama, et al., "A transgenic model of visceral obesity and the metabolic syndrome," *Science*, vol. 294, no. 5549, pp. 2166–2170, 2001.

[86] J. Berger, M. Tanen, A. Elbrecht, et al., "Peroxisome proliferator-activated receptor-γ ligands inhibit adipocyte 11β-hydroxysteroid dehydrogenase type 1 expression and activity," *Journal of Biological Chemistry*, vol. 276, no. 16, pp. 12629–12635, 2001.

[87] B. S. Cha, T. P. Ciaraldi, L. Carter, et al., "Peroxisome proliferator-activated receptor (PPAR) γ and retinoid X receptor (RXR) agonists have complementary effects on glucose and lipid metabolism in human skeletal muscle," *Diabetologia*, vol. 44, no. 4, pp. 444–452, 2001.

[88] C. Kausch, J. Krützfeldt, A. Witke, et al., "Effects of troglitazone on cellular differentiation, insulin signaling, and glucose metabolism in cultured human skeletal muscle cells," *Biochemical and Biophysical Research Communications*, vol. 280, no. 3, pp. 664–674, 2001.

[89] A. A. Parulkar, M. L. Pendergrass, R. Granda-Ayala, T. R. Lee, and V. A. Fonseca, "Nonhypoglycemic effects of thiazolidinediones," *Annals of Internal Medicine*, vol. 134, no. 1, pp. 61–71, 2001.

[90] F. Massiéra, M. Bloch-Faure, D. Ceiler, et al., "Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation," *The FASEB Journal*, vol. 15, no. 14, pp. 2727–2729, 2001.

[91] C. J. Östgren, U. Lindblad, O. Melander, A. Melander, L. Groop, and L. Råstam, "Peroxisome proliferator-activated receptor-γPro12Ala polymorphism and the association with blood pressure in type 2 diabetes: skaraborg hypertension and diabetes project," *Journal of Hypertension*, vol. 21, no. 9, pp. 1657–1662, 2003.

[92] S. J. Hasstedt, Q.-F. Ren, K. Teng, and S. C. Elbein, "Effect of the peroxisome proliferator-activated receptor-γ2 Pro12Ala variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 2, pp. 536–541, 2001.

[93] B. Isomaa, P. Almgren, T. Toumi, et al., "Cardiovascular morbidity and mortality associated with the metabolic syndrome," *Diabetes Care*, vol. 24, no. 4, pp. 683–689, 2001.

[94] J. Minamikawa, S. Tanaka, M. Yamauchi, D. Inoue, and H. Koshiyama, "Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 5, pp. 1818–1820, 1998.

[95] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[96] L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans, "Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.

[97] M. Ricote, J. Huang, L. Fajas, et al., "Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7614–7619, 1998.

[98] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[99] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[100] J. S. Welch, M. Ricote, T. E. Akiyama, F. J. Gonzalez, and C. K. Glass, "PPARγ and PPARδ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 11, pp. 6712–6717, 2003.

[101] K. J. Moore, E. D. Rosen, M. L. Fitzgerald, et al., "The role of PPAR-γ in macrophage differentiation and cholesterol uptake," *Nature Medicine*, vol. 7, no. 1, pp. 41–47, 2001.

[102] A. R. Tall, P. Costet, and N. Wang, "Regulation and mechanisms of macrophage cholesterol efflux," *Journal of Clinical Investigation*, vol. 110, no. 7, pp. 899–904, 2002.

[103] G. Chinetti, S. Lestavel, V. Bocher, et al., "PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway," *Nature Medicine*, vol. 7, no. 1, pp. 53–58, 2001.

[104] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans, "PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation," *Nature Medicine*, vol. 7, no. 1, pp. 48–52, 2001.

[105] E. Iwata, I. Yamamoto, T. Motomura, et al., "The association of Pro12Ala polymorphism in PPARγ2 with lower carotid artery IMT in Japanese," *Diabetes Research and Clinical Practice*, vol. 62, no. 1, pp. 55–59, 2003.

[106] Z. C. Yan, Z. M. Zhu, C. Y. Shen, et al., "Peroxisome proliferator-activated receptor γ C-161T polymorphism and carotid artery atherosclerosis in metabolic syndrome," *Zhonghua Yi Xue Za Zhi*, vol. 84, no. 7, pp. 543–547, 2004 (Chinese).

[107] Y. Li and M. A. Lazar, "Differential gene regulation by PPARγ agonist and constitutively active PPARγ2," *Molecular Endocrinology*, vol. 16, no. 5, pp. 1040–1048, 2002.

[108] M. J. Reginato, S. T. Bailey, S. L. Krakow, et al., "A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor γ-activating properties," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32679–32684, 1998.

[109] S. Rocchi, F. Picard, J. Vamecq, et al., "A unique PPARγ ligand with potent insulin-sensitizing yet weak adipogenic activity," *Molecular Cell*, vol. 8, no. 4, pp. 737–747, 2001.

[110] A. Shimaya, E. Kurosaki, R. Nakano, R. Hirayama, M. Shibasaki, and H. Shikama, "The novel hypoglycemic agent YM440 normalizes hyperglycemia without changing body fat weight in diabetic db/db mice," *Metabolism: Clinical and Experimental*, vol. 49, no. 3, pp. 411–417, 2000.

[111] P. Misra, R. Chakrabarti, R. K. Vikramadithyan, et al., "PAT5A: a partial agonist of peroxisome proliferator-activated receptor γ is a potent antidiabetic thiazolidinedione yet weakly adipogenic," *Journal of Pharmacology and Experimental Therapeutics*, vol. 306, no. 2, pp. 763–771, 2003.

[112] J. P. Berger, A. E. Petro, K. L. MacNaul, et al., "Distinct properties and advantages of a novel peroxisome proliferator-activated protein γ selective modulator," *Molecular Endocrinology*, vol. 17, no. 4, pp. 662–676, 2003.

[113] K. R. Kim, J. H. Lee, S. J. Kim, et al., "KR-62980: a novel peroxisome proliferator-activated receptor γ agonist with weak adipogenic effects," *Biochemical Pharmacology*, vol. 72, no. 4, pp. 446–454, 2006.

[114] K. Liu, R. M. Black, J. J. Acton III, et al., "Selective PPARγ modulators with improved pharmacological profiles," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 10, pp. 2437–2440, 2005.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 12781, 12 pages
doi:10.1155/2007/12781

Review Article

Genetic Manipulations of PPARs: Effects on Obesity and Metabolic Disease

Yaacov Barak and Suyeon Kim

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA

Received 7 August 2006; Revised 10 November 2006; Accepted 16 November 2006

Recommended by Wallace Harrington

The interest in genetic manipulations of PPARs is as old as their discovery as receptors of ligands with beneficial clinical activities. Considering the effects of PPAR ligands on critical aspects of systemic physiology, including obesity, lipid metabolism, insulin resistance, and diabetes, gene knockout (KO) in mice is the ideal platform for both hypothesis testing and discovery of new PPAR functions in vivo. With the fervent pursuit of the magic bullet to eradicate the obesity epidemic, special emphasis has been placed on the impacts of PPARs on obesity and its associated diseases. As detailed in this review, understanding how PPARs regulate gene expression and basic metabolic pathways is a necessary intermediate en route to deciphering their effects on obesity. Over a decade and dozens of genetic modifications of PPARs into this effort, valuable lessons have been learned, but we are left with more questions to be answered. These lessons and future prospects are the subject of this review.

1. PPARα

The only PPAR faithful to its acronym, PPARα, is the nuclear receptor of peroxisome proliferators—a diverse group of compounds, which in addition to toxic and carcinogenic chemicals include the lipid-lowering fibrate drugs [1]. PPARα is expressed in active metabolic tissues, including liver, heart, brown fat, and skeletal muscle, where it regulates genes that catalyze fatty acid (FA) catabolism [1, 2]. By 1995, mice homozygous for a *Ppara*-null allele were generated and found to be viable, healthy, fertile, and devoid of gross phenotypic defects under standard husbandry [3]. However, these mice could mount neither the hepatic response to peroxisome proliferators nor the induction of lipid-metabolizing enzymes by fibrates [3, 4]. These results confirmed the null nature of this allele, and obviated the need for alternative null configurations; consequently, this strain has become the exclusive animal model for studies of PPARα deficiency to date. These studies are summarized below.

1.1. *Ppara KO and obesity*

Early studies of *Ppara*-null mice reported hepatosteatosis and elevated levels of circulating triglycerides (TG) and cholesterol, as well as a significant increase in gonadal fat pad mass [5–8]. The integral role of PPARα in body fat mass determination is further cemented by the demonstration that the KO mice fail to decrease adipose tissue weight in response to hyperleptinemia [9]. However, the contribution of PPARα to total body weight is ambiguous, with conflicting reports of substantial age-related obesity [6, 7] versus no significant body weight effects in congenic 129/SvJae or C57BL/6N *Ppara*$^{-/-}$ mice [8]. The discrepant outcomes of these studies have been attributed to subtle experimental variations in the genetic background and chow composition, and suggest that the contribution of PPARα to obesity is strongly influenced by genomic and environmental contexts.

1.2. *Ppara KO and fasting*

The relatively minor phenotype of *Ppara*-null mice under standard husbandry conditions is consistent with a contingency function that comes into play under metabolic duress. Accordingly, multiple studies addressed the ability of *Ppara*-null to cope with dietary challenges. The most informative manipulation has been fasting, during which PPARα deficiency was shown to cause excessive surge in circulating FA levels, rapid hepatic and cardiac lipid accumulation, absent ketogenic response, profound hypoglycemia and hepatic glycogen depletion [10–13]. These anomalies are thought to

arise from failure of the mutant livers to catabolize adipose tissue-derived FA, which on the one hand impairs gluconeogenesis at both enzyme activity and substrate levels, and on the other hand leads to morbid accumulation of nonmetabolized lipids [14, 15]. PPARα is similarly critical for cardiac lipid oxidation, which is the main energy source for the heart during fasting and exercise; reviewed in [16]. Both constitutive and inducible expression of PPARα target genes are blunted in *Ppara*-null hearts, which exhibit abnormal TG accumulation during fasting and progressive deterioration of myofibrillar and mitochondrial integrity upon aging [10, 17]. The crucial importance of PPARα-mediated hepatic and cardiac lipid catabolism, regardless of fasting, is also evident in the severe hypoglycemia and staggering lipid accumulation in livers and hearts of *Ppara*-null mice following pharmacological inhibition of FA flux [18]. Interestingly, female and estrogen-treated male *Ppara*-null mice are significantly protected against this lethal combination of tissue hyperlipidemia and systemic hypoglycemia, implying an alternative, estrogen-dependent lipid utilization pathway [18]. While proper cardiac metabolism is disrupted in the absence of PPARα, dosage and temporal regulation of the receptor are critical, and its constitutive transgenic overexpression in cardiac muscle via the α myosin heavy chain (MHC) promoter is detrimental [19]. Hearts of *MHC-Ppara* transgenic mice exhibit a faithful phenocopy of diabetic cardiomyopathy, with increased lipid oxidation, a reciprocal decrease in glucose utilization, and symptoms of ventricular hypertrophy [19].

1.3. *Ppara KO in high-fat and cholesterol-rich diets*

The role of PPARα in the physiological outcomes of high fat diet (HFD) is not as clear as its role in fasting physiology. *Ppara*-null mice are as susceptible as *wt* mice to HFD-induced weight gain and hepatic TG accumulation, but are protected from glucocorticoid-induced hypertension [20–22]. Blunted hyperinsulinemia and improved glucose and insulin tolerance following 2-hour fasting suggested initially that HFD-fed *Ppara*-null mice are protected from insulin resistance (IR) as a result of either reduced hepatic glucose production or increased peripheral insulin sensitivity [20]. However, this report has been contested by a study that found little difference in hyperinsulinemia and peripheral glucose uptake during euglycemic clamp of HFD-fed *wt* versus *Ppara*-null mice in the nonfasted state [23]. This contradictory result raised the concern that the improved insulin and glucose tolerance of HFD-fed *Ppara*-null mice in the earlier studies reflects no more than their established hypoglycemic response to the fasting regimen that preceded the assays; studies that bypass this conceptual hurdle will be required to reevaluate the role of PPARα in the aftermath of HFD. Because the consequences of PPARα deficiency also include a constitutive increase in circulating cholesterol, it is equally important to test the effects of a cholesterol-rich diet in *Ppara*-null mice. Surprisingly, addition of 2% cholesterol to the chow caused fat pad weight reduction and increased de novo lipogenesis in *Ppara*-null mice, indicating that the receptor participates in basal and feedback-regulated cholesterol and triacylglycerol homeostasis in adipose tissue [24]. These activities impinge directly on the contribution of PPARα to obesity.

1.4. *Effects of Ppara KO on other tissues*

Unlike the effect of PPARα on cardiac muscle, PPARα deficiency had no significant effect on the responses of skeletal muscle to either fasting or heavy exercise, perhaps due to compensation by redundant functions of PPARδ [25]. However, transgenic overexpression of PPARα in skeletal muscle, using the muscle creatine kinase (MCK) promoter, protected mice from HFD-induced obesity, albeit at the expense of glucose intolerance and insulin resistance [26]. The proposed mechanism entails reduced insulin-stimulated glucose uptake due to repression of AMP-activated protein kinase-dependent glucose transporter gene expression by oxidized FA. The hepatocentric view of systemic PPARα effects is moderated by a recent report of increased peripheral glucose utilization in fasted *Ppara*-null mice, which was refractory to adenovirus-mediated reconstitution of hepatic PPARα [27]. Moreover, direct injection of a PPARα agonist into the lateral ventricle of *wt* mice significantly reduced whole body glucose utilization, suggesting that PPARα also functions centrally [27]. Tissue-specific *Ppara*-null mice, which have yet to be generated, would be an ideal platform to validate and further explore these intricate mechanisms of PPARα action.

2. A PANOPLY OF PPARγ KNOCKOUTS

Without detracting from the importance of PPARα and PPARδ (see below for PPARδ), the defining moment in the explosive growth of the PPAR field has been the identification of PPARγ as the high-affinity receptor of the insulin-sensitizing thiazolidinedione (TZD) drugs [28, 29]. The pivotal role of PPARγ in the adipocyte life cycle [30–34], combined with the blockbuster success of its TZD ligands in treating type II diabetes [35, 36], generated widespread enthusiasm for the prospect of solving the causal relationship between obesity and diabetes through PPARγ research. The use of gene knockout in mice presented the most logical investigative approach, leading to the generation of a dazzling array of mouse strains with genetic modifications of *Pparg*. This volume of effort reflects not only the immense biomedical significance of the gene, but also the complexity of the genetic data, which had encumbered immediate, straightforward understanding of PPARγ function in vivo and had spawned numerous alternative hypotheses. The myriad *Pparg* KO strains, and the results of their analyses, are summarized below.

Constitutive Pparg deficiency cannot be studied in adult mice due to the essential role of PPARγ in placental development, which abolishes survival beyond mid-gestation [34]. However, aggregation with tetraploid *wt* embryos provided *Pparg*-null embryos with *wt* placentas and rescued them to term [34]. In these chimeras, *Pparg*-null cells committed to the adipocyte lineage, but failed to proliferate and differentiate into *bona fide* adipocytes, and a chimeric pup that survived a few days after birth was devoid of any type

of adipose tissue [34]. This effort proved the essential role of PPARγ in early adipogenesis in vivo, but unfortunately, perinatal lethality precluded studies of this *Pparg*-null configuration beyond birth. The current availability of floxed *Pparg* alleles (see below) and epiblast-specific Cre-expressing mouse strains [37, 38] should revitalize this configuration by facilitating higher throughput generation of *Pparg*-null mice supported by *wt* placentas. Studies in progress in our lab with standard *wt/Pparg*-null chimeras, in which diploid host-derived *wt* cells coexist with *Pparg*-null ES-derived cells, confirmed the formation and subsequent arrest of *Pparg*-null adipose tissue primordia. However, here *wt* cells infiltrated the stagnant *Pparg*-null primordia and repopulated them through a previously unknown developmental feedback mechanism (S. Kim and Y. Barak, unpublished). Consequently, post-term *wt/Pparg*-null chimeras invariably possess only *wt* adipose tissue, in contrast to the random contribution of *wt* and *Pparg*-null cells to other tissues [31].

2.1. *Pparg*$^{+/-}$ mice

With *bona fide* adult *Pparg*-null mice unavailable, investigators initially turned to *Pparg*-haploinsufficient mice to explore the effects of reduced PPARγ dosage. As expected, adiposity of *Pparg*$^{+/-}$ mice was reduced, supporting the assertion that PPARγ contributes quantitatively to adipose mass [39]. However, contrary to the expectation that reduced levels of the TZD receptor will cause a parallel reduction in insulin sensitivity, *Pparg*$^{+/-}$ mice were more insulin-sensitive than *wt* controls when challenged by either HFD or aging [39–41]. This confounding observation conflicts with the monochromatic view of PPARγ as a beneficial TZD-activated insulin sensitizer and raises the counterintuitive notion that it has pathogenic activities. While the nature of these adverse properties of the receptor is unclear, one potential example of a latent pathogenic effect is the positive relationship between PPARγ dosage and adipose tissue mass, which might come into play under conditions of nutritional affluence. However, excessive reduction of PPARγ activity by treating haploinsufficient mice with a PPARγ antagonist reversed the tide and resulted in lipodystrophy and IR [42, 43].

2.2. Tissue-specific Pparg KOs

Tissue-specific Pparg KOs were subsequently developed by several groups using *Cre-loxP* methodology, with the vision of both bypassing the embryonic lethality of constitutive *Pparg* deficiency and resolving the physiological functions of PPARγ one tissue at a time [32, 44, 45]. *Pparg* has since been deleted in a substantial number of cell types, of which the most pertinent to this review are adipocytes, myocytes, and hepatocytes, and from a broader metabolic disease perspective also macrophages, pancreatic β-cells, renal collecting duct epithelia, and endothelial cells (referenced below).

2.2.1. Adipocyte-specific Pparg KO

The abundant expression of PPARγ in adipocytes indicates that its important functions in these cells extend beyond its indispensability for their formation. Moreover, the association between obesity as well as type II diabetes and the antidiabetic effect of TZDs fuel the hypothesis that PPARγ activity in adipocytes is a key to systemic insulin sensitivity. A mouse whose adipocytes lack PPARγ would provide the ultimate test for this idea. Generation of such a model was attempted using an adipocyte-specific *Fabp4*(aP2)-CRE transgene. While, as mentioned earlier, PPARγ is essential for adipocyte differentiation, the *Fabp4* promoter is activated after completion of adipogenesis, and thus allows the PPARγ-dependent formation of adipocytes prior to *Pparg* deletion [32, 45]. Contrary to a widespread, unsubstantiated concern, the *Fabp4* promoter does not drive transgene expression in macrophages or other major metabolic tissues [32], and therefore the phenotype of these mice is not muddled by gene deletion in nonadipocyte cell types. Adipocyte-specific *Pparg*-null mice exhibited rapid loss of brown adipose tissue (BAT) and subcutaneous fat [32, 45]. Astonishingly, however, white adipose tissue (WAT) retained normal mass throughout a substantial stretch of adulthood [32]. This retention occurred despite substantial cell death and extensive fibrosis and inflammatory infiltration, and resulted from both overt hypertrophy of surviving adipocytes and adipocyte regeneration [32, 45].

The tight dependence of adipocytes on PPARγ for survival and the interpretation that adipocyte regeneration mitigates lipodystrophy were unequivocally proven by studies of mice with tamoxifen-inducible adipocyte *Pparg* KO [33]. These mice carry a *loxP*-flanked (floxed) *Pparg* allele and an *Fabp4*-driven fusion of Cre with a tamoxifen-responsive estrogen receptor mutant, which translocates to the adipocyte nucleus and targets the floxed allele only in response to tamoxifen administration. Induction of Cre activity in these mice induced synchronous, near-complete loss of white and brown adipocytes within 7 days, followed by acute inflammatory infiltration of the damaged fat pads, and complete rebound of adipocyte number and adipose tissue integrity within 6 weeks of the initial insult [33]. Thus, PPARγ is essential for the survival of mature adipocytes, but a rapid and robust regenerative process mitigates a loss of fat tissue following *Pparg* deletion. Similar regenerative potential of adipose tissue was recently demonstrated in mice with inducible adipocyte apoptosis [46], buttressing the notion that fat regenerates with remarkable efficiency in response to adipocyte death, beyond the context of PPARγ deficiency. Thus, adipose tissue of *Fabp4*-Cre *Pparg*$^{fl/fl}$ mice comprised a dynamic mixture of dying *Pparg*-null adipocytes alongside repopulating *wt* adipocytes—a condition that hindered the generation of mice with adipose tissue that uniformly lacks PPARγ. As long as WAT was sustained, these mice maintained relatively normal lipid and glucose homeostasis, despite substantial reduction in circulating leptin and adiponectin and an anticipated rise in the levels of free FA [32, 45]. Moderate IR and glucose intolerance, as well as hepatomegaly, steatosis, and increased hepatic glucose production, developed only in conjunction with the eventual terminal atrophy of WAT [32]. Analyses of two independent stocks of these mice by two research teams found obvious resistance to

HFD-induced obesity, likely due to the failure to accumulate adipocytes. However, the two teams observed opposite effects on insulin sensitivity. In one study, HFD accelerated lipoatrophy and exacerbated IR [32], whereas the other study found no such degenerative effect and the mutation protected the mice from IR [45]. In summary, while reproving the critical role of WAT in systemic insulin sensitivity and the indispensability of PPARγ to adipocyte viability, the adipocyte-specific *Pparg*-null mouse fell short of a definitive demonstration that adipocyte PPARγ regulates whole body metabolism.

2.2.2. Myocyte-specific Pparg KO

The insulin sensitizing activity of PPARγ ligands and the key role of skeletal muscle in peripheral insulin sensitivity generated great interest in the hypothesis that PPARγ exerts its insulin sensitizing activity from within myocytes. However, this hypothesis was challenged by the very low basal expression of PPARγ in skeletal muscle. The issue was addressed by two parallel studies that analyzed the outcome of *Pparg* deletion in myocytes. In the first study, myocyte-specific *Pparg*-null mice generated by *MCK* promoter-driven Cre recombinase exhibited increased adiposity, elevated susceptibility to HFD-induced weight gain, and marked hepatic IR in hyperinsulinemic-euglycemic clamps [47]. However, these mice were only as sensitive to HFD-induced IR and as responsive to the insulin sensitizing effects of TZDs as *wt* mice, suggesting that muscle PPARγ is dispensable for the antidiabetic effects of PPARγ agonists. The second study used mice generated using the same Cre transgene, but a different floxed *Pparg* allele, and first addressed the controversial issue of low PPARγ expression in myocytes [48]. It demonstrated that the minute amount of *Pparg* mRNA observed in muscle extracts undergoes *MCK*-Cre-mediated recombination, and thus, unequivocally proved that the transcript originated in myocytes rather than other cell types that populate muscle tissue. Mice in this study developed insulin and glucose intolerance with age, and exhibited severely compromised insulin-stimulated muscle glucose uptake, as well as liver and adipose tissue IR. In contrast to the first study, here TZDs failed to ameliorate muscle insulin resistance, suggesting that myocyte PPARγ regulates muscle insulin sensitivity cell autonomously. While the differential sensitivity of the two strains to TZDs raises concerns about the validity of the interpretations, they are not necessarily contradictory, considering that both the metabolic challenges (HFD versus aging) and the assayed activities (Insulin tolerance tests versus muscle glucose uptake) were different in each study. Still, more definitive studies, using mice with a purer genetic background and a standardized experimental approach, are required to settle these discrepancies. Regardless of the final answer, it is clear that while PPARγ may have some metabolic functions in myocytes, these functions are not sufficiently robust to account for the systemic antidiabetic actions of TZDs.

2.2.3. Hepatocyte-specific Pparg KO

As in muscle, basal PPARγ expression in liver is minimal. However, hepatic PPARγ expression is induced substantially during steatosis. The effects of albumin Cre-mediated hepatocyte *Pparg* deficiency were studied in *wt* and two different diabetic mouse models that succumb to steatosis—*A-Zip/F* lipoatrophic mice and leptin-deficient *ob/ob* mice [49, 50]. On an otherwise *wt* background, hepatic *Pparg* deficiency caused a significant defect in TG clearance, hyperlipidemia, and increased body fat mass with age, demonstrating the importance of hepatic PPARγ for basal fat tolerance and management of adiposity [50]. On the two diabetic backgrounds deficiency of hepatocytes for *Pparg* caused marked amelioration of hepatosteatosis, but exacerbated hyperlipidemia and muscle insulin resistance [49, 50]. These traits were reversed by TZDs in *ob/ob*, but not A-Zip/F mice, suggesting that the drugs exert their effect through activation of PPARγ in adipocytes, not hepatocytes. Together, these studies indicate that hepatocyte PPARγ is required for basal fat tolerance and, in addition, for steatosis of the diabetic liver, which serves to improve TG homeostasis and dampen systemic IR. However, they also clearly indicate that hepatic PPARγ is not critical for TZD-induced insulin sensitization.

2.2.4. Other tissue-specific Pparg KOs

The relatively modest effects of PPARγ deficiencies in fat, muscle, and liver provided the impetus for broadening the analysis of *Pparg* KO to additional cell types that participate in obesity-associated metabolic complications, namely diabetes, hypertension, and atherosclerosis. The outcomes of these analyses are briefly summarized as follows.

Pparg deficiency in β-islets caused a hyperplastic response without altering glucose homeostasis, ruling out a critical function of the receptor in homeostatic functions of β cells [51].

A strong rationale for the generation and analysis of *Pparg*-null macrophages was provided by observations that TZDs induce macrophage genes that regulate lipid flux, suppress inflammatory gene expression, and ameliorate atherosclerosis [52–54]. Early studies with *Pparg*-null macrophages in culture and in vivo demonstrated that TZD effects on lipoprotein flux indeed depend on PPARγ, but several of the reported anti-inflammatory effects of TZDs are independent of PPARγ [44, 55]. Nevertheless, adaptive transfer of *Pparg*-deficient macrophages exacerbated genetic- and diet-induced atherosclerosis in recipient mice, demonstrating that PPARγ performs key anti-atherogenic functions in these cells [56, 57]. In addition, a recent, yet-to-be-published symposium talk reported that macrophage-specific *Pparg*-null mice are glucose intolerant and exhibit increased sensitivity to HFD-induced insulin resistance [58]. Thus, PPARγ orchestrates multiple beneficial activities in macrophages that could be harnessed for the development of advanced therapies for atherosclerosis.

Edema due to fluid retention is an undesired side effect of TZD treatment in diabetic patients [59]. Mice with *Pparg* KO in renal collecting duct epithelia are resistant to this TZD-borne complication, confirming that PPARγ mediates it, apparently by enhancing sodium retention [60, 61]. This activity highlights an additional mechanism through which

PPARγ may regulate plasma volume, hypertension, and cardiovascular function.

Pparg deletion in endothelial cells exacerbates both HFD-induced and salt-induced hypertension, and renders the condition nonresponsive to TZDs, demonstrating that endothelial PPARγ is critical for mitigating the effects of dietary stress on blood pressure [62].

Ablation of PPARγ in cardiomyocytes causes elevated cardiac NF-κB activity and increased expression of cardiac embryonic genes, which lead to enhanced myofibril assembly and cardiac hypertrophy but does not affect systolic function [63]. The relationship between this phenotype and the metabolic functions of PPARγ in other tissues is not entirely clear, although aspects of cardiac lipid metabolism have yet to be addressed in this mouse.

2.3. Pparg2-specific KOs

Alternative promoters give rise to several *Pparg* isoforms with distinct 5′ ends. PPARγ1 is the ubiquitous isoform, expressed in all PPARγ-expressing tissues [64]. Adipocytes express, in addition to PPARγ1, a cell-specific isoform termed PPARγ2, whose unique 5′ exon encodes a 30 residue-long N-terminal extension of the ligand-independent transactivation domain of PPARγ1 [65]. Because the placenta expresses only PPARγ1, KO of PPARγ2 could provide yet another means to bypass the lethal outcome of constitutive *Pparg* deficiency, as well as to interrogate potential unique functions of this adipocyte-specific isoform. In all, three teams have knocked out *Pparg2* using distinct targeting strategies that produced slightly different results [66–68]. Knock-in of red fluorescent protein into the *Pparg2*-specific B exon produced a clean KO of PPARγ2 while retaining normal PPARγ1 expression in adipocytes [66]. This configuration interfered with adipocyte differentiation in vitro and markedly reduced fat mass in vivo. This lipodystrophic phenotype involved significant reduction in the size, number, and TG content of brown and white adipocytes, and decreased expression of typical adipocyte markers [66]. A second knockout configuration entailed replacement of the entire B exon and flanking intronic sequences with a *lacZ-neo* cassette and resulted in a similar *Pparg2*-specific gene disruption, without affecting *Pparg1* [67]. This configuration was as detrimental to adipocyte differentiation in vitro as the previous KO configuration, but unlike that KO it had only a marginal effect on either fat mass or basal adipocyte size [67]. It is unclear whether these differences are meaningful or rather reflect minor differences in the experimental setup used by the two teams, for example in allele configuration, the genetic background of the mice, composition of the chow, or analytical methods. A third *Pparg2* targeting configuration, which resulted from an intronic neo cassette downstream of exon B, eliminated *Pparg2* expression but inadvertently altered *Pparg1* expression, abolishing it in WAT while augmenting it in BAT [68]. Mice homozygous for this modification were deemed PPARγ hypomorphs ($Pparg^{hyp/hyp}$). Unlike the first two configurations, $Pparg^{hyp/hyp}$ were subject to high mortality rate and growth retardation during infancy; survivors thrived after weaning but were substantially lipodystrophic [68]. Importantly, contrary to other models of lipodystrophy, all three *Pparg2*-null configurations, including $Pparg^{hyp/hyp}$, exhibited a surprisingly modest decrease in glucose or insulin tolerance and did not develop steatosis. The suggestion that this relatively healthy phenotype is mitigated by compensatory lipid oxidation in muscle tissue [68] has to be reconciled with the failure of a similar compensatory mechanism to offset other cases of lipodystrophy.

2.4. Knock-in of dominant-negative mutations from human patients

As if the analyses described to this point were not sufficiently counterintuitive and indecisive, mice heterozygous for *Pparg-L466A* or *Pparg-P465L*—two dominant-negative missense mutations identified in human subjects—provided further surprises. Patients carrying one allele of either mutation alongside a second *wt* allele suffer from partial congenital lipodystrophy with hallmarks of the metabolic syndrome, including dyslipidemia, early-onset type II diabetes, and hypertension [69–73]. It therefore made perfect sense to replace the mouse *Pparg* gene with similar mutations, with the obvious expectation of recapitulating the clinical phenotype. Two research teams carried out this endeavor, each knocking in one of the mutations [74, 75]. Mice homozygous for either mutation died in utero, demonstrating the null nature of the alleles. However, while mice heterozygous for either mutation exhibited moderate hypertension and anomalies of either fat distribution or adipocyte morphology, none fully recapitulated the lipodystrophic phenotype of the orthologous patients [74, 75]. Moreover, $Pparg^{P465L/+}$ mice displayed no gross changes in plasma chemistry and were in fact more glucose tolerant than *wt* mice, both basally and following HFD, just like standard *Pparg*-haploinsufficient mice [74]. In addition, although more physiological anomalies were reported for $Pparg^{L466A/+}$ mice compared to $Pparg^{P465L/+}$ mice, including elevated free FA levels, hepatic steatosis and HFD-induced insulin resistance [75], their morbidity did not amount to that of their human counterparts.

2.5. Other genetic manipulations of Pparg

In addition to the *Pparg* KO onslaught, there has been a substantial public health interest in more subtle aspects of its function. These include the effects of genetic polymorphisms and post-translational modifications, which have been linked both genetically and epidemiologically to obesity and type II diabetes in the human population [76–79]. The first reported effort that undertook this approach is the S112A point mutation, which eliminates a MAP kinase phosphorylation site that inhibits the transcriptional activation capacity and adipogenic functions of PPARγ [80]. $Pparg^{S112A/S112A}$ mice are viable and healthy, and do not display physiological anomalies under normal husbandry. However, the failure to regulate PPARγ action by phosphorylation protects these mice against HFD-induced adipocyte hypertrophy and insulin resistance [80]. These results validate the utility of

subtle structural mutations for uncovering important physiological activities of PPARγ. Informal communications with other researchers, as well as the public NIH grant database, reveal that additional genetic manipulations aimed at understanding the biological function of conserved and polymorphic sequence elements of PPARγ are currently underway in mice.

2.6. *Pparg* KOs—summary and remarks

In aggregate, a slew of attempts to generate molecular genetic models that will reveal a role for PPARγ in obesity, insulin resistance, and related metabolic disorders have yielded partial success and confounding results. Constitutive *Pparg* KO was nonviable, *Pparg* haploinsufficiency was unexpectedly beneficial, and the pathogenic effect of dominant-negative *Pparg* mutations in human patients was not faithfully replicated in mouse models. Reassuringly, chimeric mouse studies and adipocyte-specific KOs unequivocally proved the critical role of PPARγ in adipocyte differentiation and survival. However, the potential for an interpretable effect on energy metabolism was thwarted by the inability to obtain long-lasting *Pparg*-null adipocytes, which did not allow teasing out the effect of PPARγ deficiency from the general impact of lipodystrophy. Quite disappointingly, KOs in other tissues had relatively modest effects basally and latent metabolic defects in response to dietary or genetic challenges. While these studies invoked encouraging links to atherosclerosis and hypertension, none amounted to full-blown IR, let alone diabetes. These major deviations from straightforward expectations raise concerns about the applicability of genetic studies of *Pparg* in the mouse to human metabolism. However, one should be reminded that TZDs are equally potent as insulin sensitizers in both mice and humans [35, 36], highly suggestive of similar metabolic functions of PPARγ *per se* across species. A more likely explanation for the relatively benign outcomes of these studies is the inherently fickle nature of genetic, physiological, and metabolic experiments in mice. Evolution likely differentiated metabolic physiology in rodents versus humans, and although PPARγ may have the exact same function in the bigger scheme, other genes and pathways may modify the outcome. In addition, lab mice are reared in a highly controlled ambient and provided either with uniform lean chow that differs drastically from human diet, or with experimental diets that mimic our own dietary follies, but which rodents have not evolved to handle. Effects of genetic background and modifier genes on outcomes and their interpretation comprise another obstacle. On the one hand, many of the studies summarized here do not clarify the extent of genetic homogeneity of the tested cohorts, potentially obstructing minor, yet critical effects of the mutations. On the other hand, the human population is genetically diverse, and gene defects that would devastate one person could be inconsequential in another. A case in point is the dramatic exacerbating effect of a mutation in the PPP1R3A gene on the outcome of PPARG mutations in a human pedigree [72]. Genes and pathways with comparable modifying effects could compensate for the effects of *Pparg* deficiency in mice. Moreover, redundant activities of PPARγ in different tissues or an altogether misguided choice of target tissues and readouts might have further hindered interpretation. Finally, it may be time to start entertaining the notion that the problem might be with the hypothesis itself: clearly, activation of PPARγ with TZDs is a robust therapy for IR, but does this mean that the pathway is necessary for basal insulin sensitivity in mice and men?

3. PPARδ

PPARδ was initially regarded as a promising prospect for studies of obesity and associated diseases purely on the merit of its pharmaceutically accomplished homologues [64]. With pharmacological agonists and genetic manipulations of PPARδ coming to fruition in recent years, these expectations are starting to be realized, and implicate PPARδ in important aspects of obesity, energy metabolism and metabolic disease. As in *Pparg*-null mice, analysis of *Ppard* deficiency also faces the challenge of substantial embryonic mortality, albeit for completely different reasons. The nature of the challenge, the different solutions, and the associated caveats are discussed briefly as a primer to the review of phenotypes associated with *Ppard*-null and gain-of-function models.

In all, 6 *Ppard*-null configurations have been generated in mice. Three knockout strains harboring deletions or insertions that wipe out the PPARδ protein product in its entirety cause severe placental defects that lead to substantial embryonic mortality [81–83]. While there are practically no surviving homozygous null animals on the standard, C57BL/6 (B6) background, survival is increased to between 5% and 20% on outbred B6 : 129/Sv [81] or FVB : B6 backgrounds (Y. Barak, unpublished data). Unfortunately, *Ppard*-null mice and *wt* controls generated in this fashion inherently possess mixed genetic backgrounds, whose stochastic quantitative trait locus effects significantly muddle physiological data. In addition, through successive interbreeding of surviving homozygous null FVB : B6 mice over several generations, our lab has managed to generate a genetically semistable *Ppard*-null stock with approximately 50% survival (Y. Barak, unpublished data). However, while this stock provides a higher yield of *Ppard*-null mice with a relatively isogenic background, the nature of the breeding strategy hindered the generation of genetically matching *wt* controls. In a fourth *Ppard* null allele, no substantial embryonic lethality was reported [84]. However, in this allele *Ppard* was truncated 60 amino acids from its C-terminus, leaving its entire DNA-binding domain and most of its ligand-binding domain intact, and raising a reasonable concern that it is a hypomorph that enabled embryonic survival via residual PPARδ functions. Therefore, analyses of adult mice carrying this KO configuration have to be interpreted with the cautionary note that it is likely incompletely deficient for PPARδ. Finally, floxed *Ppard* alleles have been generated as well [81, 85]. These configurations enable the targeting of *Ppard* in specific tissues with the obvious caveat that Cre-mediated deletion of floxed alleles is seldom fully penetrant. To avoid confusion, the term *Ppard*-null

mice is used in the following text to describe animals with germ-line deletion of the gene in all tissues, whereas studies performed with tissue-specific *Ppard* KOs are spelled out.

3.1. Genetic manipulations of PPARδ and adipose tissue

Early studies of outbred *Ppard*-null mice under standard husbandry conditions revealed a substantial decrease in the size of BAT and WAT [81, 84]. Fat mass was not reduced in adipocyte-specific *Ppard*-null mice (floxed *Ppard x Fabp4-Cre*) [81], demonstrating that this trait is not adipocyte-autonomous, and must result from impaired PPARδ activity in other tissues. While unable to achieve normal adiposity on standard, low fat chow, *Ppard*-null mice underwent a quicker and substantially more aggressive weight gain in response to HFD compared to *wt* controls [86, 87]. These observations were complemented and extended by studies of mice expressing constitutively active PPARδ in adipose tissue [87]. In these mice, the *Fabp4* promoter drives adipocyte-specific expression of a fusion protein between the transactivation domain of the Herpes Virus VP16 protein and PPARδ (*Fabp4-VP-Ppard*), such that the latter is rendered permanently active, irrespective of endogenous ligands. When reared on standard, low-fat chow *Fabp4-VP-Ppard* mice exhibited significant reduction in body weight and in the overall mass and TG content of adipose tissue, as well as in the levels of circulating TG and free FA [87]. However, the mice were protected from the adipocyte hypertrophy, dyslipidemia, obesity, and steatosis that occur in response to either HFD or impaired leptin signaling [87]. Quelling of obesity in these mice was associated with upregulation of genes that control lipid catabolism and adaptive thermogenesis in both BAT and WAT; reassuringly, the same genes are induced in response to systemic administration of a PPARδ ligand [87]. In contrast, adipocyte-specific PPARδ deficiency compromised HFD-mediated induction of the uncoupling protein 1 gene, *Ucp1*, in BAT [87]. Combined, these two genetic extremes of deficiency versus constitutive activation identify PPARδ as a critical regulator of lipid homeostasis and adiposity.

3.2. Genetic manipulations of PPARδ and muscle

The abundant expression of PPARδ in myocytes suggests an important role in skeletal muscle [2]. Two transgenic models of muscle-specific PPARδ overexpression and one of muscle-specific *Ppard*-deficiency confirmed this notion and revealed a massive impact of PPARδ on muscle and whole body physiology. MCK promoter-driven expression of constitutively active *VP-Ppard* resulted in a dramatic type switch of muscle from type II, glycolytic fibers to type I, slow-twitch, oxidative fibers, and a staggering increase in aerobic endurance [88]. This switch was associated with activation of the typical oxidative fiber expression program, including genes that regulate lipid catabolism, mitochondrial electron transfer, oxidative metabolism, and type I contractile structures [88]. Overexpression of *wt Ppard* in skeletal muscle activates a similar expression pattern, and falls just short of inducing fiber-type switching [89]; the tamer induction of these genes in the latter mouse strain reflects the lesser activity of *wt* PPARδ compared to the VP16-fused variant. These observations were fully corroborated by skeletal muscle-specific KO of *Ppard*, which resulted in the reciprocal muscle type switch from high- to low-oxidative fibers [85]. Molecular analyses of these mice revealed that PPARδ regulates the expression of the transcriptional cofactor PGC1α, which regulates mitochondrial biogenesis and muscle type switch, providing a plausible mechanistic explanation for the basis of PPARδ function in muscle [85]. Remarkably, constitutive activity of PPARδ in muscle protected the mice from HFD-induced adipocyte hypertrophy, obesity, and IR, demonstrating the major influence of PPARδ-induced energy dissipation in muscle on systemic energy homeostasis [88]. In full agreement with these findings, muscle-specific *Ppard* deficiency resulted in obesity, adipocyte hypertrophy, and insulin resistance [85]. Moreover, the basal respiratory quotient and glucose tolerance of whole-body *Ppard*-null mice are significantly reduced in the absence of additional dietary or genetic challenges [90]. Combined, these observations indicate that enhancement of basal metabolism by PPARδ in general, and in muscle in particular, are critical for systemic energy homeostasis, and play a pivotal role in curbing obesity and its metabolic sequelae.

In addition to the gain and loss-of-function studies in skeletal muscle, loss-of-function studies revealed a critical requirement for PPARδ also in cardiac muscle. Cardiomyocyte-specific *Ppard*-null mice (floxed *Ppard x MHC-Cre*) exhibited reduced expression of genes regulating FA oxidation, accompanied by progressive cardiac lipid accumulation, cardiac hypertrophy, and dilated cardiomyopathy [91]. The mice develop typical symptoms of congestive heart failure and died within the first 10 months of life, demonstrating the vital importance of PPARδ for myocardial FA oxidation and function [91]. Considering that mice carrying germ-line *Ppard* deficiency reach old age without major incident [81, 85], the harsher phenotype of mice that lack this PPAR only in the heart requires explanation. In addition, as PPARα induces similar pathways of cardiac FA oxidation and protection from lipotoxicity (see above), it will be crucial to determine how these differ from those regulated by PPARδ, and why neither PPAR compensates for the deficiency of the other.

3.3. Genetic manipulations of PPARδ and atherosclerosis

The abundant expression of PPARδ in macrophages provided a compelling rationale to study its contribution to macrophage biology and atherosclerosis. Comparative studies of *wt* versus *Ppard*-null embryonic stem cell-derived macrophages identified very low-density lipoprotein (VLDL) as a rich source of PPARδ agonists and the gene for the lipid droplet-associated ADRP protein as a tightly regulated PPARδ target gene [82]. Combined with the observed

increases in hepatic VLDL production, circulating VLDL levels, and VLDL-associated TG in *Ppard*-null mice [86], this functional interaction suggested that PPARδ is engaged in negative feedback regulation of systemic VLDL flux. While these studies provide circumstantial support for the potential role of PPARδ in macrophage lipid metabolism, subsequent studies found no effect of PPARδ deletion or activation on cholesterol flux in macrophages [92]. In contrast, deletion of the *Ppard* gene reduced the expression of proinflammatory genes in macrophages, as did treatment with PPARδ agonists [92]. The similar effects of PPARδ deficiency and activation invoke a mechanism, in which the association of unliganded PPARδ with transcriptional corepressors promotes inflammation, which can be relieved by either ligand-mediated derepression or an outright gene KO. Most importantly, these activities have a measurable impact on atherosclerosis, and transplantation of *Ppard*-null bone marrow markedly suppressed atherosclerosis in LDL-receptor KO mice [92]. Thus, basal PPARδ activity in macrophages augments the pathogenesis of atherosclerosis, and PPARδ ligands may exert therapeutic effects by reversing, rather than enhancing, this pathogenic activity.

4. SUMMARY AND PROSPECTS

This review summarized the insights obtained into the functions of PPARs in obesity and metabolic disease through genetic manipulation of mice. For focus purposes, we excluded many of the studies that provided seminal insights into the in vivo functions of PPARs through the use of pharmacological agents; this information is available in other reviews in this volume and elsewhere.

It is evident from the studies reviewed that deficiencies or unscheduled expression of PPARα, PPARγ, and PPARδ impact multiple tissues and vital metabolic processes, and that despite their substantial homology and evidence of shared transcriptional targets, the physiological functions of each are unique. These observations are compiled in Figure 1.

Some of the conclusions that emerge from these studies are consistent and irrefutable, such as the critical role of PPARα in the fasting response, the indispensability of PPARγ for adipocyte differentiation and survival, or the role of muscle PPARδ in fiber type determination and basal oxidative metabolism. Other conclusions are solid, but could be refined and extended by further studies; examples include the antiatherosclerotic functions of PPARγ. However, many studies report data and conclusions that seem either overstated or in conflict altogether with other studies. Nevertheless, in case of studies in the latter category we tried our best to summarize the data as published, point out major discrepancies, and where possible, provide plausible explanations for disparities between reports, while leaving it to the readers to formulate their own judgment. Still, the text is likely permeated with some of our own biases, formed through informal discussions with other researchers, familiarity with the evolution of some of the concepts and hypotheses, and our own unpublished work.

As pointed out throughout this review, inconsistencies or erroneous data could readily arise from minor imperfections in the targeting strategy, inappropriate heterogeneity of the genetic background, differences in husbandry, feeding regimens and experimental protocols, and, last but not least, human error. Although these issues need to be ironed out in the long term, one may take the philosophical stance that hard-to-reproduce results are too minor to be biologically significant. This leaves us with the larger, yet-to-be-answered questions that should be addressed by genetic manipulations of PPARs in the near future.

Currently one of the biggest questions concerning PPARα is the therapeutic promise of fibrate drugs and derivatives, which have been all but neglected in recent years. Considering the unique functions of PPARα in lipid clearance and the fasting response, are there adverse metabolic conditions for which the potential of its agonists to provide an ideal treatment has been overlooked? The combined effects of *Ppara* KO and agonists on animal models of various diseases that entail altered lipid homeostasis should provide answers to this question.

For PPARγ, several mysteries beg resolution, none more important than its connection to insulin sensitization, which has thus far eluded definitive proof. The following are three examples for the many potential approaches that could be employed to address this topical issue. First, beyond its importance for adipogenesis and adipocyte viability, is adipocyte PPARγ a major player in systemic metabolism? Can we gain a molecular understanding of the death mechanism of *Pparg*-null adipocytes and use it to delete *Pparg* in these cells while averting their death? Assuming that we can devise such methods to obtain mice with viable *Pparg*-null adipocytes, what would their metabolic phenotype be? Second, we should continue to explore the contributions of PPARγ to metabolic homeostasis through its actions in additional tissues. Considering the critical role of central regulation in energy homeostasis, one glaringly neglected hypothesis is that PPARγ may also function centrally; this idea could be tested by tissue-specific *Pparg* KOs in the CNS and hypothalamic neurons. Third, we do not yet understand the mechanisms of insulin sensitization by *Pparg* haploinsufficiency. Additional in vivo experiments are required to identify the culprit tissue(s) and the target genes whose deregulation underlies this phenomenon.

PPARδ research has been lagging behind that of PPARα and PPARγ, and new findings are starting to trickle from multiple tissue-specific *Ppard* KOs. The immediate significant questions revolve around the detailed mechanistic understanding of PPARδ action in lipid and oxidative metabolism and in inflammation. Considering that *Ppard*-null mice surviving gestation are by and large healthy under standard husbandry, how important are these functions for basal health? And when these functions come into play under metabolic stress, how can they be modulated for the best possible treatment of metabolic diseases? As pointed out above, combination studies of pharmacological agonists and genetically manipulated animals will bring us several steps closer to answering these questions.

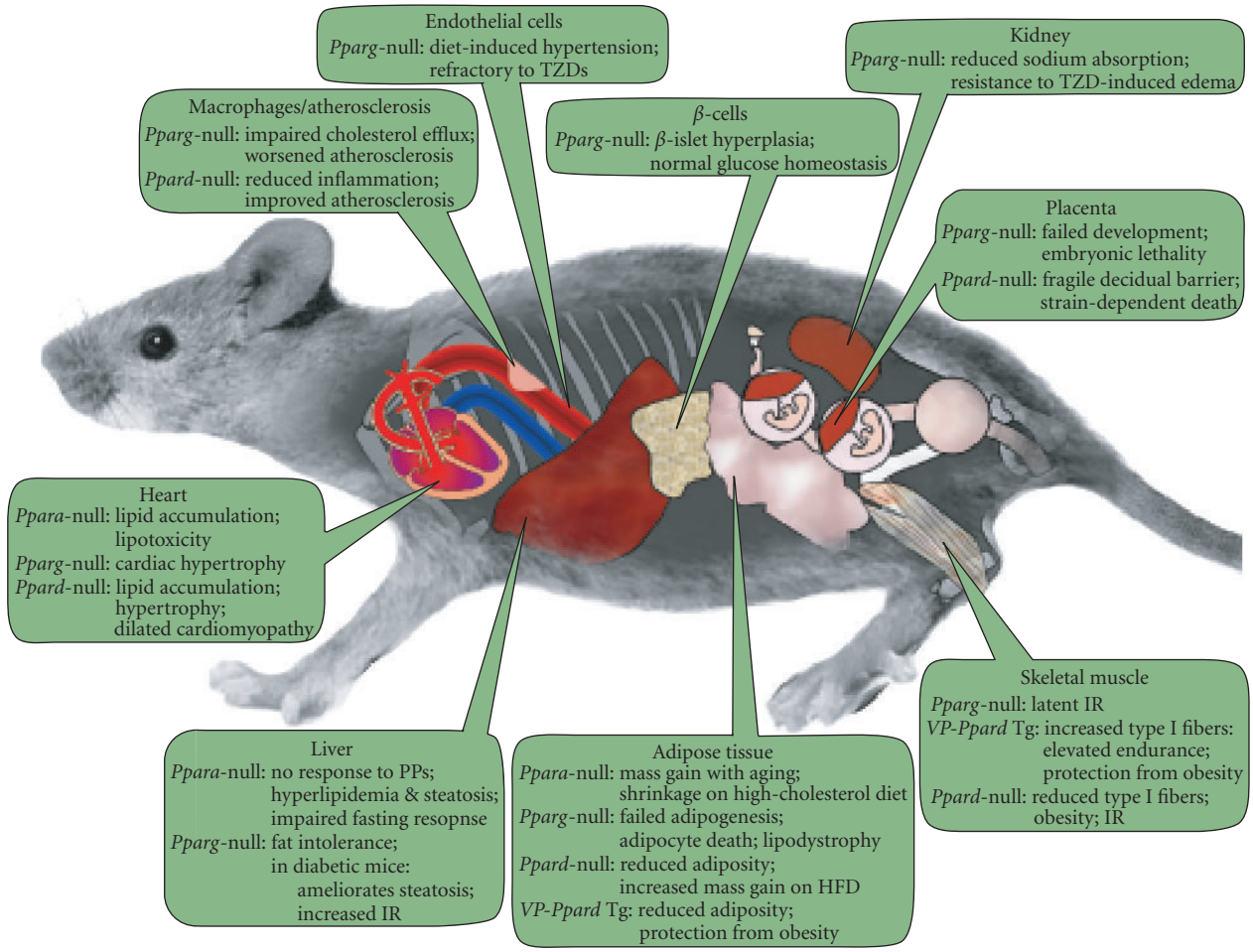


Figure 1: *Genetic manipulations of PPARs—compilation of metabolic phenotypes*. The scheme synthesizes observations from both whole-body and tissue-specific KOs.

ACKNOWLEDGMENTS

The authors wish to thank Stephen Sampson for critical reading and comments on the manuscript, Jesse Hammer for expert graphics, and Pat Cherry for administrative assistance. Y. B. is supported in part by NIH Grant HD044103 and by a grant from The March of Dimes. S. K. is a recipient of an AHA Northeast Affiliate Postdoctoral Fellowship.

REFERENCES

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[3] S. S.-T. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[4] T. Aoyama, J. M. Peters, N. Iritani, et al., "Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα)," *Journal of Biological Chemistry*, vol. 273, no. 10, pp. 5678–5684, 1998.

[5] J. M. Peters, N. Hennuyer, B. Staels, et al., "Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor α-deficient mice," *Journal of Biological Chemistry*, vol. 272, no. 43, pp. 27307–27312, 1997.

[6] P. Costet, C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau, "Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis," *Journal of Biological Chemistry*, vol. 273, no. 45, pp. 29577–29585, 1998.

[7] M. E. Poynter and R. A. Daynes, "Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-κB signaling, and reduces inflammatory cytokine production in aging," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32833–32841, 1998.

[8] T. E. Akiyama, C. J. Nicol, C. Fievet, et al., "Peroxisome proliferator-activated receptor-α regulates lipid homeostasis,

but is not associated with obesity. Studies with congenic mouse lines," *Journal of Biological Chemistry*, vol. 276, no. 42, pp. 39088–39093, 2001.

[9] Y. Lee, X. Yu, F. Gonzales, et al., "PPARα is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11848–11853, 2002.

[10] T. C. Leone, C. J. Weinheimer, and D. P. Kelly, "A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: the PPARα-null mouse as a model of fatty acid oxidation disorders," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 13, pp. 7473–7478, 1999.

[11] S. Kersten, J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1489–1498, 1999.

[12] M. C. Sugden, K. Bulmer, G. F. Gibbons, B. L. Knight, and M. J. Holness, "Peroxisome-proliferator-activated receptor-α (PPARα) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin," *Biochemical Journal*, vol. 364, no. 2, pp. 361–368, 2002.

[13] S. S.-T. Lee, W.-Y. Chan, C. K. C. Lo, D. C. C. Wan, D. S. C. Tsang, and W.-T. Cheung, "Requirement of PPARα in maintaining phospholipid and triacylglycerol homeostasis during energy deprivation," *Journal of Lipid Research*, vol. 45, no. 11, pp. 2025–2037, 2004.

[14] J. Xu, G. Xiao, C. Tirujillo, et al., "Peroxisome proliferator-activated receptor α (PPARα) influences substrate utilization for hepatic glucose production," *Journal of Biological Chemistry*, vol. 277, no. 52, pp. 50237–50244, 2002.

[15] R. H. J. Bandsma, T. H. van Dijk, A. ter Harmsel, et al., "Hepatic *de novo* synthesis of glucose-6-phosphate is not affected in PPARα-deficient mice but is preferentially directed towards hepatic glycogen stores after a short-term fast," *Journal of Biological Chemistry*, vol. 279, no. 10, pp. 8930–8937, 2004.

[16] J. M. Huss and D. P. Kelly, "Nuclear receptor signaling and cardiac energetics," *Circulation Research*, vol. 95, no. 6, pp. 568–578, 2004.

[17] K. Watanabe, H. Fujii, T. Takahashi, et al., "Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor α associated with age-dependent cardiac toxicity," *Journal of Biological Chemistry*, vol. 275, no. 29, pp. 22293–22299, 2000.

[18] F. Djouadi, C. J. Weinheimer, J. E. Saffitz, et al., "A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor α-deficient mice," *Journal of Clinical Investigation*, vol. 102, no. 6, pp. 1083–1091, 1998.

[19] B. N. Finck, J. J. Lehman, T. C. Leone, et al., "The cardiac phenotype induced by PPARα overexpression mimics that caused by diabetes mellitus," *Journal of Clinical Investigation*, vol. 109, no. 1, pp. 121–130, 2002.

[20] M. Guerre-Millo, C. Rouault, P. Poulain, et al., "PPAR-α-null mice are protected from high-fat diet-induced insulin resistance," *Diabetes*, vol. 50, no. 12, pp. 2809–2814, 2001.

[21] C. Bernal-Mizrachi, S. Weng, C. Feng, et al., "Dexamethasone induction of hypertension and diabetes is PPAR-α dependent in LDL receptor-null mice," *Nature Medicine*, vol. 9, no. 8, pp. 1069–1075, 2003.

[22] D. Patsouris, J. K. Reddy, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression," *Endocrinology*, vol. 147, no. 3, pp. 1508–1516, 2006.

[23] M. Haluzik, O. Gavrilova, and D. LeRoith, "Peroxisome proliferator-activated receptor-α deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies," *Endocrinology*, vol. 145, no. 4, pp. 1662–1667, 2004.

[24] K. K. Islam, B. L. Knight, K. N. Frayn, D. D. Patel, and G. F. Gibbons, "Deficiency of PPARα disturbs the response of lipogenic flux and of lipogenic and cholesterogenic gene expression to dietary cholesterol in mouse white adipose tissue," *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, vol. 1734, no. 3, pp. 259–268, 2005.

[25] D. M. Muoio, P. S. MacLean, D. B. Lang, et al., "Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice. Evidence for compensatory regulation by PPARδ," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26089–26097, 2002.

[26] B. N. Finck, C. Bernal-Mizrachi, D. H. Han, et al., "A potential link between muscle peroxisome proliferator-activated receptor-α signaling and obesity-related diabetes," *Cell Metabolism*, vol. 1, no. 2, pp. 133–144, 2005.

[27] C. Knauf, J. Rieusset, M. Foretz, et al., "Peroxisome proliferator-activated receptor-α-null mice have increased white adipose tissue glucose utilization, GLUT4, and fat mass: role in liver and brain," *Endocrinology*, vol. 147, no. 9, pp. 4067–4078, 2006.

[28] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[29] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[30] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[31] E. D. Rosen, P. Sarraf, A. E. Troy, et al., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[32] W. He, Y. Barak, A. Hevener, et al., "Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15712–15717, 2003.

[33] T. Imai, R. Takakuwa, S. Marchand, et al., "Peroxisome proliferator-activated receptor γ is required in mature white and brown adipocytes for their survival in the mouse," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 13, pp. 4543–4547, 2004.

[34] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[35] J. M. Olefsky and A. R. Saltiel, "PPARγ and the treatment of insulin resistance," *Trends in Endocrinology and Metabolism*, vol. 11, no. 9, pp. 362–368, 2000.

[36] J. Plutzky, "The potential role of peroxisome proliferator-activated receptors on inflammation in type 2 diabetes mellitus and atherosclerosis," *The American Journal of Cardiology*, vol. 92, no. 4, supplement 1, pp. 34–41, 2003.

[37] M. D. Tallquist and P. Soriano, "Epiblast-restricted Cre expression in MORE mice: a tool to distinguish embryonic vs. extra-embryonic gene function," *Genesis*, vol. 26, no. 2, pp. 113–115, 2000.

[38] S. Hayashi, P. Lewis, L. Pevny, and A. P. McMahon, "Efficient gene modulation in mouse epiblast using a *Sox2Cre* transgenic mouse strain," *Mechanisms of Development*, vol. 119, supplement 1, pp. S97–S101, 2002.

[39] N. Kubota, Y. Terauchi, H. Miki, et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[40] P. D. G. Miles, Y. Barak, W. He, R. M. Evans, and J. M. Olefsky, "Improved insulin-sensitivity in mice heterozygous for PPAR-γ deficiency," *Journal of Clinical Investigation*, vol. 105, no. 3, pp. 287–292, 2000.

[41] P. D. G. Miles, Y. Barak, R. M. Evans, and J. M. Olefsky, "Effect of heterozygous PPARγ deficiency and TZD treatment on insulin resistance associated with age and high-fat feeding," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 284, no. 3, pp. E618–E626, 2003.

[42] T. Yamauchi, H. Waki, J. Kamon, et al., "Inhibition of RXR and PPARγ ameliorates diet-induced obesity and type 2 diabetes," *Journal of Clinical Investigation*, vol. 108, no. 7, pp. 1001–1013, 2001.

[43] T. Yamauchi, J. Kamon, H. Waki, et al., "The mechanisms by which both heterozygous peroxisome proliferator-activated receptor γ (PPARγ) deficiency and PPARγ agonist improve insulin resistance," *Journal of Biological Chemistry*, vol. 276, no. 44, pp. 41245–41254, 2001.

[44] T. E. Akiyama, S. Sakai, G. Lambert, et al., "Conditional disruption of the peroxisome proliferator-activated receptor γ gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux," *Molecular and Cellular Biology*, vol. 22, no. 8, pp. 2607–2619, 2002.

[45] J. R. Jones, C. Barrick, K.-A. Kim, et al., "Deletion of PPARγ in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 17, pp. 6207–6212, 2005.

[46] U. B. Pajvani, M. E. Trujillo, T. P. Combs, et al., "Fat apoptosis through targeted activation of caspase 8: a new mouse model of inducible and reversible lipoatrophy," *Nature Medicine*, vol. 11, no. 7, pp. 797–803, 2005.

[47] A. W. Norris, L. Chen, S. J. Fisher, et al., "Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones," *Journal of Clinical Investigation*, vol. 112, no. 4, pp. 608–618, 2003.

[48] A. L. Hevener, W. He, Y. Barak, et al., "Muscle-specific Pparg deletion causes insulin resistance," *Nature Medicine*, vol. 9, no. 12, pp. 1491–1497, 2003.

[49] K. Matsusue, M. Haluzik, G. Lambert, et al., "Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes," *Journal of Clinical Investigation*, vol. 111, no. 5, pp. 737–747, 2003.

[50] O. Gavrilova, M. Haluzik, K. Matsusue, et al., "Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass," *Journal of Biological Chemistry*, vol. 278, no. 36, pp. 34268–34276, 2003.

[51] E. D. Rosen, R. N. Kulkarni, P. Sarraf, et al., "Targeted elimination of peroxisome proliferator-activated receptor γ in β cells leads to abnormalities in islet mass without compromising glucose homeostasis," *Molecular and Cellular Biology*, vol. 23, no. 20, pp. 7222–7229, 2003.

[52] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[53] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[54] A. C. Li, K. K. Brown, M. J. Silvestre, T. M. Willson, W. Palinski, and C. K. Glass, "Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice," *Journal of Clinical Investigation*, vol. 106, no. 4, pp. 523–531, 2000.

[55] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans, "PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation," *Nature Medicine*, vol. 7, no. 1, pp. 48–52, 2001.

[56] A. Chawla, W. A. Boisvert, C.-H. Lee, et al., "A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis," *Molecular Cell*, vol. 7, no. 1, pp. 161–171, 2001.

[57] V. R. Babaev, P. G. Yancey, S. V. Ryzhov, et al., "Conditional knockout of macrophage PPARγ increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 8, pp. 1647–1653, 2005.

[58] G. Pascual, M. Ricote, A. Hevener, et al., "Anti-inflammatory and antidiabetic roles of PPARγ in macrophages," in *Proceedings of Keystone Symposia: Adipogenesis, Obesity and Inflammation*, Vancouver, British Columbia, Canada, January 2006, 004a.

[59] N. K. Hollenberg, "Considerations for management of fluid dynamic issues associated with thiazolidinediones," *American Journal of Medicine*, vol. 115, no. 8, supplement 1, pp. 111–115, 2003.

[60] H. Zhang, A. Zhang, D. E. Kohan, R. D. Nelson, F. J. Gonzalez, and T. Yang, "Collecting duct-specific deletion of peroxisome proliferator-activated receptor γ blocks thiazolidinedione-induced fluid retention," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 26, pp. 9406–9411, 2005.

[61] Y. Guan, C. Hao, D. R. Cha, et al., "Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption," *Nature Medicine*, vol. 11, no. 8, pp. 861–866, 2005.

[62] C. J. Nicol, M. Adachi, T. E. Akiyama, and F. J. Gonzalez, "PPARγ in endothelial cells influences high fat diet-induced hypertension," *American Journal of Hypertension*, vol. 18, no. 4, pp. 549–556, 2005.

[63] S. Z. Duan, C. Y. Ivashchenko, M. W. Russell, D. S. Milstone, and R. M. Mortensen, "Cardiomyocyte-specffic knockout and agonist of peroxisome proliferator-activated receptor-γ both induce cardiac hypertrophy in mice," *Circulation Research*, vol. 97, no. 4, pp. 372–379, 2005.

[64] S. A. Kliewer, B. M. Forman, B. Blumberg, et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[65] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an

adipocyte enhancer," *Genes and Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[66] J. Zhang, M. Fu, T. Cui, et al., "Selective disruption of PPARγ2 impairs the development of adipose tissue and insulin sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 29, pp. 10703–10708, 2004.

[67] G. Medina-Gomez, S. Virtue, C. Lelliott, et al., "The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-γ2 isoform," *Diabetes*, vol. 54, no. 6, pp. 1706–1716, 2005.

[68] H. Koutnikova, T.-A. Cock, M. Watanabe, et al., "Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPARγ hypomorphic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 24, pp. 14457–14462, 2003.

[69] I. Barroso, M. Gurnell, V. E. F. Crowley, et al., "Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension," *Nature*, vol. 402, no. 6764, pp. 880–883, 1999.

[70] A. K. Agarwal and A. Garg, "A novel heterozygous mutation in peroxisome proliferator-activated receptor-γ gene in a patient with familial partial lipodystrophy," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 1, pp. 408–411, 2002.

[71] R. A. Hegele, H. Cao, C. Frankowski, S. T. Mathews, and T. Leff, "PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy," *Diabetes*, vol. 51, no. 12, pp. 3586–3590, 2002.

[72] D. B. Savage, M. Agostini, I. Barroso, et al., "Digenic inheritance of severe insulin resistance in a human pedigree," *Nature Genetics*, vol. 31, no. 4, pp. 379–384, 2002.

[73] M. Gurnell, D. B. Savage, V. K. K. Chatterjee, and S. O'Rahilly, "The metabolic syndrome: peroxisome proliferator-activated receptor γ and its therapeutic modulation," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 6, pp. 2412–2421, 2003.

[74] Y.-S. Tsai, H.-J. Kim, N. Takahashi, et al., "Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARγ," *Journal of Clinical Investigation*, vol. 114, no. 2, pp. 240–249, 2004.

[75] B. D. Freedman, E.-J. Lee, Y. Park, and J. L. Jameson, "A dominant negative peroxisome proliferator-activated receptor-γ knock-in mouse exhibits features of the metabolic syndrome," *Journal of Biological Chemistry*, vol. 280, no. 17, pp. 17118–17125, 2005.

[76] E. Hu, J. B. Kim, P. Sarraf, and B. M. Spiegelman, "Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ," *Science*, vol. 274, no. 5295, pp. 2100–2103, 1996.

[77] M. Ristow, D. Muller-Wieland, A. Pfeiffer, W. Krone, and C. R. Kahn, "Obesity associated with a mutation in a genetic regulator of adipocyte differentiation," *New England Journal of Medicine*, vol. 339, no. 14, pp. 953–959, 1998.

[78] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[79] D. Altshuler, J. N. Hirschhorn, M. Klannemark, et al., "The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes," *Nature Genetics*, vol. 26, no. 1, pp. 76–80, 2000.

[80] S. M. Rangwala, B. Rhoades, J. S. Shapiro, et al., "Genetic modulation of PPARγ phosphorylation regulates insulin sensitivity," *Developmental Cell*, vol. 5, no. 4, pp. 657–663, 2003.

[81] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[82] A. Chawla, C.-H. Lee, Y. Barak, et al., "PPARδ is a very low-density lipoprotein sensor in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 3, pp. 1268–1273, 2003.

[83] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[84] J. M. Peters, S. S.-T. Lee, W. Li, et al., "Growths, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[85] M. Schuler, F. Ali, C. Chambon, et al., "PGC1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes," *Cell Metabolism*, vol. 4, no. 5, pp. 407–414, 2006.

[86] T. E. Akiyama, G. Lambert, C. J. Nicol, et al., "Peroxisome proliferator-activated receptor β/δ regulates very low density lipoprotein production and catabolism in mice on a Western diet," *Journal of Biological Chemistry*, vol. 279, no. 20, pp. 20874–20881, 2004.

[87] Y.-X. Wang, C.-H. Lee, S. Tiep, et al., "Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity," *Cell*, vol. 113, no. 2, pp. 159–170, 2003.

[88] Y.-X. Wang, C.-L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[89] S. Luquet, J. Lopez-Soriano, D. Holst, et al., "Peroxisome proliferator-activated receptor δ controls muscle development and oxidative capability," *FASEB Journal*, vol. 17, no. 15, pp. 2299–2301, 2003.

[90] C.-H. Lee, P. Olson, A. Hevener, et al., "PPARδ regulates glucose metabolism and insulin sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 9, pp. 3444–3449, 2006.

[91] L. Cheng, G. Ding, Q. Qin, et al., "Cardiomyocyte-restricted peroxisome proliferator-activated receptor-δ deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy," *Nature Medicine*, vol. 10, no. 11, pp. 1245–1250, 2004.

[92] C.-H. Lee, A. Chawla, N. Urbiztondo, et al., "Transcriptional repression of atherogenic inflammation: modulation by PPARδ," *Science*, vol. 302, no. 5644, pp. 453–457, 2003.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 86394, 6 pages
doi:10.1155/2007/86394

Review Article

Metabolic Functions of Peroxisome Proliferator-Activated Receptor β/δ in Skeletal Muscle

Céline Gaudel and Paul A. Grimaldi

INSERM U636, Centre de Biochimie, UFR Sciences, Université de Nice Sophia Antipolis, Parc Valrose, 06108 Nice, France

Received 13 September 2006; Revised 16 November 2006; Accepted 21 November 2006

Recommended by Wallace Harrington

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that act as lipid sensors and adapt the metabolic rates of various tissues to the concentration of dietary lipids. PPARs are pharmacological targets for the treatment of metabolic disorders. PPARα and PPARγ are activated by hypolipidemic and insulin-sensitizer compounds, such as fibrates and thiazolidinediones. The roles of PPARβ/δ in metabolic regulations remained unclear until recently. Treatment of obese monkeys and rodents by specific PPARβ/δ agonists promoted normalization of metabolic parameters and reduction of adiposity. Recent evidences strongly suggested that some of these beneficial actions are related to activation of fatty acid catabolism in skeletal muscle and also that PPARβ/δ is involved in the adaptive responses of skeletal muscle to environmental changes, such as long-term fasting or physical exercise, by controlling the number of oxidative myofibers. These observations indicated that PPARβ/δ agonists might have therapeutic usefulness in metabolic syndrome by increasing fatty acid consumption in skeletal muscle and reducing obesity.

1. INTRODUCTION

The prevalence of adult obesity and obesity-associated metabolic disorders, including insulin resistance, glucose intolerance, hypertension, and dyslipidemia, has reached epidemic proportions in industrialized countries. The causes of the increase of this cluster of pathologies, known as the metabolic syndrome, are multiple and not totally elucidated. However, it is accepted that environmental factors, such as excess of food intake and lack of physical exercise, that characterize western lifestyle and lead to lipid homeostasis imbalance, are major contributors in the development of these pathologies. Lipid homeostasis requires a strict equilibrium between lipid availability and lipid consumption. In the normal situation, fatty acids coming either from food or from hepatic lipogenesis are utilized as energetic substrates in heart and skeletal muscles. Adipose tissue plays a central role in lipid homeostasis and can manage a transient increase in lipid availability by increasing the amount of stored triacylglycerol. However, long-term excess of dietary lipids and/or decrease of energy expenditure create a profound disturbance in this physiological equilibrium leading to a permanent increase in fatty acid availability and, on a long-term basis, to accumulation of triacylglycerol and other lipids in various tissues, such as adipose, liver, pancreas, and skeletal muscle. Such a lipid deposition leads to impairment of insulin responsiveness and metabolic dysfunction [1]. During the last decade, it has been demonstrated that adipocyte hypertrophy, a typical hallmark of adult obesity, results in a profound alteration of adipokine production and impairs the normal crosstalk between adipose tissue and the other organs increasing the metabolic disorders [2]. Several evidences clearly indicated that reducing lipid contents in blood and insulin-sensitive tissues is a crucial challenge to prevent metabolic syndrome. To reach this goal, lifestyle intervention has been shown to be an efficient strategy. For instance, weight loss, leading to a normalization of adipocyte size and adipokine secretion, and recurring physical exercise, promoting increment of energy expenditure in skeletal muscle and heart, have strong beneficial effects on insulin resistance and type 2 diabetes in human [3]. Because changing western lifestyle is very doubtful, pharmaceutical approaches mimicking the metabolic actions of weight loss and/or physical exercise should be of great interest. During the last 15 years, our knowledge of the molecular basis of lipid homeostasis regulation has been considerably improved and numerous

studies have particularly demonstrated the roles of the peroxisome proliferator-activated receptors (PPARs) in the control of lipid metabolism, providing new ideas about the pharmacological treatment of metabolic syndrome.

2. PPARs: LIPID-ACTIVATED TRANSCRIPTION FACTORS AND REGULATORS OF LIPID METABOLISM

PPARs are ligand-activated transcription factors that belong to the nuclear receptor superfamily and play multiple physiological roles in several tissues. Three PPAR isotypes, α (NR1C1), β/δ (NR1C2), and γ (NR1C3), have been described so far. Each of the PPAR isotypes is encoded in a separate gene and exhibits tissue-selective expression patterns. PPARα is mainly expressed in liver, heart, kidney, small intestine, and brown adipose tissue [4]. Several forms of PPARγ have been identified with distinct expression patterns. PPARγ2 is almost exclusively found in white and brown adipose tissues, while PPARγ1 is expressed in several other tissues and cell types including intestine, placenta, and macrophages [5]. PPARβ/δ has a broad expression pattern in adult mammals, but it is abundantly expressed in small intestine, skeletal and cardiac muscles, brain, and adipose tissue [6, 7].

PPARs are organized in different domains. The amino-terminal domain is poorly conserved between the three isotypes and contains a ligand-independent transactivation function. The central domain, which is highly conserved, brings the capacity of DNA binding. The carboxyl-terminal region contains the ligand-binding domain and confers the ligand-dependent transactivation function. X-ray crystal structure analyses have revealed some important differences in the ligand-binding pocket of PPAR isotypes [8, 9]. These differences explain why PPAR isotypes can bind a large diversity of molecules and also display a relative selectivity for both natural and synthetic ligands.

PPARs heterodimerize with the retinoid X receptor (RXR, NR2B) and bind to a specific DNA responsive element, called peroxisome proliferator response element (PPRE), found in a large number of genes encoding proteins involved in a variety of functions, including lipid and carbohydrate metabolisms, inflammation, cell proliferation, and differentiation [10, 11].

An important mark of PPAR transcriptional regulation is the interaction with cofactors. The unliganded PPAR/RXR heterodimer interacts with corepressors that exert transcriptional repression. It has been proposed that binding of the ligand promotes a conformational change that is permissive for interactions with coactivator proteins allowing nucleosome remodeling and activation of the transcription of target genes [8, 12]. Several corepressors and coactivators able to interact in a selective manner with the various PPAR isotypes have been described. Some of these cofactors are expressed in a tissue-specific manner and are controlled by physiological status in a given tissue. This selectivity of interaction could explain the differential tissue-specific transcriptional activities of the various PPARs and the activity level of a specific isotype depending upon the expression level of the cofactors in a given tissue or physiological situation.

It is now established that PPARs are lipid sensors and adapt the metabolic rates of various tissues to the concentration of dietary lipids. This role is related to the capacity of the various PPAR isotypes to bind fatty acids and fatty acid derivatives and to regulate the expression of several genes implicated in fatty acid uptake, handling, and metabolism in various tissues. Long-chain fatty acids, either saturated or unsaturated, appeared almost equally active for the three PPAR isotypes and, interestingly, the metabolism of the fatty acid is not required, as 2-bromopalmitate, a nonmetabolized fatty acid, appeared to be a potent PPAR agonist in preadipose cells [13].

Several fatty acid derivatives have been shown to be PPAR agonists. These molecules appeared to be more selective for the PPAR isotypes than fatty acids. For instance, the 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) is a selective PPARγ agonist [14], leukotriene B4 and oleylethanolamide are activating selectively the α isotype [15, 16], and the prostacyclin is more active on PPARβ/δ than on the other isotypes [17]. However, as it is not possible to estimate the actual concentrations of fatty acids and fatty acid derivatives within the nuclear compartment, the physiological implication of these molecules as endogenous PPAR ligands remains an open question.

Due to their potential therapeutic interest for the treatment of metabolic disorders, several classes of PPAR synthetic ligands have been developed. Fibrates, used from several years as hypolididemic compounds, are specific ligands/activators of PPARα [4]. Lipid lowering action of fibrates is mainly due to their capacity to upregulate, through PPARα activation, several genes involved in hepatic fatty acid oxidation mimicking the effects of fasting that increases PPARα expression in liver [18].

Thiazolidinediones [19] that are potent and specific activators of the γ isotype are used as insulin sensitizers. This action is paradoxically related to the adipogenic action of PPARγ. It has been shown that thiazolidinediones promote a remodeling of adipose tissue by the recruitment of new and metabolically active adipocytes. These new adipocytes have beneficial effects by increasing the storage capacity of fatty acids and by normalizing adipokine secretion [20].

More recently, compounds able to specifically bind and activate PPARβ/δ have been developed and it has been shown that such compounds have beneficial metabolic effects in obese animals [21, 22]. The availability of these potent and specific agonists and the construction of appropriate cellular and animal models revealed the important roles of this PPAR isotype in lipid metabolism, especially in skeletal muscle, and pointed out the nuclear receptor as a potential target for the pharmacological treatment of metabolic syndrome.

Many studies revealed that PPARβ/δ agonists could be effective compounds to normalize several biological parameters perturbed during metabolic syndrome. Some of these studies were conducted by using the GW1516 compound that activates PPARβ/δ at very low concentrations both in vitro and in vivo with a 1000-fold selectivity over the other PPAR isotypes [23]. An interesting study by Oliver et al. has

evidenced the beneficial actions of GW1516 administration in insulin-resistant obese monkeys [21]. Indeed, a 4-week treatment with the PPARβ/δ agonist increased high-density lipoprotein cholesterol, decreased low-density lipoprotein cholesterol, reduced the levels of small and dense low-density lipoproteins, and normalized insulin and triglyceride blood levels. Moreover, it was reported that the same molecule reduced adiposity and improved insulin responsiveness in diet-induced and genetically obese mice [22, 24].

The mechanisms involved in these beneficial actions of PPARβ/δ agonist administration to obese animals are not completely elucidated and, as the nuclear receptor is broadly expressed, it is likely that these actions are involving several tissues. However, during the last past years, several experimental evidences coming from both cell culture and in vivo studies have indicated that PPARβ/δ plays a central role in the regulation of lipid metabolism and adaptive development in skeletal muscle and that responses of this tissue could explain some of the antidiabetic and lipid-lowering actions of PPARβ/δ agonists in obese animals.

3. PPARβ/δ: REGULATORY ROLES IN MUSCLE METABOLISM AND PHYSIOLOGY

PPARβ/δ is several-fold more abundant than the other PPAR isotypes in rodent and human muscles [25]. Moreover, we have shown that long-term fasting [26] and endurance training [27], two physiological situations characterized by an increase in muscle fatty acid catabolism, increased PPARβ/δ mRNA and protein contents in mouse skeletal muscle. A similar PPARβ/δ upregulation was observed in human muscle after either long-term or short-term moderate exercise training [28–30].

Skeletal muscle accounts for about 40% of the body mass and, in this tissue, energy expenditure, insulin sensitivity, and fuel preference are highly affected by muscle work and myofiber composition [31, 32]. Depending upon their physiological roles, the different muscles contain variable percentages of specific myofibers that differ in both contractile and metabolic properties. Type 2b myofibers express fast isoforms of contractile proteins and synthesize ATP mainly from anaerobic glycolysis. Type 2a myofibers express fast contractile proteins, but contain more mitochondria, and are able to synthesize ATP from oxidation of glucose and fatty acids. Type 1 myofibers also have an oxidative metabolism and express the slow isoforms of contractile proteins. For instance, soleus muscle, which is implicated in endurance works, contains almost exclusively type 1 and type 2a oxidative myofibers, while the white gastrocnemius contains a majority of type 2b glycolytic myofibers and is implicated in short-term and intense exercise. Importantly, the myofiber composition of a given muscle is not fixed and is modified in some physiological or pathological situations. Endurance training promotes a fiber-type transition in human and rodents. In human muscle, moderate exercise induces a transition from type 2b to type 2a phenotype [33], while a more intense exercise is required for a transition toward type 1 phenotype [34]. Voluntary exercise increases type 2a myofiber percentage in several mouse muscles with or without hyperplasia, that is, increment in total myofiber number [35]. Sedentary life and type 2 diabetes lead to the opposite phenotype with a reduction of oxidative phenotype of various muscles [36, 37].

3.1. PPARβ/δ regulates fatty acid burning in skeletal muscle

Muoio et al. reported that exposure of differentiated human or rat L6 myotubes to a highly selective PPARβ/δ agonist or to a specific PPARα agonist equally increased fatty acid oxidation and induced expression of several lipid regulatory genes, such as uncoupling protein 3 (UCP3), pyruvate dehydrogenase kinase 4 (PDK4), and carnitine palmitoyltransferase 1 (CPT1). These observations suggested a redundancy in the regulatory functions of both PPAR isotypes on fatty acid metabolism in cultured myotubes [38]. To directly establish the implication of PPARβ/δ in the control of lipid metabolism in muscle cells, we conducted gain-of-function and loss-of-function studies by overexpressing either native or dominant negative forms of the nuclear receptor in C2C12 myogenic cells. We showed that exposure of differentiated C2C12 myotubes to 2-bromopalmitate, a non-metabolized fatty acid, or to GW0742, a specific PPARβ/δ agonist, upregulated expression of genes implicated in fatty acid uptake, handling, and metabolism, such as Fatty Acid Translocase (FAT/CD36), heart-Fatty Acid Binding Protein (h-FABP), and CPT1. Furthermore, the direct implication of PPARβ/δ in these regulations was established by the demonstration that the responses were, respectively, enhanced in PPARβ/δ-overexpressing cells and almost completely abolished in cells expressing the dominant negative form of PPARβ/δ [26]. A microarray expression profiling study confirmed these findings and showed that in L6 myotubes, activation of PPARβ/δ upregulated expression of a large panel of genes that control fatty acid transport, β-oxidation, mitochondrial respiration, and energy uncoupling [22]. Interestingly, Dressel et al. demonstrated that the various PPAR isotypes regulated different metabolic pathways in differentiated C2C12 cells. They reported that PPARβ/δ controlled fatty acid catabolism, while PPARα was involved in the control of fructose uptake and glycogen metabolism, and PPARγ controlled expression of genes implicated in glucose uptake and lipid synthesis [39].

Next to these data obtained with cultured myotubes, it was reported that administration of PPARβ/δ agonist upregulated expression of several genes implicated in lipid metabolism and fatty acid catabolism and reduced lipid content in mouse skeletal muscle [22].

The demonstration that PPARβ/δ agonists induced fatty acid burning in muscle, explains, at least partly, the beneficial effects of such treatment in obese animals, as it is well established that fatty acid catabolism is reduced in muscles from diabetic and obese animals and that lipid deposition is leading to insulin resistance, especially in muscle tissues [1, 36, 37]. Moreover, the generation of transgenic models for a muscle-specific overexpression of PPARβ/δ revealed

another important and interesting function of the nuclear receptor in muscle physiology that could be very important for the understanding of the mechanisms implicated in the beneficial effects of PPARβ/δ activation.

3.2. Roles of PPARβ/δ in lipid metabolism and adaptive responses of skeletal muscle

To further investigate the roles of PPARβ/δ in muscle physiology, we have generated an animal model allowing a skeletal muscle-specific overexpression of the nuclear receptor [27]. In such an animal model, the PPARβ/δ protein content was increased by 4- to 6-fold early after birth in all types of myofibers, that is, oxidative and glycolytic, fast- and slow-twitch. Histological analysis revealed that the number of type 2a myofibers, that is, oxidative fast twitch, was increased in muscles from PPARβ/δ-overexpressing animals when compared to their control littermates. In tibialis anterior muscle and, to a lesser extent, in soleus muscle, this remodeling was due to an increase in total myofiber number, with a specific increase of type 2a myofibers, while in other muscles, such as plantaris and EDL, the increase in type 2a myofiber number was only due to conversion of type 2b to type 2a myofibers. These observations were confirmed by the demonstration that PPARβ/δ overexpression led to increased expression of genes implicated in fatty acid catabolism, such as citrate synthase, h-FABP, and UCP-2.

Another group investigated the effects of muscle-specific expression of a constitutively active PPARβ/δ (VP16-PPARβ/δ) mutant form. Such animals displayed a more pronounced phenotype characterized by an increase of slow-twitch myofiber number in all types of muscles, including predominantly fast-twitch muscles [40]. The discrepancy between the two animal models could be due to the fact that the VP16-PPARβ/δ has a strongest transcriptional activity and upregulates expression of genes that are not affected by overexpression of the wild type PPARβ/δ. For instance, PGC-1, which plays a crucial role in conversion of fast-twitch to slow-twitch myofibers [41], is upregulated in muscles from VP16-PPARβ/δ mice [40] but is unchanged in muscles from PPARβ/δ-overexpressing animals [27]. However, it appeared that overexpression of either native or constitutively active PPARβ/δ forms has beneficial metabolic effects in mice by reducing adiposity, lowering lipid contents in several organs, and increasing insulin responsiveness [27, 40].

Collectively, these findings strongly suggested that overexpression and/or activation of PPARβ/δ mimics the actions of physical exercise on muscle remodeling and metabolism, at least in mouse. Several experimental evidences favor the hypothesis that PPARβ/δ plays a central role in adaptive response of skeletal muscle to endurance exercise. Daily moderate swimming exercise promoted PPARβ/δ upregulation in mouse skeletal muscle [27]. This increased expression requires several weeks of training, while it has been reported that in human muscle, a similar change in PPARβ/δ mRNA abundance takes place after shorter exercise period [28]. Moreover, VP16-PPARβ/δ mice display increased resistance to fatigue and running performance than their control littermates [40]. The molecular mechanisms that lead to the increased expression of PPARβ/δ in skeletal muscle during endurance training remain to be elucidated. Similarly, the molecular and cellular events that link the expression and activation levels of PPARβ/δ to myoblast proliferation and oxidative fiber typing remain to be characterized. However, it can be proposed that upregulation of the nuclear receptor is one of the first events leading to changes in the oxidative fiber number, while activation of PPARβ/δ, by natural or synthetic ligands, controls the degree of conversion of fast-twitch to slow-twitch phenotype.

4. CONCLUSIONS

During the few past years, the knowledge of physiological functions of PPARβ/δ has considerably increased and it is now established that specific agonists of the nuclear receptor may have therapeutic usefulness in metabolic syndrome. The actions of PPARβ/δ in skeletal muscle, that is, oxidative myofiber remodeling and increase of fatty acid burning capacity, may explain the beneficial effects of specific agonists on obesity and insulin resistance by limiting substrate availability for lipid synthesis and accumulation in adipose tissue and other insulin sensitive tissues. The muscle remodeling induced by PPARβ/δ activation may also affect the endocrine functions of skeletal muscle. It is now established that physical exercise is increasing fatty acid burning, but it is also changing the secretion level of muscle cytokines, called myokines, that control metabolic responses of other tissues, including adipose tissue [42]. Further studies are required to investigate the regulatory functions of PPARβ/δ activation on myokine production. Future work is also needed to clarify the roles of PPARβ/δ in other tissues that express the nuclear receptor at high levels, such as heart, intestine and brain, in order to prevent any side effects of PPARβ/δ activation.

Very importantly, level of experimental evidence is still restrained to animal models and a direct extrapolation of data obtained with rodent or primate models to the human context is risky as there are great differences in metabolic regulations between species. Clinical trials have been initiated and will provide important data regarding efficiency, tolerance, and safety in human for some PPARβ/δ agonists. The outcome of such clinical trials is eagerly awaited to confirm the regulatory roles of PPARβ/δ in human muscle physiology and metabolism.

ACKNOWLEDGMENTS

The work performed in the author's laboratory is funded by the Institut National de la Santé et de la Recherche Médicale, the Programme National de Recherche sur le Diabète (no. A04074AS) from the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche, and the Programme Cardiovasculaire, Obésité et Diabète from the Agence Nationale de la Recherche (ANR-05-PCOD-012).

REFERENCES

[1] R. H. Unger and L. Orci, "Lipotoxic diseases of nonadipose tissues in obesity," *International Journal of Obesity*, vol. 24, supplement 4, pp. S28–S32, 2000.

[2] M. Fasshauer and R. Paschke, "Regulation of adipocytokines and insulin resistance," *Diabetologia*, vol. 46, no. 12, pp. 1594–1603, 2003.

[3] W. C. Knowler, E. Barrett-Connor, S. E. Fowler, et al., "Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin," *New England Journal of Medicine*, vol. 346, no. 6, pp. 393–403, 2002.

[4] I. Issemann, R. A. Prince, J. D. Tugwood, and S. Green, "The peroxisome proliferator-activated receptor: retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs," *Journal of Molecular Endocrinology*, vol. 11, no. 1, pp. 37–47, 1993.

[5] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes and Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[6] E.-Z. Amri, F. Bonino, G. Ailhaud, N. A. Abumrad, and P. A. Grimaldi, "Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors," *Journal of Biological Chemistry*, vol. 270, no. 5, pp. 2367–2371, 1995.

[7] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[8] R. T. Nolte, G. B. Wisely, S. Westin, et al., "Ligand binding and co-activator assembly of the peroxisome proliferator- activated receptor-γ," *Nature*, vol. 395, no. 6698, pp. 137–143, 1998.

[9] H. E. Xu, M. H. Lambert, V. G. Montana, et al., "Molecular recognition of fatty acids by peroxisome proliferator-activated receptors," *Molecular Cell*, vol. 3, no. 3, pp. 397–403, 1999.

[10] L. Michalik and W. Wahli, "Peroxisome proliferator-activated receptors: three isotypes for a multitude of functions," *Current Opinion in Biotechnology*, vol. 10, no. 6, pp. 564–570, 1999.

[11] P. Tontonoz, E. Hu, J. Devine, E. G. Beale, and B. M. Spiegelman, "PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene," *Molecular and Cellular Biology*, vol. 15, no. 1, pp. 351–357, 1995.

[12] S. Surapureddi, S. Yu, H. Bu, et al., "Identification of a transcriptionally active peroxisome proliferator-activated receptor α-interacting cofactor complex in rat liver and characterization of PRIC285 as a coactivator," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11836–11841, 2002.

[13] P. A. Grimaldi, S. M. Knobel, R. R. Whitesell, and N. A. Abumrad, "Induction of aP2 gene expression by nonmetabolized long-chain fatty acids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 22, pp. 10930–10934, 1992.

[14] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-deoxy-$\Delta^{12,\,14}$–prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[15] P. R. Devchand, H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli, "The PPARα-leukotriene B_4 pathway to inflammation control," *Nature*, vol. 384, no. 6604, pp. 39–43, 1996.

[16] J. Fu, S. Gaetani, F. Oveisi, et al., "Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α," *Nature*, vol. 425, no. 6953, pp. 90–93, 2003.

[17] H. Lim, R. A. Gupta, W.-G. Ma, et al., "Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ," *Genes and Development*, vol. 13, no. 12, pp. 1561–1574, 1999.

[18] T. Lemberger, R. Saladin, M. Vázquez, et al., "Expression of the peroxisome proliferator-activated receptor α gene is stimulated by stress and follows a diurnal rhythm," *Journal of Biological Chemistry*, vol. 271, no. 3, pp. 1764–1769, 1996.

[19] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[20] T. Kadowaki, K. Hara, T. Yamauchi, Y. Terauchi, K. Tobe, and R. Nagai, "Molecular mechanism of insulin resistance and obesity," *Experimental Biology and Medicine*, vol. 228, no. 10, pp. 1111–1117, 2003.

[21] W. R. Oliver Jr., J. L. Shenk, M. R. Snaith, et al., "A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 9, pp. 5306–5311, 2001.

[22] T. Tanaka, J. Yamamoto, S. Iwasaki, et al., "Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15924–15929, 2003.

[23] M. L. Sznaidman, C. D. Haffner, P. R. Maloney, et al., "Novel selective small molecule agonists for peroxisome proliferator-activated receptor δ (PPARδ)—synthesis and biological activity," *Bioorganic and Medicinal Chemistry Letters*, vol. 13, no. 9, pp. 1517–1521, 2003.

[24] Y.-X. Wang, C.-H. Lee, S. Tiep, et al., "Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity," *Cell*, vol. 113, no. 2, pp. 159–170, 2003.

[25] E. Chevillotte, J. Rieusset, M. Roques, M. Desage, and H. Vidal, "The regulation of uncoupling protein-2 gene expression by ω-6 polyunsaturated fatty acids in human skeletal muscle cells involves multiple pathways, including the nuclear receptor peroxisome proliferator-activated receptor β," *Journal of Biological Chemistry*, vol. 276, no. 14, pp. 10853–10860, 2001.

[26] D. Holst, S. Luquet, V. Nogueira, K. Kristiansen, X. Leverve, and P. A. Grimaldi, "Nutritional regulation and role of peroxisome proliferator-activated receptor δ in fatty acid catabolism in skeletal muscle," *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1633, no. 1, pp. 43–50, 2003.

[27] S. Luquet, J. López-Soriano, D. Holst, et al., "Peroxisome proliferator-activated receptor δ controls muscle development and oxidative capability," *The FASEB Journal*, vol. 17, no. 15, pp. 2299–2301, 2003.

[28] M. J. Watt, R. J. Southgate, A. G. Holmes, and M. A. Febbraio, "Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) α and δ and PPAR coactivator 1α in human skeletal muscle, but not lipid regulatory genes," *Journal of Molecular Endocrinology*, vol. 33, no. 2, pp. 533–544, 2004.

[29] D. J. Mahoney, G. Parise, S. Melov, A. Safdar, and M. A. Tarnopolsky, "Analysis of global mRNA expression in human

skeletal muscle during recovery from endurance exercise," *The FASEB Journal*, vol. 19, no. 11, pp. 1498–1500, 2005.

[30] T. Fritz, D. K. Kramer, H. K. R. Karlsson, et al., "Low-intensity exercise increases skeletal muscle protein expression of PPARδ and UCP3 in type 2 diabetic patients," *Diabetes/Metabolism Research and Reviews*, vol. 22, no. 6, pp. 492–498, 2006.

[31] R. A. DeFronzo, R. Gunnarsson, O. Bjorkman, M. Olsson, and J. Wahren, "Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus," *Journal of Clinical Investigation*, vol. 76, no. 1, pp. 149–155, 1985.

[32] L. J. Goodyear and B. B. Kahn, "Exercise, glucose transport, and insulin sensitivity," *Annual Review of Medicine*, vol. 49, pp. 235–261, 1998.

[33] P. Andersen and J. Henriksson, "Training induced changes in the subgroups of human type II skeletal muscle fibres," *Acta Physiologica Scandinavica*, vol. 99, no. 1, pp. 123–125, 1977.

[34] E. Jansson and L. Kaijser, "Muscle adaptation to extreme endurance training in man," *Acta Physiologica Scandinavica*, vol. 100, no. 3, pp. 315–324, 1977.

[35] D. L. Allen, B. C. Harrison, A. Maass, M. L. Bell, W. C. Byrnes, and L. A. Leinwand, "Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse," *Journal of Applied Physiology*, vol. 90, no. 5, pp. 1900–1908, 2001.

[36] J. Mercier, A. Perez-Martin, X. Bigard, and R. Ventura, "Muscle plasticity and metabolism: effects of exercise and chronic diseases," *Molecular Aspects of Medicine*, vol. 20, no. 6, pp. 319–373, 1999.

[37] C. J. Tanner, H. A. Barakat, G. L. Dohm, et al., "Muscle fiber type is associated with obesity and weight loss," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 282, no. 6, pp. E1191–E1196, 2002.

[38] D. M. Muoio, P. S. MacLean, D. B. Lang, et al., "Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice. Evidence for compensatory regulation by PPARδ," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26089–26097, 2002.

[39] U. Dressel, T. L. Allen, J. B. Pippal, P. R. Rohde, P. Lau, and G. E. O. Muscat, "The peroxisome proliferator-activated receptor β/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells," *Molecular Endocrinology*, vol. 17, no. 12, pp. 2477–2493, 2003.

[40] Y.-X. Wang, C.-L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[41] J. Lin, H. Wu, P. T. Tarr, et al., "Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres," *Nature*, vol. 418, no. 6899, pp. 797–801, 2002.

[42] J. López-Soriano, C. Chiellini, M. Maffei, P. A. Grimaldi, and J. M. Argilés, "Roles of skeletal muscle and peroxisome proliferators-activated receptors in the development and treatment of obesity," *Endocrine Reviews*, vol. 27, no. 3, pp. 318–329, 2006.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 74364, 12 pages
doi:10.1155/2007/74364

Review Article

PPARs in the Control of Uncoupling Proteins Gene Expression

Francesc Villarroya, Roser Iglesias, and Marta Giralt

Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona 585 08007, Spain

Received 31 July 2006; Revised 18 October 2006; Accepted 18 October 2006

Recommended for Publication by Sander Kersten

Uncoupling proteins (UCPs) are mitochondrial membrane transporters involved in the control of energy conversion in mitochondria. Experimental and genetic evidence relate dysfunctions of UCPs with metabolic syndrome and obesity. The PPAR subtypes mediate to a large extent the transcriptional regulation of the UCP genes, with a distinct relevance depending on the UCP gene and the tissue in which it is expressed. UCP1 gene is under the dual control of PPARγ and PPARα in relation to brown adipocyte differentiation and lipid oxidation, respectively. UCP3 gene is regulated by PPARα and PPARδ in the muscle, heart, and adipose tissues. UCP2 gene is also under the control of PPARs even in tissues in which it is the predominantly expressed UCP (eg, the pancreas and liver). This review summarizes the current understanding of the role of PPARs in UCPs gene expression in normal conditions and also in the context of type-2 diabetes or obesity.

CURRENT KNOWLEDGE OF THE BIOLOGY OF MITOCHONDRIAL UNCOUPLING PROTEINS

Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner mitochondrial membrane. The first member of the family, UCP1, is expressed in brown adipocytes and it confers on brown adipose tissue its thermogenic capacity. UCP1 confers to the mitochondrial inner membrane an enhanced conductivity to protons, thus resulting in the uncoupling of the respiratory chain and heat production. This action of UCP1 in brown adipose tissue constitutes the main molecular basis for nonshivering thermogenesis in rodents in response to cold exposure and diet. The thermogenic activity of brown fat is mainly regulated by norepinephrine released from the sympathetic nervous system innervating the tissue, acting through β-adrenergic, cAMP-dependent pathways. Accumulating pieces of evidence over more than two decades have indicated that energy expenditure processes elicited by UCP1 are involved in the control of energy balance, and that UCP1 activity in brown adipose tissue may provide the basis for diet-induced thermogenesis. In fact, obesity models in rodents are in most cases associated with low levels and activity of UCP1 in brown fat. Less clear is the role of UCP1 in human obesity, taking into account the residual amounts of brown adipocytes in adult humans. However, sensitive methodologies based on RT-PCR have revealed that remnant UCP1-expressing cells are widespread among the white adipose depots of human adults. Furthermore, genetic evidence of the association of UCP1 gene polymorphisms with disturbances of body weight in humans keeps the debate on the physiological role of UCP1 in adults ongoing [1]. The discovery in 1997 of two proteins highly similar to UCP1, named UCP2 and UCP3, with a high level of expression in humans, suggested the possibility that the role of UCP1 in the control of energy expenditure was played in humans by these two novel proteins. A decade later, the precise roles of UCP2 and UCP3 remain a matter of debate [2–4]. Like UCP1, UCP2 and UCP3 lower the mitochondrial membrane protomotive potential, but it is unclear whether dissipation of metabolic energy as heat is their primary biological function. However, their capacity to protect against obesity has been demonstrated, at least for UCP3, in experimental settings based on transgenic mice overexpressing the protein in muscle [5]. The specific involvement of UCP2 and UCP3 in the control of reactive oxygen species production or in fatty acid oxidation has been proposed. In any case, genetic approaches in humans have highlighted the involvement of both proteins in metabolic regulation and in associated disturbances such as diabetes and obesity [6].

The transcriptional control of gene expression of UCP1, UCP3, and, to a minor extent, of UCP2 determines the levels of the corresponding proteins in tissues and cells. Research in

recent years has identified peroxisome proliferator-activated receptors (PPARs) as pivotal actors in the control of transcription of the UCP genes. As well as providing a basis for insight into the regulation of transcription of UCP genes in response to physiological ligands of PPARs, an understanding of the precise mechanisms and the PPAR subtypes involved in this regulation would provide the possibility of the development of pharmacological approaches to modulate the levels of UCPs, given the availability of drugs acting selectively on PPAR subtypes, such as fibrates and thiazolidinediones.

PPARS IN THE CONTROL OF THE UCP1 GENE, BROWN ADIPOCYTE DIFFERENTIATION, AND ENERGY EXPENDITURE

The UCP1 gene is a target of dual regulation by PPARγ and PPARα in brown adipose tissue

Brown adipose tissue and white adipose tissue have distinct metabolic functions. In contrast to the role of white adipose tissue as a site of energy storage, brown fat dissipates metabolic energy as heat, thus promoting energy expenditure. Whereas large amounts of white adipose tissue are associated with obesity, the development of high levels as well as high activity of brown adipose tissue is usually associated with a reduction in body weight. However, brown adipocytes and white adipocytes share multiple metabolic features and gene expression patterns, such as those related to lipid storage. They also share key transcriptional factors that mediate their differentiation process; namely, PPARγ and CCAAT-enhancer binding-protein α (C/EBPα). In fact, all three PPARs are expressed in brown fat [7], and their relative roles in regulating brown fat thermogenesis and in UCP1 gene expression will be discussed.

PPARγ is highly expressed both in brown and white adipocytes. Activation of PPARγ induces brown and white adipocytes differentiation by regulating the expression of genes involved in adipogenesis and lipid storage, whereas PPARγ-null cells cannot differentiate into adipocytes [8]. Mice that specifically lack PPARγ in adipose tissues have reduced adiposity and compromised survival of mature brown and white adipocytes [9, 10]. Furthermore, the transcription factor C/EBPα, which is necessary for white adipose tissue development in mice [11], also has a critical role in brown adipocyte differentiation during perinatal development, although later on C/EBPβ and C/EBPδ can functionally replace C/EBPα [12]. C/EBPα (and also C/EBPβ and C/EBPδ) function synergistically with PPARγ to regulate genes expressed in both brown and white adipocytes [13], but also the brown fat-specific UCP1 gene [14–16]. In fact, the transcription of the UCP1 gene is tightly regulated during brown adipocyte differentiation and in response to thermogenic activation. The 5′-flanking regions of the rat, mouse, and human UCP1 genes share a common genomic structure: a proximal regulatory region and an upstream enhancer located at -2 kb for review, see [17]. The proximal regulatory promoter contains C/EBP-regulated sites and the main cAMP-regulatory element [14, 18, 19]. The UCP1 gene distal enhancer includes a complex organization of nuclear receptor binding sites which mediate the transcriptional activation of the UCP1 gene by retinoids, thyroid hormones, PPAR agonists, and also cAMP, probably through induction of the PPAR coactivator-1α (PGC-1α) [18, 20–25].

PGC-1α was first identified as a PPARγ-interacting protein displaying preferential expression in mature brown adipocytes rather than white adipocytes [26]. The expression of PGC-1α is highly induced in brown fat in response to thermogenic activation via cAMP-signaling pathways [15, 26]. PGC-1α has been proposed to be essential for brown adipocyte differentiation and induction of the UCP1 gene [26]. As previously mentioned, UCP1 is uniquely present in brown adipocytes, where it is highly expressed as it may account for up to 8% of the mitochondrial protein (and mitochondrial protein represents 50% of total protein). Brown adipocytes, unlike white adipocytes, also possess powerful fatty acid oxidation machinery as evidenced by the abundance of mitochondria, a high level of expression of PPARα and a high activity of fatty acid oxidation pathways. PGC-1α can activate all of these key components of the thermogenic program through coactivation of PPARγ and PPARα (see below), or of transcription factors such as nuclear respiratory factor-1 [24, 26, 27]. In this way, forced expression of PGC-1α in white adipocytes induces mitochondrial biogenesis and expression of UCP1 [26–28]. In contrast, PGC-1β, another coactivator highly similar to PGC-1α, is only involved in controlling mitochondrial biogenesis together with PGC-1α [29]. Furthermore, loss of PGC-1α does not alter "in vitro" brown adipocyte differentiation but completely blunts the thermogenic induction via cAMP of the UCP1 gene and other thermogenic and mitochondrial genes [29].

Thiazolidinediones, drugs specifically activating PPARγ, have an overall effect of promoting adipogenesis, but have also been reported to induce mitochondrial biogenesis [30] besides their direct effect upon UCP1 transcription via PPARγ activation (see above). This induction of "brown fat-like" features by thiazolidinediones entails direct upregulation of transcription of the PGC1α gene by PPARγ in adipocytes [31]. This induction of PGC1α is amplified by an autoregulatory loop mediated by the coactivation of PPARγ action on PGC1α gene transcription by PGC1α itself [31], similarly to PGC1α coactivation with PPARγ in the promoters of other genes such as UCP1 [24].

In summary, the available data point to a function of PGC1α in orchestrating the regulation of mitochondrial biogenesis and UCP1 gene induction during brown adipocyte differentiation. Regarding UCP1 gene transcription, coactivation with PPARγ is probably involved in mediating this effect of PGC-1α. However, the thermogenic activation of mature brown adipocytes results in a negative regulation of PPARγ, thus suggesting that PPARγ may not be essential for UCP1 gene expression in already differentiated brown adipocytes recently reviewed in [32].

Since PPARα is preferentially expressed in brown adipocytes as compared to white adipocytes, it can be expected that it is mainly through PPARα that the UCP1 gene is induced in mature brown adipocytes. Agonists of either

PPARγ or PPARα can induce UCP1 gene expression both in brown fat "in vivo" and in brown adipocytes "in vitro" [24, 33, 34]. Furthermore, the PPAR-response element of the UCP1 gene enhancer can bind either PPARγ or PPARα [24]. PGC-1α also coactivates PPARα-dependent regulation of the UCP1 gene [24]. Although basal expression of UCP1 mRNA in brown fat from PPARα-null mice is not altered [35], there is an impaired activation of UCP1 gene expression in PPARα-null mice in several physiological situations associated with cold stress (our unpublished observations). Furthermore, genetic analyses revealed that PPARα gene expression is associated with UCP1 gene induction [36].

Likewise, PGC1α can cooperate with PPARα in the transcriptional control of genes for fatty acid catabolism in brown fat. Activation of brown fat thermogenesis, which is mediated by cAMP-dependent pathways, rapidly induces lipolysis of the stored triglycerides. Released fatty acids, in addition to being the major substrate for thermogenesis and the inducers of UCP1 uncoupling activity through direct interaction with the UCP1 protein in the inner mitochondrial membrane [37], may also act as PPAR-activators. Thus, the PGC-1α/PPARα interaction can coordinately regulate gene expression required for active thermogenesis, including fatty acid oxidation, in mature brown adipocytes.

Whether PPARδ, the third PPAR subtype, can also play a direct role in the regulation of UCP1 gene expression has not been clearly elucidated. Transgenic mice overexpressing an active form of PPARδ in adipose tissues displayed reduced accumulation of triglycerides both in white fat and brown fat [38]. However, only the size of white depots was reduced. UCP1 and genes involved in fatty acid catabolism were moderately induced in brown fat and highly induced in white fat in these mice. However, neither induction of the endogenous UCP1 gene in primary murine brown adipocytes by the PPARδ-specific GW501516 ligand nor PPARδ-dependent regulation of the UCP1 gene promoter has been observed in brown adipocytes in culture (our unpublished observations).

Rexinoid-dependent UCP1 gene regulation in brown adipose tissue

Both white and brown adipose tissues contain retinoic acid receptor (RAR) and retinoid X receptor (RXR) subtypes with distinct relative abundances. Retinoic- and rexinoid-dependent pathways of regulation in adipose tissues have previously been extensively reviewed [39, 40].

Retinoic acid acting via RAR has long been recognized as a potent inhibitor of the differentiation of preadipocytes into white and brown adipocytes [41, 42]. However, when retinoic acid acts upon already differentiated brown adipocytes, it dramatically increases UCP1 gene expression through a direct transcriptional effect (see below) [21]. The action of retinoic acid in promoting UCP1 gene expression has been confirmed "in vivo" by pharmacological treatment and by vitamin A supplementation of the diet [43, 44]. However, the biological significance of this powerful retinoic acid-dependent regulation of the UCP1 gene in response to RAR activation remains unknown.

Retinoic acid stimulates UCP1 gene transcription through a complex "retinoid-responsive region" in the distal enhancers of the rat or human UCP1 genes [21, 23]. Both RAR- and RXR-binding sites in the enhancer contribute to the retinoic acid effects [45]. Induction of UCP1 gene expression by retinoic acid does not require PGC1α [29]. The UCP1 gene is a direct target of specific RXR activators through RXR-containing heterodimers that bind to the enhancer region of the UCP1 gene [45]. Phytanic acid (3, 7, 11, 15-tetramethylhexadecanoic acid), which is a derivative of the phytol side chain of chlorophyll, has been reported to be a natural ligand of RXR subtypes [46], but also to be a direct activator of PPARα [47]. Phytanic acid induces UCP1 gene expression through the RXR-binding sites in the UCP1 gene enhancer [48].This may be closely related to thermogenic activation, as phytanic acid accumulates in the brown adipose tissue fat stores and is released as a free acid when lipolysis is active in the tissue owing to thermogenic stimuli. In these conditions, phytanic acid can act as a signaling molecule linking lipolysis with enhanced synthesis of UCP1 protein to favor thermogenesis [49].

In summary, as depicted in Figure 1, the expression of the UCP1 gene is directly regulated by PPARs in association with adipogenic differentiation (via PPARγ) and in coordination with induction of gene expression for the fatty acid oxidation required for active thermogenesis (via PPARα). Whether these PPAR/rexinoid-dependent pathways can affect energy expenditure in adult humans remains to be determined. Although the amounts of UCP1-expressing brown adipocytes are low in adult humans, UCP1 gene expression can be reactivated in several conditions such as high exposure to catecholamines released by pheochromocytomas [50], or chronic treatment with antiretroviral drugs [51]. Future research will be required to determine whether PPAR agonists and/or retinoids cause similar activation, considering that they are powerful activators of human UCP1 gene transcription "in vitro" [23].

PPARα AND PPARδ CONTROL UCP3 GENE EXPRESSION IN SKELETAL MUSCLE AND HEART

Free fatty acids are major inducers of UCP3 gene expression in skeletal muscle and heart

Initial studies on the regulation of UCP3 gene expression in skeletal muscle, its main site of expression, revealed that transcript levels of UCP3 were dramatically influenced by the availability of free fatty acids to the tissue both in rodents and humans. This explained the rise in UCP3 mRNA in muscle after starvation, an observation initially considered as a paradox at the time when UCP3 was expected to have a role similar to UCP1 in the promotion of energy expenditure [52]. Today, we know that UCP3 mRNA levels are systematically upregulated in association with any physiological or experimental rise in circulating free fatty acids, either when they originate from lipolysis in white fat (starvation or exercise) or from the diet (high-fat diet) [53–55]. The increase

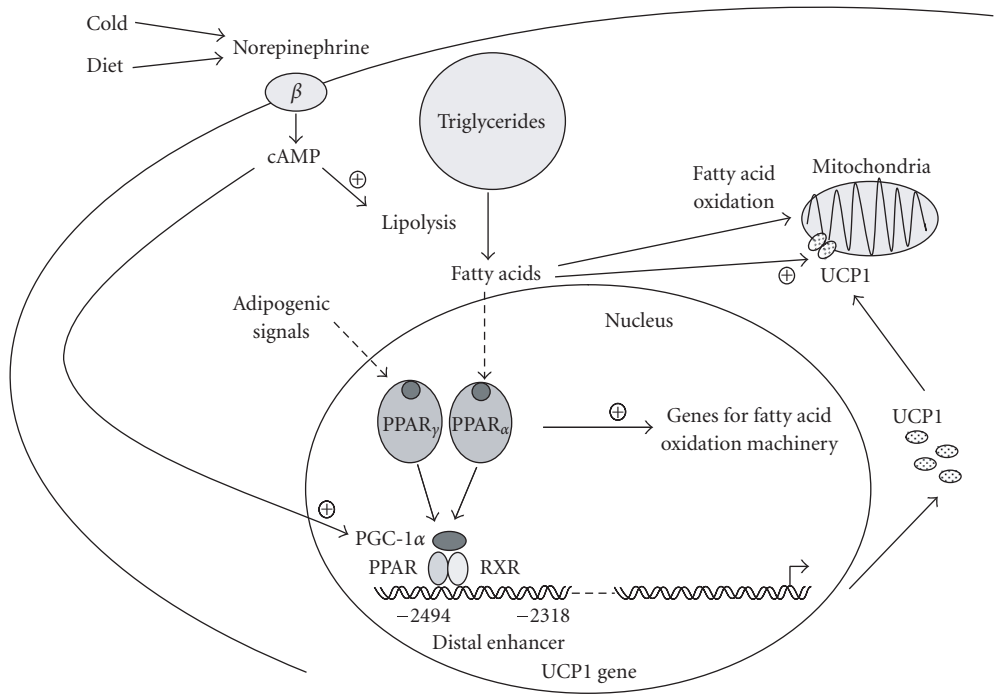


Figure 1: Schematic representation of the regulation of UCP1 gene expression by ligand-dependent activation of PPARα and PPARγ, and coactivation by PGC-1α. The diagram shows the PPAR response element in the rat UCP1 gene enhancer (24). Major features of the transcriptional regulation of the mouse and human UCP1 genes appear to be similar (16, 23). During brown adipocyte differentiation, adipogenic signals activate transcription of the UCP1 gene through PPARγ and coactivation by PGC-1α, in concert with overall induction of adipocyte differentiation towards the brown fat lineage. In response to thermogenic simuli on mature brown adipocytes, activation of PPARα by lipolysis-derived fatty acids contributes to the coordination of UCP1 gene transcription (thermogenesis) with the lipid oxidation pathways providing metabolic fuel for oxidation.

in free fatty acids due to the initiation of milk (a fat-rich diet) intake also causes a dramatic rise in UCP3 mRNA after birth [56]. The opposite situation also occurs: a drop in free fatty acid levels such as that occurring in lactating dams is associated with a decrease in UCP3 transcript in muscle [57]. Studies in humans confirmed the regulation of UCP3 mRNA expression by fatty acids in human skeletal muscle and the heart [58, 59].

Several studies have indicated that favoring the intracellular presence of free fatty acids stimulates UCP3 gene expression. Thus, overexpression of lipoprotein lipase in muscle leads to a rise in UCP3 mRNA, surely due to the enhancement in local free fatty acid availability via hydrolysis of triglycerides [60]. Moreover, when intracellular fatty acid oxidation is blocked by the use of etomoxir, an inhibitor of carnitine palmitoyl transferase-1, UCP3 transcript levels rise also [61].

PPARα and PPARδ, mediators of the fatty acid-dependent control of UCP3 transcription in skeletal muscle and heart

Multiple lines of evidence have shown that PPARα plays a major role in the induction of the UCP3 gene in response to fatty acids. Acute treatment of mice pups with the specific activator of PPARα Wy 14643 mimics the postnatal skeletal muscle UCP3 gene induction caused by fatty acids coming from milk [56]. A single injection of this drug to adult lactating mice also induces UCP3 mRNA expression [57]. Moreover, PPARα-null mice show reduced levels of UCP3 gene expression and impaired response to starvation in the heart [62–64]. This does not occur in skeletal muscle in adult PPARα-null mice, possibly due to compensatory up-regulation of the UCP3 gene by PPARδ (see below). However, PPARα-null mice neonates display lowered UCP3 gene expression both in skeletal muscle and in the heart [65]. On the other hand, transcriptomic analysis of muscle or heart from transgenic mice which overexpress PPARα specifically in these tissues revealed that UCP3 mRNA is among the most intensely induced gene transcripts [66, 67]. This occurs in concert with induction of many other genes involved in fatty acid oxidation. Thus, the UCP3 gene appears to be part of the cluster of PPARα-regulated, fatty acid catabolism-related genes in the muscle and heart. Regardless of the information provided by experimental approaches directly addressing the issue of the biological function of UCP3, these observations strongly suggest that UCP3 function is likely to be related to fatty acid metabolism in these tissues.

Despite all these lines of evidence, reports on the effects of chronic treatment with fibrates, which are potential activators of PPARα in muscle, have led to variable results; from unchanged expression of the UCP3 gene using Wy 14643

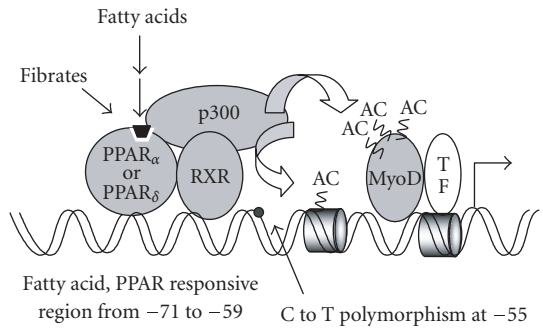


Figure 2: Schematic representation of the regulation of UCP3 gene transcription by PPARs. The proximal region responsive to PPARα and PPARδ activation via PPAR/RXR heterodimers is shown. The −55 C to T polymorphism is adjacent to this region. MyoD and TFs indicate the binding of MyoD and of basal transcription factors, respectively, close to the site of transcription initiation. P300, the main coactivator linking ligand-dependent activation of PPARs with transcriptional activation is shown. AC indicates the acetylation sites involved in transcriptional activation.

[33] to upregulation using bezafibrate [68]. The reasons for this variability in response to chronic treatment as opposed to the systematic upregulation observed in acute, single-injection treatment with fibrates are unclear. Perhaps the hypolipidemic consequences of chronic fibrate treatment, including reductions in the levels of circulating fatty acids, may counteract the direct positive effects of the drugs on the UCP3 gene.

Studies in cell culture have been also less conclusive in relation to the role of PPARα in the control of UCP3 gene expression. Myogenic cells in culture express very low levels of UCP3 relative to muscle "in vivo" [69] and, when they were exposed to fibrates, PPARδ-dependent activation appears to have a more powerful effect on UCP3 gene induction than does PPARα activation [70, 71]. However, the significance of these observations for "in vivo" regulation of the UCP3 gene is unclear because myogenic cell lines such as C2C12 or L6 show abnormally reduced expression of PPARα relative to that in skeletal muscle. Thus, a low sensitivity of the UCP3 gene (and other PPARα-target genes) to PPARα activators is anticipated in such cell systems [71, 72].

The capacity of PPARδ to activate UCP3 in muscle and the heart has been demonstrated also using "in vivo" approaches. Similar to PPARα overexpressing mouse models, overexpression of PPARδ in muscle obtained via transgenic mice revealed that UCP3 is among the genes most sensitive to induction [73, 74]. Moreover, a mouse model of targeted disruption of PPARδ specifically in the heart revealed a reduction in UCP3 levels [75]. The recent availability of drugs acting specifically on PPARδ confirmed "in vivo" and "in vitro" the sensitivity of the UCP3 gene to activation via PPARδ. Thus, chronic treatment of mice with a PPARδ activator induces UCP3 gene expression in concert with other genes of lipid metabolism [76, 77]. Therefore, the dual regulation of the UCP3 gene by PPARα and PPARδ in muscle and heart is shared by many genes involved in fatty acid oxidation and again suggests the involvement of UCP3 in biological functions related to fatty acid catabolism.

Most of the above conclusions arising from studies on experimental animals or human volunteers have been confirmed by studies directly addressing the transcriptional control of the human and mouse UCP3 gene promoter in muscle cells. Both PPARα and PPARδ activate the UCP3 gene promoter and mediate transcriptional responsiveness to fatty acids and to drugs specifically activating both PPAR subtypes. This occurs due to the presence of a PPAR-responsive element in the proximal region of the UCP3 promoter [65, 78]. Moreover, RXR activators (rexinoids) activate UCP3 gene transcription via ligand-dependent activation of the RXR moiety of the PPARα/RXR or PPARδ/RXR heterodimers binding to the promoter. Interestingly, PPAR-dependent activation of the UCP3 gene requires MyoD, which acts as a transcription factor permissive for basal and PPAR-dependent regulation of the UCP3 gene in muscle cells. Coactivators such as p300 mediate this functional relationship between MyoD and PPAR-dependent regulation of the UCP3 gene [78].

The control of UCP3 gene transcription by PPAR/RXR heterodimers, which retain the capacity for ligand-dependent activation of the RXR moiety [78], explains the sensitivity of UCP3 gene expression to 9-cis retinoic acid in myogenic cells [69] and to dietary vitamin A supplementation or acute retinoic acid-treatment [79]. However, it should be taken into account that RAR-dependent pathways of regulation are also active on the UCP3 gene promoter [69]. On the other hand, although RXR has been proposed to be able to mediate transcriptional regulation through binding itself to fatty acids, UCP3 gene promoter studies appeared to exclude the possibility that RXR plays this role at the UCP3 gene [65].

Moreover, dozens of reports in recent years have indicated a positive association between a C to T polymorphism in the human UCP3 gene promoter and body weight disturbances or insulin resistance [80]. This C to T change has been reported to modulate the relative levels of UCP3 transcripts in muscle from Pima Indians [81]. UCP3 promoter analysis revealed that the site of this polymorphism is adjacent to the PPARα/δ-responsive element (see Figure 2), although no direct effects on promoter activity dependent on the presence of C or T have been demonstrated to date [78].

On the other hand, the potential role of PPARγ in the control of UCP3 in the muscle or heart is unclear. Contradictory results have been reported on the action of thiazolidinediones on UCP3 gene expression in myogenic cells, from inhibition [82] to stimulation [83]. Treatment with thiazolidinediones "in vivo" also led to variable effects depending on the type of thiazolidinedione or the length of treatment [33, 57, 84, 85]. Mice with a muscle-specific PPARγ deletion show unaltered UCP3 gene expression [86]. In these mice, treatment with rosiglitazone or troglitazone leads to a reduction in UCP3 mRNA levels whatever the genotype, thus indicating that the effects of thiazolidinediones on the UCP3 gene are likely to be PPARγ-independent [86]. This is in agreement with UCP3 gene promoter studies indicating a lack of sensitivity to PPARγ at least in the context of myogenic cells [65, 78].

In summary, PPARα and PPARδ are major regulators of UCP3 gene expression in skeletal muscle and the heart, as they appear to mediate the powerful physiological regulation of these genes by fatty acids. The physiological role of UCP3 in relation to fatty acids is unclear. However, the available data indicate that, when the muscle or heart is challenged by an overload of fatty acids, UCP3 may act to favor fatty acid metabolism in such a way that minimizes toxicity and mitochondrial production of reactive oxygen species. Pharmacological activation of PPARα and PPARδ via fibrates may then favor these physiological functions in muscle. Type 2 diabetes, and ultimately obesity or metabolic syndrome, may be related to the appearance of insulin resistance in muscle as a consequence of defective handling of fatty acids. The action of PPARs on the control of UCP3 gene expression may represent a potential tool to prevent the negative effects of high exposure of muscle to fatty acids, although further research will be required to more firmly establish this possibility.

FATTY ACIDS AND PPARS IN THE CONTROL OF UCP2 GENE EXPRESSION IN SKELETAL MUSCLE AND HEART

The expression of the UCP2 gene shares with UCP3 being stimulated by fatty acids in skeletal muscle and heart, as well as being a target of PPARα and PPARδ-dependent activation in these tissues. However, several evidences indicate that fatty acid-dependent activation of UCP2 gene transcription is more complex, and involves also PPARα and PPARδ-independent mechanisms. The relative roles of these PPAR-independent mechanisms may be different depending on the tissue in which UCP2 is expressed, and, for instance, they are especially relevant in heart or other tissues such as the liver (see below). Direct effects of PPARδ activators on UCP2 mRNA expression have been demonstrated in human myotubes [87], and direct analysis of regulation of the UCP2 gene promoter in muscle cells indicated that PPARγ and their ligands induce promoter activity. However, no direct binding of PPARγ could be detected, thus raising the possibility of an indirect effect [88].

PPARS IN THE CONTROL OF UCP3 AND UCP2 GENE EXPRESSION IN ADIPOSE TISSUES

As previously mentioned, UCP3 is highly expressed in brown adipose tissue and to a very minor extent in white fat, whereas UCP2 is expressed in both types of adipose tissue. As in the muscle or heart, drugs activating PPARα or PPARδ induce UCP3 gene expression in brown fat, both as a result of acute, single-dose treatment, and after chronic treatment [33, 34].

The high expression of PPARγ in adipose tissues, in contrast with that in muscle, together with the sensitivity of the UCP3 and UCP2 genes to the PPARα and PPARδ subtypes raised the question of the capacity of PPARγ activation to affect UCP3 and UCP2 gene expression in adipose cells. The effects of chronic treatment with rosiglitazone, a thiazolidinedione capable of activating PPARγ, have been reported to involve a robust induction [89], a moderate increase [90] or even no change [33] in UCP3 mRNA levels in white adipose tissue. The reasons for these discrepancies are unclear and different doses or rodent species and strains used may be the basis of the different findings. It should be noted that, as mentioned for UCP1, any treatment of mice or cells driving the white fat phenotype into a brown fat-like phenotype or generally promoting brown fat differentiation may result in increased UCP3 gene expression in white adipose depots. This UCP3 mRNA induction in white adipose depots could be just one more symptom of the acquisition of "brown fat-like" features, considering the plasticity of adipose depots in rodents. Rosiglitazone treatment "in vivo" may exert these overall effects and its action on UCP3 gene expression may depend on the extent of alterations in the brown versus white pattern of gene expression.

Concerning UCP2, chronic thiazolidinedione treatment in rodents has also been reported to increase [33] or to not affect [90] UCP2 gene expression in white fat, whereas increased expression of UCP2 mRNA has been observed in subcutaneous adipose tissue from human patients treated with rosiglitazone [91]. A moderate induction of UCP2 mRNA has also been reported in cell cultures of white adipocytes [92]. In the context of white adipogenic cell lines, PPARγ and their ligands induce UCP2 promoter activity in the absence of direct binding and via E-box elements in the proximal region of the promoter [88]. In brown adipocytes, rosiglitazone as well as activators of PPAR common to the PPARα and PPARδ subtypes induce UCP2 mRNA expression. However, 9-cis retinoic acid and selective activators of RXR were the most powerful in inducing UCP2 mRNA expression, most probably due to their capacity to activate the dimers of RXR with PPARs or with other permissive nuclear receptors [93].

On the other hand, adipose tissues contain large amounts of endogenous triglycerides, which are capable of resulting in the local generation of free fatty acids after lipolysis. PPAR receptors can provide a mechanism for responsiveness of UCP2 and UCP3 expression to intracellularly derived fatty acids. Thus, a cross-talk between adrenergic regulation of adipose tissue lipolysis and PPAR mechanisms of induction

of gene expression of UCP2 and UCP3 may occur as mentioned above for UCP1, especially in response to noradrenergic stimulus in brown adipocytes.

ROLE OF PPARS IN THE CONTROL OF UCP2 GENE EXPRESSION IN PANCREATIC β-CELLS

Studies in UCP2-null mice have revealed that UCP2 exerts substantial negative control over glucose-stimulated insulin secretion [94]. Thus, UCP2 expression may play an important role in the pathogenesis of diabetes. UCP2 expression is stimulated by high glucose and/or high free fatty acid levels both "in vivo" and "in vitro", as well as being increased in animal models of type 2 diabetes. On the other hand, a genetic deficiency of UCP2 improves β-cell function in animal models as well as in "in vitro" models of glucotoxicity and lipotoxicity in β-cells reviewed in [95].

It has been demonstrated that exposure to fatty acids increases transcription of the UCP2 gene in human and rodent cells representative of adipocytes and myocytes (see above), as well as in pancreatic β-cell-derived cell lines (INS-1 cells). An enhancer region has been identified between -86 to -44 of the mouse UCP2 gene. This enhancer contains Sp1 elements, sterol regulatory element (SRE), and double E-box elements all clustered together and is responsible for basal and fatty acid-stimulated transcription. The response to fatty acids appears to be mediated by sterol regulatory element binding proteins (SREBPs) binding to the SRE [96]. This enhancer is not conserved in the human UCP2 promoter but two E-box motifs at -911 to -906 and -743 to -738 have been identified as being responsible for the SREBP activation of human UCP2 gene transcription in INS-1E cells [97]. However, despite the important pathophysiological implications, the mechanisms by which chronic exposure to fatty acids increases UCP2 expression in pancreatic β-cells have not been completely characterized, and in addition to SREBP proteins, PPAR receptors and the G protein-coupled receptor GPR40 could be implicated.

All PPAR subtypes are expressed in pancreatic β-cells [98]. Although their roles in β-cell function remain poorly understood, several lines of evidence suggest that PPARα may be implicated in the modulation of insulin secretion: (i) fatty acids stimulate the expression of PPARα and its target genes in islets [98]; (ii) clofibrate treatment or PPARα overexpression in INS-1cells induce UCP2 expression, increase fatty acid oxidation, and decrease basal and glucose-stimulated insulin secretion [99]; (iii) in wild-type mice, starvation increases islet PPARα and UCP2 expression, which may contribute to decreased insulin secretion, whereas fasted PPARα null-mice display increased plasma insulin levels and enhanced glucose-induced insulin secretion [100]. Thus, pancreatic PPARα signaling appears to be significant "in vivo" and, when PPARα is activated due to elevated fatty acid levels, as in obesity, it may contribute to glucose intolerance and β-cell dysfunction.

Contradictory data have been reported on the effects of PPARγ on UCP2 expression in β-cells. It has been described that overexpression of PPARγ causes upregulation of UCP2 expression and suppresses glucose-stimulated insulin secretion [101]. In contrast, the increase in UCP2 expression induced by chronic exposure of pancreatic islets to palmitate is prevented by addition of rosiglitazone, and this treatment also normalizes insulin secretion [102]. No direct binding of PPARγ to the enhancer in the mouse UCP2 gene has been observed. Thus, the effects on UCP2 expression may be produced by indirect mechanisms [88].

GPR40 has been recently identified as a G protein-coupled receptor selectively expressed in β-cells and activated by fatty acids. GPR40-null mice develop neither hyperinsulinemia nor glucose intolerance when challenged with a chronic high-fat diet. In contrast, transgenic mice overexpressing GPR40 in β-cells are glucose intolerant and show impaired glucose-stimulated insulin secretion. In addition, in pancreatic islets of these mice, the mRNA levels of PPARα, SREBP1c, and UCP2 are increased. Thus GPR40 may play a key role in the development of diabetes and could be implicated in the upregulation of PPARα signaling in insulin-resistant conditions [103].

PPARS AND UCP GENE EXPRESSION IN THE LIVER

The liver is the organ in which expression of UCPs is the lowest, in basal conditions. Only minor expression of UCP2 can be detected in the adult liver, and it is mainly due to high expression in Kupffer cells [104]. However, in situations of metabolic stress, UCP2 expression is induced in the liver, and enhanced expression appears mainly in hepatocytes [105].

Increased UCP2 mRNA expression in the liver has been reported in response to starvation, but also in obese, leptin-deficient conditions, and in rodents treated with a high-fat diet [35, 106, 107]. However, the increase in UCP2 expression is not necessarily related to obesity and insulin resistance, as a high fish-oil diet, which does not result in significant weight gain, is more effective in increasing UCP2 levels than is a high safflower oil-based diet [108]. Thus, it has been suggested that fatty acids might be key factors determining the control of UCP2 expression in the liver, regardless of whether they are associated with high lipolysis in situations of starvation or the opposite, high fatty acid levels as in obesity. PPAR signaling is a candidate for mediation of this regulation. In fact, PPARα expression increases in the liver during fasting [35] and in several models of murine obesity [106]. Chronic treatment of rodents with PPARα agonists such as fenofibrate or Wy 14643 increases hepatic UCP2 mRNA expression [105–108]. UCP2 mRNA levels are also upregulated in cultured hepatocytes in response to polyunsaturated fatty acids, Wy 14643 or fenofibrate [105, 109]. However, there is some data suggesting the existence of signaling mechanisms other than through PPARα. For instance, the increase in liver UCP2 expression induced by starvation is preserved in PPARα-null animals [35]. It has been suggested that PPARδ may contribute to the regulation of UCP2 gene expression in PPARα-deficient mice [110]. Regulation via PPARγ must be also considered as UCP2 is induced by the PPARγ activator troglitazone in cultured hepatocytes. However, the PPARα activator Wy 14643 was a more powerful inducer of UCP2 gene

expression in hepatic cells [109]. Despite the very low expression of PPARγ in the liver under basal conditions, it is increased in obesity, in insulin resistance, and after a high-fat diet [106, 107]. PPARγ is highly expressed in liver from PPARα null-mice fed a high-fat diet, and this is associated with an induction of UCP2 gene expression [107]. Moreover, adenoviral-induced overexpression of PPARγ in the liver of PPARα null-mice causes a dramatic increase in UCP2 mRNA levels [107]. Thus, the available data suggests a major role for PPARα in the regulation of UCP2 expression in the liver whereas, in some particular pathophysiological situations, additional pathways may be involved; mainly PPARδ and PPARγ as well as possibly other transcription factors.

Among UCP gene regulation in the liver, most attention has been focused in UCP2, as other UCP genes are silent in this tissue. However, it has been described that chronic fenofibrate administration to mice or rats induces "de novo" UCP3 expression in the liver [108, 111]. Recently, it has been demonstrated that the appearance of UCP3 transcripts is accompanied by the presence of the UCP3 protein in the mitochondrial fraction. In fact, genes involved in fatty acid oxidation and preferentially expressed in muscle, such as carnitine palmitoyl-transferase I-b, are also induced in the liver as a consequence of fenofibrate treatment [112]. Interestingly, although this treatment also upregulates UCP2 mRNA levels, UCP2 protein was not detectable, most likely due to the presence of an inhibitory post-translational mechanism. Thus, in the absence of UCP2 protein, the uncoupling effects detected in liver mitochondria after fenofibrate treatment are presumably attributable to UCP3 [112]. The results of chronic fenofibrate treatment stress the importance of post-translational mechanisms of regulation of UCP2 gene expression in the liver, in agreement with previous reports in other systems [113].

CONCLUSIONS AND PERSPECTIVES

Intensive research efforts over recent decades have established that PPARs are major controllers of UCPs gene expression. Different PPAR subtypes are preferentially involved in the control of each UCP gene depending on the UCP gene or the main tissue of expression. The control of UCPs genes by PPAR subtypes either provides tissue-specific regulation of UCPs gene transcription, as seen in UCP1 control by PPARγ, or regulates the responsiveness of UCPs genes to metabolic challenges, as seen in the control of the UCP3 gene by PPARα and PPARδ in the muscle and heart. The precise identification of mechanisms or PPAR subtypes involved in the control of UCP genes may be of utmost relevance in the foreseeable pharmacological approaches aimed at influencing metabolic disturbances involving skeletal muscle (ie, UCP3 gene control) or at modulating pancreatic insulin secretion (ie, UCP2 control in the pancreas). This research can be expected to have a high impact in the near future in relation to obesity and metabolic syndrome. Other issues poorly explored to date, as for instance the role of PPAR-dependent regulation of UCP2 gene expression in macrophages, cells expressing high levels of UCP2 [114] and highly sensitive to PPARs [115], would be important to further establish the mechanisms of PPAR action in inflammatory processes, including the chronic inflammation present in obesity. A new transgenic mouse model with a specific deletion of PPARγ in macrophages has already been developed [116] which may be useful in exploring the role of PPARγ in this cell type. We should expect much new data in the next years on the role of PPAR subtypes in obesity and metabolic syndrome, and on the role of disturbances in PPAR-mediated control of UCPs gene expression in these pathologies.

ACKNOWLEDGMENTS

This work is supported by Grants SAF 2005-01722 (Ministerio de Educación y Ciencia, Spain) and FIS-PI052336 (Ministerio de Sanidad. FIS, Spain).

REFERENCES

[1] M. Del Mar Gonzalez-Barroso, D. Ricquier, and A.-M. Cassard-Doulcier, "The human uncoupling protein-1 gene (UCP1): present status and perspectives in obesity research," *Obesity Reviews*, vol. 1, no. 2, pp. 61–72, 2000.

[2] S. Rousset, M.-C. Alves-Guerra, J. Mozo, et al., "The biology of mitochondrial uncoupling proteins," *Diabetes*, vol. 53, no. suppl 1, pp. S130–S135, 2004.

[3] J. Nedergaard, D. Ricquier, and L. P. Kozak, "Uncoupling proteins: current status and therapeutic prospects," *EMBO Reports*, vol. 6, no. 10, pp. 917–921, 2005.

[4] M. D. Brand and T. C. Esteves, "Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3," *Cell Metabolism*, vol. 2, no. 2, pp. 85–93, 2005.

[5] J. C. Clapham, J. R. S. Arch, H. Chapman, et al., "Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean," *Nature*, vol. 406, no. 6794, pp. 415–418, 2000.

[6] S. Krauss, C.-Y. Zhang, and B. B. Lowell, "The mitochondrial uncoupling-protein homologues," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 3, pp. 248–261, 2005.

[7] A. Valmaseda, M. C. Carmona, M. J. Barberá, et al., "Opposite regulation of PPAR-α and -γ gene expression by both their ligands and retinoic acid in brown adipocytes," *Molecular and Cellular Endocrinology*, vol. 154, no. 1-2, pp. 101–109, 1999.

[8] E. D. Rosen, C. J. Walkey, P. Puigserver, and B. M. Spiegelman, "Transcriptional regulation of adipogenesis," *Genes and Development*, vol. 14, no. 11, pp. 1293–1307, 2000.

[9] W. He, Y. Barak, A. Hevener, et al., "Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15712–15717, 2003.

[10] T. Imai, R. Takakuwa, S. Marchand, et al., "Peroxisome proliferator-activated receptor γ is required in mature white and brown adipocytes for their survival in the mouse," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 13, pp. 4543–4547, 2004.

[11] H. G. Linhart, K. Ishimura-Oka, F. DeMayo, et al., "C/EBPα is required for differentiation of white, but not brown, adipose tissue," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 22, pp. 12532–12537, 2001.

[12] M. C. Carmona, R. Iglesias, M.-J. Obregón, G. J. Darlington, F. Villarroya, and M. Giralt, "Mitochondrial biogenesis and thyroid status maturation in brown fat require CCAAT/enhancer-binding protein α," *Journal of Biological Chemistry*, vol. 277, no. 24, pp. 21489–21498, 2002.

[13] Z. Wu, E. D. Rosen, R. Brun, et al., "Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity," *Molecular Cell*, vol. 3, no. 2, pp. 151–158, 1999.

[14] P. Yubero, C. Manchado, A.-M. Cassard-Doulcier, et al., "CCAAT/enhancer binding proteins α and β are transcriptional activators of the brown fat uncoupling protein gene promoter," *Biochemical and Biophysical Research Communications*, vol. 198, no. 2, pp. 653–659, 1994.

[15] M. C. Carmona, E. Hondares, M. L. Rodriguez de la Concepcion, et al., "Defective thermoregulation, impaired lipid metabolism, but preserved adrenergic induction of gene expression in brown fat of mice lacking C/EBPβ," *Biochemical Journal*, vol. 389, no. 1, pp. 47–56, 2005.

[16] I. B. Sears, M. A. MacGinnitie, L. G. Kovacs, and R. A. Graves, "Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor γ," *Molecular and Cellular Biology*, vol. 16, no. 7, pp. 3410–3419, 1996.

[17] D. Ricquier and F. Bouillaud, "The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP," *Biochemical Journal*, vol. 345, no. 2, pp. 161–179, 2000.

[18] U. C. Kozak, J. Kopecky, J. Teisinger, S. Enerback, B. Boyer, and L. P. Kozak, "An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene," *Molecular and Cellular Biology*, vol. 14, no. 1, pp. 59–67, 1994.

[19] P. Yubero, M. J. Barberá, R. Alvarez, et al., "Dominant negative regulation by c-Jun of transcription of the uncoupling protein-1 gene through a proximal cAMP-regulatory element: a mechanism for repressing basal and norepinephrine-induced expression of the gene before brown adipocyte differentiation," *Molecular Endocrinology*, vol. 12, no. 7, pp. 1023–1037, 1998.

[20] A.-M. Cassard-Doulcier, C. Gelly, N. Fox, et al., "Tissue-specific and β-adrenergic regulation of the mitochondrial uncoupling protein gene: control by cis-acting elements in the 5′-flanking region," *Molecular Endocrinology*, vol. 7, no. 4, pp. 497–506, 1993.

[21] R. Alvarez, J. de Andres, P. Yubero, et al., "A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene," *Journal of Biological Chemistry*, vol. 270, no. 10, pp. 5666–5673, 1995.

[22] R. Rabelo, C. Reyes, A. Schifman, and J. E. Silva, "Interactions among receptors, thyroid hormone response elements, and ligands in the regulation of the rat uncoupling protein gene expression by thyroid hormone," *Endocrinology*, vol. 137, no. 8, pp. 3478–3487, 1996.

[23] M. del Mar Gonzalez-Barroso, C. Pecqueur, C. Gelly, et al., "Transcriptional activation of the human *ucp1* gene in a rodent cell line. Synergism of retinoids, isoproterenol, and thiazolidinedione is mediated by a multipartite response element," *Journal of Biological Chemistry*, vol. 275, no. 41, pp. 31722–31732, 2000.

[24] M. J. Barberá, A. Schlüter, N. Pedraza, R. Iglesias, F. Villarroya, and M. Giralt, "Peroxisome proliferator-activated receptor activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell," *Journal of Biological Chemistry*, vol. 276, no. 2, pp. 1486–1493, 2001.

[25] W. Cao, K. W. Daniel, J. Robidoux, et al., "p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene," *Molecular and Cellular Biology*, vol. 24, no. 7, pp. 3057–3067, 2004.

[26] P. Puigserver, Z. Wu, C. W. Park, R. Graves, M. Wright, and B. M. Spiegelman, "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis," *Cell*, vol. 92, no. 6, pp. 829–839, 1998.

[27] Z. Wu, P. Puigserver, U. Andersson, et al., "Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1," *Cell*, vol. 98, no. 1, pp. 115–124, 1999.

[28] C. Tiraby, G. Tavernier, C. Lefort, et al., "Acquirement of brown fat cell features by human white adipocytes," *Journal of Biological Chemistry*, vol. 278, no. 35, pp. 33370–33376, 2003.

[29] M. Uldry, W. Yang, J. St-Pierre, J. Lin, P. Seale, and B. M. Spiegelman, "Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation," *Cell Metabolism*, vol. 3, no. 5, pp. 333–341, 2006.

[30] L. Wilson-Fritch, S. Nicoloro, M. Chouinard, et al., "Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone," *The Journal of Clinical Investigation*, vol. 114, no. 9, pp. 1281–1289, 2004.

[31] E. Hondares, O. Mora, P. Yubero, et al., "Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1α gene transcription: an autoregulatory loop controls PGC-1α expression in adipocytes via peroxisome proliferator-activated receptor-γ coactivation," *Endocrinology*, vol. 147, no. 6, pp. 2829–2838, 2006.

[32] J. Nedergaard, N. Petrovic, E. M. Lindgren, A. Jacobsson, and B. Cannon, "PPARγ in the control of brown adipocyte differentiation," *Biochimica et Biophysica Acta*, vol. 1740, no. 2, pp. 293–304, 2005.

[33] L. J. Kelly, P. P. Vicario, G. M. Thompson, et al., "Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression," *Endocrinology*, vol. 139, no. 12, pp. 4920–4927, 1998.

[34] N. Pedraza, G. Solanes, R. Iglesias, M. Vazquez, M. Giralt, and F. Villarroya, "Differential regulation of expression of genes encoding uncoupling proteins 2 and 3 in brown adipose tissue during lactation in mice," *Biochemical Journal*, vol. 355, no. pt 1, pp. 105–111, 2001.

[35] S. Kersten, J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1489–1498, 1999.

[36] B. Xue, A. Coulter, J. S. Rim, R. A. Koza, and L. P. Kozak, "Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots," *Molecular and Cellular Biology*, vol. 25, no. 18, pp. 8311–8322, 2005.

[37] D. G. Nicholls, S. A. Cunningham, and E. Rial, "The bioenergetic mechanisms of brown adipose tissue mitochondria," in *Brown Adipose Tissue*, P. Trayhurn and D. G. Nicholls, Eds., pp. 52–85, Edward Arnold, London, UK, 1986.

[38] Y. X. Wang, C. H. Lee, S. Tiep, et al., "Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity," *Cell*, vol. 113, no. 2, pp. 159–170, 2003.

[39] F. Villarroya, M. Giralt, and R. Iglesias, "Retinoids and adipose tissues: metabolism, cell differentiation and gene

expression," *International Journal of Obesity and Related Metabolic Disorders*, vol. 23, no. 1, pp. 1–6, 1999.

[40] F. Villarroya, R. Iglesias, and M. Giralt, "Retinoids and retinoid receptors in the control of energy balance: novel pharmacological strategies in obesity and diabetes," *Current Medicinal Chemistry*, vol. 11, no. 6, pp. 795–805, 2004.

[41] A. Chawla and M. A. Lazar, "Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 5, pp. 1786–1790, 1994.

[42] P. Puigserver, F. Vazquez, M. L. Bonet, C. Pico, and A. Palou, "In vitro and in vivo induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid," *Biochemical Journal*, vol. 317, no. pt 3, pp. 827–833, 1996.

[43] M. V. Kumar, G. D. Sunvold, and P. J. Scarpace, "Dietary vitamin A supplementation in rats: suppression of leptin and induction of UCP1 mRNA," *Journal of Lipid Research*, vol. 40, no. 5, pp. 824–829, 1999.

[44] M. L. Bonet, J. Oliver, C. Pico, et al., "Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression," *Journal of Endocrinology*, vol. 166, no. 3, pp. 511–517, 2000.

[45] R. Alvarez, M. Checa, S. Brun, et al., "Both retinoic-acid-receptor- and retinoid-X-receptor-dependent signalling pathways mediate the induction of the brown-adipose-tissue-uncoupling-protein-1 gene by retinoids," *Biochemical Journal*, vol. 345, no. pt 1, pp. 91–97, 2000.

[46] S. Kitareewan, L. T. Burka, K. B. Tomer, et al., "Phytol metabolites are circulating dietary factors that activate the nuclear receptor RXR," *Molecular Biology of the Cell*, vol. 7, no. 8, pp. 1153–1166, 1996.

[47] P. Ellinghaus, C. Wolfrum, G. Assmann, F. Spener, and U. Seedorf, "Phytanic acid activates the peroxisome proliferator-activated receptor α (PPARα) in sterol carrier protein 2-/ sterol carrier protein x-deficient mice," *Journal of Biological Chemistry*, vol. 274, no. 5, pp. 2766–2772, 1999.

[48] A. Schluter, M. J. Barberá, R. Iglesias, M. Giralt, and F. Villarroya, "Phytanic acid, a novel activator of uncoupling protein-1 gene transcription and brown adipocyte differentiation," *Biochemical Journal*, vol. 362, no. pt 1, pp. 61–69, 2002.

[49] A. Schluter, M. Giralt, R. Iglesias, and F. Villarroya, "Phytanic acid, but not pristanic acid, mediates the positive effects of phytol derivatives on brown adipocyte differentiation," *FEBS Letters*, vol. 517, no. 1–3, pp. 83–86, 2002.

[50] F. Bouillaud, F. Villarroya, E. Hentz, S. Raimbault, A. M. Cassard, and D. Ricquier, "Detection of brown adipose tissue uncoupling protein mRNA in adult patients by a human genomic probe," *Clinical Science*, vol. 75, no. 1, pp. 21–27, 1988.

[51] M. L. Rodriguez de la Concepcion, J. C. Domingo, P. Domingo, M. Giralt, and F. Villarroya, "Uncoupling protein 1 gene expression implicates brown adipocytes in highly active antiretroviral therapy-associated lipomatosis," *AIDS*, vol. 18, no. 6, pp. 959–960, 2004.

[52] D. S. Weigle, L. E. Selfridge, M. W. Schwartz, et al., "Elevated free fatty acids induce uncoupling protein 3 expression in muscle: a potential explanation for the effect of fasting," *Diabetes*, vol. 47, no. 2, pp. 298–302, 1998.

[53] S. Brun, M. C. Carmona, T. Mampel, et al., "Uncoupling protein-3 gene expression in skeletal muscle during development is regulated by nutritional factors that alter circulating non-esterified fatty acids," *FEBS Letters*, vol. 453, no. 1-2, pp. 205–209, 1999.

[54] P. Schrauwen, M. K. Hesselink, I. Vaartjes, et al., "Effect of acute exercise on uncoupling protein 3 is a fat metabolism-mediated effect," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 282, no. 1, pp. E11–E17, 2002.

[55] C. J. Chou, M. C. Cha, D. W. Jung, C. N. Boozer, S. A. Hashim, and F. X. Pi-Sunyer, "High-fat diet feeding elevates skeletal muscle uncoupling protein 3 levels but not its activity in rats," *Obesity Research*, vol. 9, no. 5, pp. 313–319, 2001.

[56] S. Brun, M. C. Carmona, T. Mampel, et al., "Activators of peroxisome proliferator-activated receptor-α induce the expression of the uncoupling protein-3 gene in skeletal muscle: a potential mechanism for the lipid intake-dependent activation of uncoupling protein-3 gene expression at birth," *Diabetes*, vol. 48, no. 6, pp. 1217–1222, 1999.

[57] N. Pedraza, G. Solanes, M. C. Carmona, et al., "Impaired expression of the uncoupling protein-3 gene in skeletal muscle during lactation: fibrates and troglitazone reverse lactation-induced downregulation of the uncoupling protein-3 gene," *Diabetes*, vol. 49, no. 7, pp. 1224–1230, 2000.

[58] O. Boss, E. Bobbioni-Harsch, F. Assimacopoulos-Jeannet, et al., "Uncoupling protein-3 expression in skeletal muscle and free fatty acids in obesity," *Lancet*, vol. 351, no. 9120, p. 1933, 1998.

[59] A. J. Murray, R. E. Anderson, G. C. Watson, G. K. Radda, and K. Clarke, "Uncoupling proteins in human heart," *Lancet*, vol. 364, no. 9447, pp. 1786–1788, 2004.

[60] D. Kratky, J. G. Strauss, and R. Zechner, "Tissue-specific activity of lipoprotein lipase in skeletal muscle regulates the expression of uncoupling protein 3 in transgenic mouse models," *Biochemical Journal*, vol. 355, no. pt 3, pp. 647–652, 2001.

[61] A. Cabrero, M. Alegret, R. Sanchez, T. Adzet, J. C. Laguna, and M. Vazquez, "Uncoupling protein-3 mRNA up-regulation in C2C12 myotubes after etomoxir treatment," *Biochimica et Biophysica Acta*, vol. 1532, no. 3, pp. 195–202, 2001.

[62] M. E. Young, S. Patil, J. Ying, et al., "Uncoupling protein 3 transcription is regulated by peroxisome proliferator-activated receptor (α) in the adult rodent heart," *FASEB Journal*, vol. 15, no. 3, pp. 833–845, 2001.

[63] D. M. Muoio, P. S. MacLean, D. B. Lang, et al., "Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice. Evidence for compensatory regulation by PPAR δ," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26089–26097, 2002.

[64] A. J. Murray, M. Panagia, D. Hauton, G. F. Gibbons, and K. Clarke, "Plasma free fatty acids and peroxisome proliferator-activated receptor α in the control of myocardial uncoupling protein levels," *Diabetes*, vol. 54, no. 12, pp. 3496–3502, 2005.

[65] N. Pedraza, M. Rosell, J. Villarroya, et al., "Developmental and tissue-specific involvement of PPAR α in the control of mouse uncoupling protein-3 gene expression," *Endocrinology*, vol. 147, no. 10, pp. 4695–704, 2006.

[66] B. N. Finck, J. J. Lehman, T. C. Leone, et al., "The cardiac phenotype induced by PPARα overexpression mimics that caused by diabetes mellitus," *Journal of Clinical Investigation*, vol. 109, no. 1, pp. 121–130, 2002.

[67] B. N. Finck, C. Bernal-Mizrachi, D. H. Han, et al., "A potential link between muscle peroxisome proliferator-activated receptor-α signaling and obesity-related diabetes," *Cell Metabolism*, vol. 1, no. 2, pp. 133–144, 2005.

[68] A. Cabrero, G. Llaverias, N. Roglans, et al., "Uncoupling protein-3 mRNA levels are increased in white adipose tissue

and skeletal muscle of bezafibrate-treated rats," *Biochemical and Biophysical Research Communications*, vol. 260, no. 2, pp. 547–556, 1999.

[69] G. Solanes, N. Pedraza, R. Iglesias, M. Giralt, and F. Villarroya, "The human uncoupling protein-3 gene promoter requires MyoD and is induced by retinoic acid in muscle cells," *FASEB Journal*, vol. 14, no. 14, pp. 2141–2143, 2000.

[70] I. Nagase, S. Yoshida, X. Canas, et al., "Up-regulation of uncoupling protein 3 by thyroid hormone, peroxisome proliferator-activated receptor ligands and 9-cis retinoic acid in L6 myotubes," *FEBS Letters*, vol. 461, no. 3, pp. 319–322, 1999.

[71] C. Son, K. Hosoda, J. Matsuda, et al., "Up-regulation of uncoupling protein 3 gene expression by fatty acids and agonists for PPARs in L6 myotubes," *Endocrinology*, vol. 142, no. 10, pp. 4189–4194, 2001.

[72] I. Lopez-Solache, V. Marie, E. Vignault, A. Camirand, and J. E. Silva, "Regulation of uncoupling protein-2 mRNA in L6 myotubules: I: thiazolidinediones stimulate uncoupling protein-2 gene expression by a mechanism requiring ongoing protein synthesis and an active mitogen-activated protein kinase," *Endocrine*, vol. 19, no. 2, pp. 197–208, 2002.

[73] Y. X. Wang, C. L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[74] S. Luquet, J. Lopez-Soriano, D. Holst, et al., "Peroxisome proliferator-activated receptor δ controls muscle development and oxidative capability," *FASEB Journal*, vol. 17, no. 15, pp. 2299–2301, 2003.

[75] L. Cheng, G. Ding, Q. Qin, et al., "Cardiomyocyte-restricted peroxisome proliferator-activated receptor-δ deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy," *Nature Medicine*, vol. 10, no. 11, pp. 1245–1250, 2004.

[76] U. Dressel, T. L. Allen, J. B. Pippal, P. R. Rohde, P. Lau, and G. E. Muscat, "The peroxisome proliferator-activated receptor β/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells," *Molecular Endocrinology*, vol. 17, no. 12, pp. 2477–2493, 2003.

[77] T. Tanaka, J. Yamamoto, S. Iwasaki, et al., "Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15924–15929, 2003.

[78] G. Solanes, N. Pedraza, R. Iglesias, M. Giralt, and F. Villarroya, "Functional relationship between MyoD and peroxisome proliferator-activated receptor-dependent regulatory pathways in the control of the human uncoupling protein-3 gene transcription," *Molecular Endocrinology*, vol. 17, no. 10, pp. 1944–1958, 2003.

[79] F. Felipe, M. L. Bonet, J. Ribot, and A. Palou, "Up-regulation of muscle uncoupling protein 3 gene expression in mice following high fat diet, dietary vitamin A supplementation and acute retinoic acid-treatment," *International Journal of Obesity and Related Metabolic Disorders*, vol. 27, no. 1, pp. 60–69, 2003.

[80] F. Villarroya, "Mitochondrial uncoupling protein-3: beyond thermoregulation," in *Recent Research Developments in Biochemistry*, S. Pandalai, Ed., vol. 3, pp. 691–704, Research Signpost, Kerala, India, 2002.

[81] P. Schrauwen, J. Xia, K. Walder, S. Snitker, and E. Ravussin, "A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians," *International Journal of Obesity and Related Metabolic Disorders*, vol. 23, no. 12, pp. 1242–1245, 1999.

[82] A. Cabrero, M. Alegret, R. M. Sanchez, T. Adzet, J. C. Laguna, and M. Vazquez, "Down-regulation of uncoupling protein-3 and -2 by thiazolidinediones in C2C12 myotubes," *FEBS Letters*, vol. 484, no. 1, pp. 37–42, 2000.

[83] C. S. Hwang and M. D. Lane, "Up-regulation of uncoupling protein-3 by fatty acid in C2C12 myotubes," *Biochemical and Biophysical Research Communications*, vol. 258, no. 2, pp. 464–469, 1999.

[84] T. Shimokawa, M. Kato, Y. Watanabe, et al., "In vivo effects of pioglitazone on uncoupling protein-2 and -3 mRNA levels in skeletal muscle of hyperglycemic KK mice," *Biochemical and Biophysical Research Communications*, vol. 251, no. 1, pp. 374–378, 1998.

[85] B. Brunmair, F. Gras, L. Wagner, et al., "Expression of uncoupling protein-3 mRNA in rat skeletal muscle is acutely stimulated by thiazolidinediones: an exercise-like effect?" *Diabetologia*, vol. 47, no. 9, pp. 1611–1614, 2004.

[86] A. L. Hevener, W. He, Y. Barak, et al., "Muscle-specific Pparg deletion causes insulin resistance," *Nature Medicine*, vol. 9, no. 12, pp. 1491–1497, 2003.

[87] E. Chevillotte, J. Rieusset, M. Roques, M. Desage, and H. Vidal, "The regulation of uncoupling protein-2 gene expression by ω-6 polyunsaturated fatty acids in human skeletal muscle cells involves multiple pathways, including the nuclear receptor peroxisome proliferator-activated receptor β," *Journal of Biological Chemistry*, vol. 276, no. 14, pp. 10853–10860, 2001.

[88] A. V. Medvedev, S. K. Snedden, S. Raimbault, D. Ricquier, and S. Collins, "Transcriptional regulation of the mouse uncoupling protein-2 gene," *Journal of Biological Chemistry*, vol. 276, no. 14, pp. 10817–10823, 2001.

[89] J. Matsuda, K. Hosoda, H. Itoh, et al., "Increased adipose expression of the uncoupling protein-3 gene by thiazolidinediones in Wistar fatty rats and in cultured adipocytes," *Diabetes*, vol. 47, no. 11, pp. 1809–1814, 1998.

[90] V. Emilsson, J. O'Dowd, S. Wang, et al., "The effects of rexinoids and rosiglitazone on body weight and uncoupling protein isoform expression in the Zucker fa/fa rat," *Metabolism*, vol. 49, no. 12, pp. 1610–1615, 2000.

[91] J. E. Digby, V. E. Crowley, C. P. Sewter, J. P. Whitehead, J. B. Prins, and S. O'Rahilly, "Depot-related and thiazolidinedione-responsive expression of uncoupling protein 2 (UCP2) in human adipocytes," *International Journal of Obesity and Related Metabolic Disorders*, vol. 24, no. 5, pp. 585–592, 2000.

[92] J. Aubert, O. Champigny, P. Saint-Marc, et al., "Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells," *Biochemical and Biophysical Research Communications*, vol. 238, no. 2, pp. 606–611, 1997.

[93] M. C. Carmona, A. Valmaseda, R. Iglesias, et al., "9-cis retinoic acid induces the expression of the uncoupling protein-2 gene in brown adipocytes," *FEBS Letters*, vol. 441, no. 3, pp. 447–450, 1998.

[94] C. Y. Zhang, G. Baffy, P. Perret, et al., "Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes," *Cell*, vol. 105, no. 6, pp. 745–755, 2001.

[95] B. B. Lowell and G. I. Shulman, "Mitochondrial dysfunction and type 2 diabetes," *Science*, vol. 307, no. 5708, pp. 384–387, 2005.

[96] A. V. Medvedev, J. Robidoux, X. Bai, et al., "Regulation of the uncoupling protein-2 gene in INS-1 β-cells by oleic acid,"

Journal of Biological Chemistry, vol. 277, no. 45, pp. 42639–42644, 2002.

[97] H. Oberkofler, K. Klein, T. K. Felder, F. Krempler, and W. Patsch, "Role of the peroxisome proliferator-activated receptor-γ coactivator-1α in the transcriptional regulation of the human uncoupling protein 2 gene in INS-1E cells," *Endocrinology*, vol. 147, no. 2, pp. 966–976, 2006.

[98] Y. T. Zhou, M. Shimabukuro, M. Y. Wang, et al., "Role of peroxisome proliferator-activated receptor-α in disease of pancreatic β cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8898–8903, 1998.

[99] K. Tordjman, K. N. Standley, C. Bernal-Mizrachi, et al., "PPARα suppresses insulin secretion and induces UCP2 in insulinoma cells," *Journal of Lipid Research*, vol. 43, no. 6, pp. 936–943, 2002.

[100] S. Gremlich, C. Nolan, R. Roduit, et al., "Pancreatic islet adaptation to fasting is dependent on peroxisome proliferator-activated receptor-α transcriptional upregulation of fatty acid oxidation," *Endocrinology*, vol. 146, no. 1, pp. 375–382, 2005.

[101] E. Ito, S. Ozawa, K. Takahashi, et al., "PPAR-γ overexpression selectively suppresses insulin secretory capacity in isolated pancreatic islets through induction of UCP-2 protein," *Biochemical and Biophysical Research Communications*, vol. 324, no. 2, pp. 810–814, 2004.

[102] J. Tian, G. Li, Y. Gu, et al., "Role and mechanism of rosiglitazone on the impairment of insulin secretion induced by free fatty acids on isolated rat islets," *Chinese Medical Journal*, vol. 119, no. 7, pp. 574–580, 2006.

[103] P. Steneberg, N. Rubins, R. Bartoov-Shifman, M. D. Walker, and H. Edlund, "The FFA receptor GRP40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse," *Cell metabolism*, vol. 1, no. 4, pp. 245–258, 2005.

[104] D. Larrouy, P. Laharrague, G. Carrera, et al., "Kupffer cells are a dominant site of uncoupling protein 2 expression in rat liver," *Biochemical and Biophysical Research Communications*, vol. 235, no. 3, pp. 760–764, 1997.

[105] T. Nakatani, N. Tsuboyama-Kasaoka, M. Takahashi, S. Miura, and O. Ezaki, "Mechanism for peroxisome proliferator-activated receptor-α activator-induced up-regulation of UCP2 mRNA in rodent hepatocytes," *Journal of Biological Chemistry*, vol. 277, no. 11, pp. 9562–9569, 2002.

[106] R. A. Memon, L. H. Tecott, K. Nonogaki, et al., "Up-regulation of peroxisome proliferator-activated receptor (PPAR-α) and PPAR-γ messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-α-responsive adipose tissue-specific genes in the liver of obese diabetic mice," *Endocrinology*, vol. 141, no. 11, pp. 4021–4031, 2000.

[107] D. Patsouris, J. K. Reddy, M. Muller, and S. Kersten, "Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression," *Endocrinology*, vol. 147, no. 3, pp. 1508–1516, 2006.

[108] N. Tsuboyama-Kasaoka, M. Takahashi, H. Kim, and O. Ezaki, "Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice," *Biochemical and Biophysical Research Communications*, vol. 257, no. 3, pp. 879–885, 1999.

[109] M. B. Armstrong and H. C. Towle, "Polyunsaturated fatty acids stimulate hepatic UCP-2 expression via a PPARα-mediated pathway," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 281, no. 6, pp. E1197–E1204, 2001.

[110] H. J. Grav, K. J. Tronstad, O. A. Gudbrandsen, et al., "Changed energy state and increased mitochondrial β-oxidation rate in liver of rats associated with lowered proton electrochemical potential and stimulated uncoupling protein 2 (UCP-2) expression," *Journal of Biological Chemistry*, vol. 278, no. 33, pp. 30525–30533, 2003.

[111] A. Lanni, F. P. Mancini, L. Sabatino, et al., "De novo expression of uncoupling protein 3 is associated to enhanced mitochondrial thioesterase-1 expression and fatty acid metabolism in liver of fenofibrate-treated rats," *FEBS Letters*, vol. 525, no. 1–3, pp. 7–12, 2002.

[112] E. Silvestri, P. De Lange, M. Moreno, et al., "Fenofibrate activates the biochemical pathways and the novo expression of genes related to lipid handling and uncoupling protein-3 functions in liver of normal rats," *Biochimica et Biophysica Acta*, vol. 1757, no. 5-6, pp. 486–495, 2006.

[113] C. Hurtaud, C. Gelly, F. Bouillaud, and C. Lévi-Meyrueis, "Translation control of UCP2 synthesis by upstream open reading frame," *Cellular and Molecular Life Sciences*, vol. 63, no. 15, pp. 1780–1789, 2006.

[114] C. Fleury, M. Neverova, S. Collins, et al., "Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia," *Nature Genetics*, vol. 15, no. 3, pp. 269–272, 1997.

[115] A. C. Li and W. Palinski, "Peroxisome proliferators-activated receptors: how their effects in macrophages can lead to the development of new drug therapy against atherosclerosis," *Annual Review of Pharmacology and Toxicology*, vol. 46, pp. 1–39, 2006.

[116] T. E. Akiyama, S. Sakai, G. Lambert, et al., "Conditional disruption of the peroxisome proliferator-activated receptor γ gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux," *Molecular and Cellular Biology*, vol. 22, no. 8, pp. 2607–2619, 2002.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 94156, 12 pages
doi:10.1155/2007/94156

Review Article

Therapeutic Potential of Retinoid X Receptor Modulators for the Treatment of the Metabolic Syndrome

Jane A. Pinaire and Anne Reifel-Miller

Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 2 November 2006; Revised 4 January 2007; Accepted 4 January 2007

Recommended by Sander Kersten

The increasing prevalence of obesity is a fundamental contributor to the growing prevalence of the metabolic syndrome. Rexinoids, a class of compounds that selectively bind and activate RXR, are being studied as a potential option for the treatment of metabolic syndrome. These compounds have glucose-lowering, insulin-sensitizing, and antiobesity effects in animal models of insulin resistance and type 2 diabetes. However, undesirable side effects such as hypertriglyceridemia and suppression of the thyroid hormone axis also occur. This review examines and compares the effects of four RXR-selective ligands: LGD1069, LG100268, AGN194204, and LG101506, a selective RXR modulator. Similar to selective modulators of other nuclear receptors such as the estrogen receptor (SERMs), LG101506 binding to RXR selectively maintains the desirable characteristic effects of rexinoids while minimizing the undesirable effects. These recent findings suggest that, with continued research efforts, RXR-specific ligands with improved pharmacological profiles may eventually be available as additional treatment options for the current epidemic of obesity, insulin resistance, type 2 diabetes, and all of the associated metabolic sequelae.

1. INTRODUCTION

The nuclear receptor (NR) family of transcription factors (also referred to as the steroid/thyroid hormone receptor superfamily) is quite large with approximately 150 proteins. This large superfamily may be categorized into three subgroups: *classic hormone receptors* such as the glucocorticoid, estrogen, retinoic acid, thyroid hormone, and vitamin D receptors; *"sensor" receptors* such as the peroxisome proliferator-activated receptors (PPARs), the liver X receptor (LXR), the farnesol X receptor (FXR), and the retinoid X receptor (RXR); and *orphan receptors* such as apolipoprotein A-I regulatory protein-1 (ARP-1) and chicken ovalbumin upstream promoter transcription factor (COUP-TF). In general, these categories describe characteristics of ligand binding to these receptors. Ligands specific for the orphan receptors, if any exist at all, have not been identified to date. Classic hormone receptors bind specific ligands with high affinity. On the other hand, a broad range of lipophilic molecules is thought to bind to the "sensor" receptors, generally with broader specificity and lower affinity as compared to hormones that bind to the classical hormone receptors. The majority of the members of this family regulates transcription of target genes by binding as homodimers or heterodimers to specific DNA sequences, called hormone response elements (HREs) or nuclear receptor responsive elements (NR-REs). For the classic hormone receptors and the sensor receptors, ligand binding induces (or stabilizes) DNA binding and modulation of target gene transcription [1].

RXR is considered "promiscuous" because it forms heterodimers with several other family members that can be further classified as permissive or nonpermissive binding partners. Heterodimers formed by RXR and permissive binding partners (PPARs, LXR, and FXR) can be activated by RXR-specific ligands or by ligands specific for the binding partner. Heterodimers formed by RXR and nonpermissive partners (vitamin D and thyroid hormone receptors) can only be activated by ligands specific for the partner, but not by ligands specific for RXR [1]. However, recent evidence suggests that the concept of NR "permissivity" may require reexamination. For example, RXR has been considered a silent partner in the RXR:TR heterodimer, yet recent data indicate that this is not always true and the ability of RXR to influence the activity of the heterodimer may depend on factors such as tissue specificity, the cellular environment, or the ability of various RXR ligands to recruit coactivator or corepressor

complexes to the region of the NRRE [2–4]. On the other hand, the activity of the RXR:FXR heterodimer, previously considered permissive, has recently been shown to be antagonized by ligand binding to RXR. These findings have led to the term "conditionally permissive" in describing NR heterodimers containing RXR [5].

Recent reviews have highlighted the important role of transcription and various transcription factor families, including the NRs, in the regulation of intermediary metabolism [1, 6]. Shulman and Mangelsdorf [7] recently reviewed literature demonstrating that the metabolic syndrome could be treated by altering the activity of NR heterodimers containing RXR and partners PPAR, LXR, FXR, and TR by using ligands specific for PPAR, LXR, FXR, and TR. The aim of our review, on the other hand, is to summarize available data suggesting that the metabolic syndrome could potentially be treated by altering the activity of RXR homo- and heterodimers with rexinoids, a class of compounds that bind selectively to RXR. We begin with an overview of the metabolic syndrome, its strong association with obesity and type 2 diabetes, and its prevalence. We then focus on the metabolic effects and potential therapeutic use of RXR-selective ligands. The last section discusses the utility and safety of these compounds for the treatment of the metabolic syndrome.

2. THE METABOLIC SYNDROME

The metabolic syndrome (also called metabolic syndrome X, syndrome X, insulin resistance syndrome, insulin resistance/hyperinsulinemia syndrome, or metabolic cardiovascular syndrome) has been extensively reviewed (see [8–11] and references therein). Briefly, insulin resistance is associated with a cluster of metabolic abnormalities that increases the risk for type 2 diabetes, cardiovascular and renal diseases, as well as some forms of cancer [8]. This cluster of metabolic abnormalities is strongly associated with obesity, predominantly visceral (abdominal) obesity, and physical inactivity and includes the following: some degree of impaired glucose homeostasis, atherogenic dyslipidemia, hypertension, an enhanced procoagulant state, and increased expression of inflammatory markers. The relationship between obesity, insulin resistance, and cardiovascular disease, recently reviewed by Reaven et al. [9] and Grundy [10], is complicated, exemplified by the fact that not all overweight or obese individuals develop insulin resistance and its associated metabolic abnormalities. However, there is little doubt that the increasing prevalence of overweight and obesity is a fundamental contributor to the rising prevalence of the metabolic syndrome (insulin resistance) and type 2 diabetes [12–14].

In a recent review, Reaven [8] differentiated the terms "insulin resistance syndrome" and "metabolic syndrome." The insulin resistance syndrome, according to Reaven, is a term used to describe a cluster of metabolic abnormalities and related outcomes that occur more commonly in insulin-resistant/hyperinsulinemic individuals, and is not meant to identify a specific clinical entity, nor does it refer to a specific clinical diagnosis. On the other hand, the term "metabolic syndrome" is more often considered a diagnostic tool useful in the clinic for identifying individuals, presumably individuals who are insulin-resistant, who are at increased risk for cardiovascular disease and other associated outcomes. Indeed, in an effort to standardize the diagnostic criteria for the metabolic syndrome, definitions have recently been put forth by multiple national and international organizations: WHO [15, 16], ATP III [17], ACE [18], IDF [19]. The definitions are summarized in Table 1, and their utility and limitations have been compared elsewhere [20]. The clinical measures used in the definitions of the "metabolic syndrome" are typically tests that are feasible and realistic for routine clinical practice. Using the ATP III criteria, the prevalence of the metabolic syndrome has continued to increase among US adults from 28.0% as reported in the NHANES III (1988–1994) to 31.9% in the NHANES 1999-2000 [21]. Most importantly, a recent 2004 analysis revealed that the prevalence of metabolic syndrome in US children and adolescents reached 38.7% in moderately obese children and 49.7% in severely obese children [22]. These data are sobering in light of the prevalence of metabolic syndrome reported in adolescents from NHANES III (1988–1994), 6.8% among overweight adolescents and 28.7% among obese adolescents [23].

Although the definitions for the clinical diagnosis of the metabolic syndrome continue to evolve, researchers and clinicians agree on the fundamental concept of the insulin resistance/metabolic syndrome as a cluster of metabolic derangements that increase risk for type 2 diabetes, cardiovascular disease, renal disease, and other associated outcomes.

3. THE RETINOID-X-RECEPTOR

The NR family of transcription factors has been extensively reviewed [1]. Briefly, there are 3 RXR isotypes: RXRα, RXRβ, and RXRγ. Each isotype is encoded by a distinct gene, and each gene is capable of generating at least 2 distinct transcripts due to alternative promoter utilization and alternative splicing. The isotypes exhibit different patterns of tissue-specific expression. RXRα is expressed in liver, kidney, spleen, placenta, and the epidermis; RXRβ is expressed ubiquitously; and RXRγ is expressed in skeletal muscle and cardiac muscle, the anterior pituitary, and to a lesser extent in brain. Importantly, the pattern of tissue-specific expression varies widely during development. The homology of the isotypes suggests that these receptors regulate common target sequences and respond to common ligands [24, 25].

Consistent with other members of the NR family, each of the RXR isotypes has a modular domain structure [1, 24–26]. The most highly conserved region, region C, is the DNA-binding domain containing 2 zinc finger motifs, the hallmark characteristic of members of this transcription factor family. Region E, the second most conserved region, contains the ligand binding domain (LBD), the primary dimerization domain, and the ligand-dependent transcriptional activation function (AF-2). Furthermore, the LBD is the region of the receptor that binds transcriptional corepressor or coactivator complexes that mediate the effect of the receptor on

Table 1: Comparison of definitions of the metabolic syndrome.

Metabolic parameter	WHO [15, 16]	ATP III [17]	ACE‡ [18]	IDF [19]
Elevated TG (mg/dL)	≥ 150	≥ 150	≥ 150	≥ 150 or treatment for elevated TG
Low HDL-C* (mg/dL)	< 39 (female) < 35 (male)	< 50 (female) < 40 (male)	< 50 (female) < 40 (male)	< 50 (female) < 40 (male) or treatment for low HDL-C
Elevated blood pressure (mm Hg)	≥ 140/90	≥ 130/85	≥ 130/85	≥ 130/85 or treatment for previously diagnosed HTN
Elevated fasting glucose (mg/dL)	—	≥ 110	110–125	≥ 100 or previously diagnosed diabetes
Elevated 2-hour post-challenge glucose (mg/dL)	—	—	> 140	—
Waist circumference* (cm)	—	> 88 (female) > 102 (male)	—	—
Waist-to-hip ratio*	> 0.85 (female) > 0.90 (male)	—	—	—
High BMI	> 30 kg/m2	—	Obesity is included in a list of factors that increase the likelihood of insulin resistance**	—
Microalbuminuria	≥ 20 μg/min or albumin-to-creatinine ratio ≥ 30 mg/g	—	—	—
Definition	Diabetes, impaired fasting glucose, impaired glucose tolerance, or insulin resistance plus 2 or more of the above	Three or more of the above	Risk factors** plus two or more of the above	Central obesity (ethnic-specific cut points) ≥ 94 cm (female) ≥ 80 cm (male) plus two or more of the above

Table modified from [12, 21]. Abbreviations: WHO, World Health Organization; ATP III, National Cholesterol Education Program Adult Treatment Panel III; ACE, American College of Endocrinology; IDF, International Diabetes Federation; HTN, hypertension.
‡ ACE uses term "insulin resistance syndrome"
*Gender-specific parameters
**Risk factors include overweight (BMI > 25 kg/m2 or waist circumference > 40 inches for men and > 35 inches for women), sedentary lifestyle, age > 40 years, non-Caucasian, family history of type 2 diabetes, hypertension, or cardiovascular disease, and personal history of polycystic ovarian syndrome, gestational diabetes, acanthosis nigricans, or nonalcoholic steatohepatitis.

transcription. The N-terminal A/B region contains a ligand-independent transcriptional activation function (AF-1). The D region is the hinge domain, allowing the DNA binding domain and the LBD to rotate. RXR homodimers and heterodimers bind to DNA targets comprised of 2 consensus hexamer motifs, or half sites, such as PuG(G/T)TCA(X), separated by a short spacer. The arrangement of these half sites and the length of the spacing between them determine the specificity of the response elements for RXR homo- or heterodimers [1, 24–26].

RXR has been shown to have diverse physiological functions using RXR knockout (KO) mouse models (reviewed in [24, 25]). Loss of RXRα results in much more severe phenotype than the loss of either RXRβ or RXRγ. Loss of RXRα function in the mouse germ line results in embryonic lethality (E13.5–16.5) due to defects of the cardiac ventricles and placenta, as well as ocular abnormalities. Additional functions of RXRα have been identified when the receptor has been selectively deleted from specific tissues in mature animals. Loss of RXRα function in adipose tissue results in altered preadipocyte differentiation and resistance to obesity, though this is thought to be attributable to the absence of functional RXRα/PPARγ heterodimers in adipose tissue. Loss of RXRα function in skin results in multiple phenotypic characteristics such as alopecia, hair follicle degeneration, and dermal cysts, and although some of these phenotypic characteristics are similar to the loss of function of the vitamin D receptor (VDR−/−), there are abnormalities in epidermal proliferation and differentiation in the RXRα−/− model that are not accounted for in the VDR−/− model. Loss of RXRα in the liver perturbs multiple metabolic pathways mediated by LXRα, PPARα, CARβ, PXR, and FXR. Interestingly, many, but not all, of the defects in lipid metabolism in liver-specific RXRα−/− mice are similar to those in LXR−/− mice, suggesting that RXRα:LXR plays an important role in hepatic lipid metabolism. The phenotype of RXRα−/− in liver also shows similarities to PPARα−/− mice, suggesting that the phenotype in each is attributable to a loss of functional RXRα:PPARα heterodimers. Furthermore, when RXRα is absent in the liver of adult mice,

the regenerative capacity of hepatocytes is impaired, and the hepatocytes have a shorter lifespan compared to wild-type animals. Absence of RXRα in prostate produces a marked alteration in the profile of secretory proteins as well as preneoplastic lesions (reviewed in [24, 25]).

Loss of either RXRβ or RXRγ in the mouse germ line is not embryonic lethal. Approximately 50% of RXR$\beta-/-$ mice die before or at birth, but the animals that do survive appear to be normal except that the males are sterile. RXR$\gamma-/-$ mice develop normally and appear similar to wild-type animals, except they have higher serum T4 and TSH levels and higher metabolic rates compared to wild-type animals (reviewed in [24, 25]).

Because RXR has been shown to play a role in diverse physiological processes including cell proliferation, differentiation, and apoptosis and metabolism, RXR isotypes have been referred to as "master regulators" [24, 25]. Therefore, compounds that alter the activity of RXR also have the potential to alter multiple physiological and metabolic pathways, with the potential of both beneficial and deleterious effects. Indeed, in animal models of insulin resistance and diabetes, rexinoids have been shown to have beneficial glucose-lowering, insulin-sensitizing, and antiobesity effects, while at the same time raising triglyceride (TG) levels and suppressing the thyroid hormone axis, side effects that have limited the development of these compounds as therapeutic agents for the treatment of type 2 diabetes and insulin resistance. In the following sections we review the metabolic effects of four different rexinoids, one of which is a selective RXR modulator.

4. EFFICACY OF REXINOIDS AS THERAPEUTIC AGENTS

4.1. LGD1069 (Bexarotene; Targretin, Ligand Pharmaceuticals Inc., Calif, USA)

LGD1069 was the first compound found to be a potent and highly specific ligand for RXR [27]. LGD1069 has poor binding affinity for RAR isoforms α, β, or γ (Kd >1000 nM for all isoforms). On the other hand, LGD1069 binds with high affinity to RXR α, β, and γ: Kd values are 14 ± 3, 21 ± 4, and 29 ± 7 nM, respectively, see [28]. LGD1069 is used clinically for the treatment of cutaneous T-cell lymphoma, though its use for treating other cancers is currently being investigated [29]. LGD1069 has been shown to lower glucose and insulin levels in the ob/ob mouse to degrees similar to rosiglitazone (ROSI) [30]. At 53 days of age, the average fasting glucose and insulin concentrations in ob/ob mice are 262 mg/dL and 12–18 ng/ml, respectively. Over the course of 2 weeks, this hyperglycemia and hyperinsulinemia continues to worsen. Treatment of 53-day old female ob/ob mice with LGD1069 resulted in a 45% reduction of fasting plasma glucose (55% reduction with ROSI) and a 30% reduction of fasting plasma insulin (15% reduction with ROSI) [30]. Most of the published data regarding LGD1069 describes effects on lipids and TG, and that data is reviewed in following sections.

4.2. LG100268

As a selective ligand for RXR, LG100268 (LG268) has poor binding affinity for RAR isoforms α, β, or γ (Ki >1000 nM for all isoforms). On the other hand, LG268 binds with high affinity to RXR α, β, and γ: Ki values are 3.2, 6.2, and 9.7 nM, respectively, see [31].

4.2.1. Effects on glucose and insulin resistance

LG268 has also been shown to lower glucose and insulin levels in the ob/ob mouse. Treatment of 53-day old female ob/ob mice with LG268 for 2 weeks resulted in a 48% reduction of fasting plasma glucose (55% reduction with ROSI) and a 59% reduction of fasting plasma insulin (15% reduction with ROSI) [30]. A glucose/insulin tolerance test showed significant reductions of both the area under the glucose curve and the area under the insulin curve with LG268, and insulin resistance was reduced approximately 75% [30].

By the age of 16 weeks, the C57BL/KsJ strain of db/db mice demonstrates a number of characteristics of progressed type 2 diabetes and pancreatic β-cell dysfunction; compared to 6-week old animals, serum insulin levels are nearly 10-fold lower and serum glucose levels and glycohemoglobin are nearly doubled. Serum glucose, glycohemoglobin, and fibrinogen, commonly elevated in insulin resistance, were reduced similarly by ROSI and LG268, and insulin content in pancreatic islets increased significantly with both compounds. The effects of ROSI and LG268 on body and organ weights were compared. Between weeks 14 and 16, mean body weight change in vehicle-, ROSI-, and LG268-treated animals was −2.7, +3.2, and +0.5 grams, respectively. Unlike ROSI, LG268 did not induce adipose tissue hypertrophy. Furthermore, LG268 produced several effects on the liver not observed with ROSI: hepatomegaly, increased peroxisome number, fatty infiltration, and induced expression of microsomal lauric acid hydroxylase. Therefore, compared with ROSI, LG268 had very similar effects on glucose-lowering, pancreatic insulin content, and serum fibrinogen levels, but distinctly different effects on body weight and liver, effects likely mediated via the RXR:PPARα pathway [32].

To further distinguish the mechanism of rexinoids as glucose-lowering and insulin sensitizing compounds from that of TZDs, Ahuja et al. examined the effect of LG268 on mRNA levels of three TZD-inducible genes shown to contain a PPRE for the RXR:PPARγ heterodimer: mitochondrial carnitine palmitoyl transferase (MCPT), steroyl coenzyme A desaturase 1 (SCD1), and fatty acid translocase (FAT). Message levels were compared in adipose tissue, skeletal muscle, and liver. To summarize, both compounds induced mRNA of all three genes: ROSI induced message levels in adipose tissue and LG268 induced the mRNAs in liver. The compounds had similar actions in skeletal muscle. Similar to what was reported by Lenhard et al. [32], ROSI and LG268 have similar effects on glucose-lowering, though their mechanisms may be quite distinct. The authors concluded that rexinoids do not function as simple "TZD mimetics" in vivo, as previously suggested, and though the RXR:PPAR heterodimer has

been shown to be permissive using in vitro transfection experiments with synthetic reporter constructs, rexinoids have a pharmacological profile distinct from TZDs [33].

To further investigate how rexinoids and TZDs differ in mediating their glucose-lowering and insulin-sensitizing effects, Shen et al. compared the effects of ROSI and LG268 on insulin signaling in muscle of db/db mice [34]. As reported by the other studies, ROSI and LG268 had similar effects on glucose-lowering. However, ROSI and LG268 were demonstrated to have distinctly different effects on components of the insulin signaling pathway in muscle. In quadricep, neither compound had any effect on insulin receptor mRNA or protein levels, or on insulin receptor tyrosine phosphorylation. However, while ROSI increased expression (mRNA and protein) of c-Cbl-associated protein (CAP), LG268 induced insulin-stimulated tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1). In extensor digitorum longus (EDL) muscle, ROSI induced both basal and insulin-stimulated tyrosine phosphorylation of C-Cbl, while LG268 induced insulin-stimulated Akt phosphorylation. In addition, LG268 suppressed IRS-1 Ser307 phosphorylation, which has been implicated in insulin resistance. Taken together, these data provided clear evidence that TZDs and rexinoids exert their effects through different mechanisms. In muscle, TZDs mediated their effects through the CAP/c-Cbl pathway, and rexinoids exerted their effects through the IRS-1/Akt pathway, suggesting that LG268 does not mediate its effects in muscle via binding to the RXR:PPARγ heterodimer. Although LXR ligands have effects similar to rexinoids, the authors point out that this too is unlikely because LXR ligands had no effect on Akt phosphorylation in these animals. Therefore, the authors speculate that LG268 may mediate its effects in muscle through PPARα or PPARδ, or possibly through the RXR:RXR homodimer [34].

4.2.2. Effects on obesity

Three studies using the Zucker fatty rat model have demonstrated antiobesity effects of rexinoids [35–37]. Emilsson et al. compared the effects of ROSI and LG268 on food consumption, body weight, and the expression of uncoupling protein (UCP) isoforms UCP-1, UCP-2, and UCP-3 [37]. ROSI significantly increased food consumption, but had no significant effect on body weight change. In contrast, LG268 significantly decreased food consumption and body weight. UCP-1 mRNA in brown adipose tissue (BAT) was significantly induced by LG268, but not by ROSI. Neither ROSI nor LG268 had any effect on UCP-2 mRNA in BAT, white adipose tissue (WAT), muscle, or brain. UCP-3 mRNA was induced by ROSI and LG268 in BAT and WAT, but in muscle only ROSI induced UCP-3. Thus, in the Zucker fatty rat, rexinoids have antiobesity effects, whereas TZDs do not. Furthermore, the authors speculated that LG268 may promote thermogenesis due to the upregulation of UCP-1 in BAT [37].

To further investigate the mechanisms underlying the antiobesity effects of rexinoids, Ogilvie et al. [36] evaluated body weight and cumulative food consumption over 42 days in female Zucker fatty rats treated with ROSI or LG268. LG268 significantly reduced body weight and cumulative food consumption whereas ROSI increased both body weight and consumed food. Body composition analysis revealed that ROSI increased fat mass, but LG268 reduced fat mass in both fatty and lean animals. Neither compound had an effect on lean body mass. The effects of ROSI and LG268 on adipogenesis and apoptosis in subcutaneous and mesenteric (ovarian) fat were compared. In ovarian fat, there was no difference between ROSI and LG268 in their effects on apoptosis and adipogenesis. In contrast, in subcutaneous fat, LG268 more strongly induced apoptosis and was a weaker inducer of adipogenesis compared with ROSI, suggesting that the LG268-mediated reduction of fat mass was at least in part due to increased apoptosis in subcutaneous adipose tissue. To determine whether decreased food consumption and body weight observed with LG268 treatment was due to adverse toxicological effects, dynamic feeding behavior before, during, and after treatment with LG268 was examined. The authors hypothesized that if LG268 had adverse toxicological effects, the animals would forgo meals (decreased meal frequency); on the other hand, if LG268 truly had antiobesity properties, the animals would become satiated after consuming less food (decreased food consumption). Indeed, LG268 was associated with decreased meal size. Finally, because RXR is present in hypothalamic satiety centers, the authors tested whether LG268 acts centrally in the brain. Injection of LG268 directly into the cerebral ventricles produced reductions of cumulative body weight gain and daily food consumption similar to that observed with oral administration of LG268, suggesting that LG268 mediated its antiobesty effects through the central nervous system. Most surprising was the observation that the TG-raising effect of LG268 (discussed in greater detail below) was abolished with ICV administration. These observations led the authors to conclude that LG268 not only regulates feeding behavior and body weight in a manner completely distinct from TZDs, through the central nervous system, but that the antiobesity action of rexinoids may be separable from its effects on TG [36].

4.3. AGN194204

As a selective ligand for RXR, AGN194204 has poor binding affinity for RAR isoforms α, β, or γ (Kd > 30 K nM for all isoforms). On the other hand, AGN194204 binds with high affinity to RXR α, β, and γ: Kd values are 0.4, 3.6, and 3.8 nM, respectively, see [38].

4.3.1. Effects on glucose and insulin resistance

Li et al. [39] examined the metabolic effects of AGN194204 in female Zucker diabetic fatty rats (ZFF rats). After being fed a high-fat diet (48% fat and 16% protein) for 3-4 weeks, these animals were treated with troglitazone (TROG) or AGN194204. AGN194204 and TROG had very similar effects on lowering serum glucose and insulin. A hyperinsulinemic-euglycemic clamp indicated that the insulin-sensitizing effects of AGN194204 occurred predominantly in the liver, while TROG exerted its insulin-sensitizing effect in the liver

Table 2: Summary of the effects of RXR-specific ligands on glucose, insulin resistance, and obesity.

RXR ligand	Effects on glucose [references]	Effects on insulin or insulin resistance [references]	Effects on body weight [references]	Effects on insulin signalling [references]
LGD1069	↓ [30]	↓ [30]	No change [30]	
LG100268	↓ [30, 32–34, 40, 41]	No change [32] ↓ [30, 33–36, 40]	No change [30, 32, 34] ↓ [35–37, 40]	IRS-1/Akt pathway and decreased IRS-1 Ser307 phosphorylation in muscle [34]
AGN194204	↓ [39]	↓ [39]	No change [42]; ↑ [39]	↑ IRS-2 protein expression before and after insulin treatment; increase in insulin-stimulated Akt phophorylation in liver [39]
LG101506	↓ [40]	↓ [40]	No change [40]	

and in peripheral tissues. The effects of AGN194204 and TROG on components of the insulin signaling pathway in liver and skeletal muscle were compared. In liver, AGN194204 increased IRS-2 protein levels before and after treatment with insulin, and increased insulin-stimulated Akt phosphorylation following insulin treatment. In skeletal muscle, TROG stimulated Akt phosphorylation and produced a small but consistent increase in IRS-1 protein before and after insulin treatment. These results are consistent with liver being the primary target of insulin-sensitizing effect of AGN194204, and with skeletal muscle being an important target of the insulin-sensitizing effects of TROG.

4.4. LG101506

LG101506 (LG1506) has poor binding affinity for RAR isoforms α, β, or γ: Ki values are 2746 ± 395, 3516 ± 420, and $> 10\,000$ nM, respectively. On the other hand, LG1506 binds with high affinity to RXR α, β, and γ: Ki values are 3.0 ± 0.8, 9.0 ± 1.7, and 11.0 ± 3.6, respectively, see [40].

4.4.1. Effects on glucose and insulin resistance

Unlike other RXR-selective ligands, LG1506 binding to the RXR receptor induces a conformation that results in selective activation of RXR:PPARγ, RXR:PPARα, and RXR:PPARδ, but not RXR:RAR, RXR:LXR, or RXR:FXR heterodimers [40]. The glucose-lowering and insulin-sensitizing effects of LG1506 were comparable to those of LG268 and ROSI in the female Zucker fa/fa rat. However, when administered as a single agent, LG1506 had no significant effect on body weight, while, as reported previously, body weight was increased with ROSI and decreased by LG268. When coadministered, however, LG1506 completely blocked the weight gain observed with ROSI.

4.5. Summary of the effects of rexinoids on glucose, insulin resistance, and obesity

The effects of RXR-specific ligands on glucose, insulin resistance, and obesity are summarized in Table 2. Review of the literature describing the antidiabetic effects of rexinoids shows that RXR-selective ligands do not mediate their glucose-lowering and insulin-sensitizing effects by merely "mimicking" the effects of TZDs. Initially thought to synergize the actions of TZDs through binding to the RXR component of the RXR:PPARγ heterodimer, recent evidence clearly demonstrates that these two classes of compounds mediate their actions via distinct mechanisms. Both Shen et al. [34] and Li et al. [39] have demonstrated that rexinoids and TZDs act through distinct pathways of insulin signaling. In addition, while both rexinoids and TZDs remodel adipose tissue, rexinoids have antiobesity properties whereas TZDs have the opposite effect. Futhermore, Ogilvie et al. [36] have demonstrated that the rexinoid LG268 mediates its antiobesity effects through the central nervous system. While the rexinoid LG1506 was shown to be weight-neutral when administered as a single agent in the Zucker fa/fa rat model [40], it completely blocked weight gain when coadministered with ROSI. Therefore, compared to TZDs, glucose-lowering, insulin-sensitizing agents currently used for the treatment of insulin resistance and type 2 diabetes, the rexinoids, with further development, may eventually offer the advantage of promoting weight loss, though being weight-neutral would be an advantage over TZDs. Furthermore, if rexinoids were to be used in combination with TZDs, this may offer even greater efficacy and limit the weight gain characteristic of TZDs.

5. SAFETY OF REXINOIDS AS THERAPEUTIC AGENTS

5.1. Elevation of triglyceride levels

Plasma triglyceride levels are maintained at normal levels in fasted animals due to the established equilibrium between the rate of hepatic secretion of very low density lipoproteins (VLDL) versus the rate of VLDL clearance. VLDL particles are cleared by lipoprotein lipase (LPL) activity present in the tissue vascular beds and hepatic lipase present in the liver [43].

Hypertriglyceridemia was initially seen in patients receiving retinoic acid isomers that are known to activate both RARs and RXRs. Administration of RAR-specific retinoids results in hypertriglyceridemia in rodents [44] and in humans [45]. Similarly, patients in a phase I clinical trial for advanced cancer treated with the RXR-selective agonist LG1069 experienced elevated triglyceride levels [46, 47].

5.2. LGD1069

Recent studies have investigated the physiological and molecular bases of the hypertriglyceridemia associated with LGD1069 treatment [41, 48, 49]. Treatment of ZDF rats with a broad range of LGD1069 doses (0.3, 1, 3, 10, or 30 mg/kg) for 14 days produced a nearly linear dose-dependent increase in serum TG levels, with no change in total cholesterol levels. Lipoprotein profile analysis revealed no significant change in the IDL or LDL fractions with LGD1069 treatment, and no significant change in the HDL fraction, though HDL particles were less heterogeneous and more bulky relative to control. However, LG1069 significantly increased the VLDL fraction. The authors investigated whether LG1069 reduced VLDL clearance by affecting lipoprotein lipase (LPL). LPL activity was potently suppressed by LG1069 in heart and skeletal muscle, with skeletal muscle being the most sensitive. Importantly, this suppression of LPL activity in skeletal muscle and cardiac tissue did not correspond to reduced LPL mRNA levels, as LG1069 had no effects on LPL mRNA levels in either tissue. On the other hand, LG1069 had no effect on LPL mRNA or activity in adipose tissue. The authors proposed that the hypertriglyceridemia observed in LG1069-treated animals was due to elevated VLDL caused by a primary defect in LPL-dependent catabolism. Furthermore, the authors speculated that skeletal muscle in particular may be an important rexinoid target tissue [41].

In contrast, when Ouamrane et al. [48] treated male C57BL/6J mice with LGD1069 or fenofibrate, no effect of LGD1069 on serum TG levels was observed. As expected, fenofibrate decreased serum TG approximately 50%, and no change in serum cholesterol was observed with either treatment. When this experiment was repeated using age-matched male PPARα-deficient mice (C57BL/6J background), LG1069 increased serum TG levels approximately 3-fold and the TG-lowering effect of fenofibrate was abolished. To further examine the involvement of PPARα in the effects of LGD1069, the ability of LGD1069 to induced hepatomegaly was examined, as was the ability of LGD1069 to induce mRNA of genes known to be responsive to peroxisome proliferators (PPs): CYP4A (cytochrome P450 4A) and PDK4 (pyruvate dehydrogenase kinase 4). In wild-type animals, LG1069 and fenofibrate induced CYP4A mRNA and hepatomegaly. In PPARα-deficient animals, the ability of fenfibrate to induce hepatomegaly was lost while the ability of LG1069 to induce hepatomegaly remained intact, though both fenofibrate and LG1069 failed to induce CYP4A in liver. In wild-type animals, LG1069 induced PDK4 mRNA in both heart and kidney, whereas LGD1069 induced PDK4 mRNA only in the heart tissue of PPARα-deficient animals. These observations led the authors to propose that rexinoids such as LGD1069 mediate their physiological effects through both PPARα-dependent pathways (induction of CYP4A mRNA in liver and PDK4 mRNA in kidney) and PPARα-independent pathways (hepatomegaly and induction of PDK4 mRNA in cardiac tissue). Still, the exact mechanism explaining why LGD1069 increased serum TG by 3-fold in the PPARα-deficient animals and not at all in the wild-type animals remained unclear. The authors speculated that two separate pathways may account for the effects of LGD1069 on serum TG in this animal model: a PPARα-independent pathway may account for the TG-raising effect of LGD1069, while a PPARα-dependent pathway would decrease serum TG via activation of the RXR:PPARα heterodimer. Thus, TG levels in the wild-type animal would reflect the relative activity of both pathways [48].

Although LGD1069 elevates serum TG in ZDF rats, very recent data demonstrate that LGD1069 inhibits atherosclerosis progression in the apolipoprotein E2 knockin (Apo E2-KI) mouse model [49]. Compared with the Apo E knockout (KO) model, which is characterized by isolated hypercholesterolemia, the Apo E2-KI mouse model develops a mixed dyslipidemia (elevated TG and hypercholesterolemia) more commonly found in humans. Female Apo E2-KI mice (C57BL6 background) were fed a Western-style diet (0.2% cholesterol and 21% fat) supplemented with or without LG1069 (0.018% wt/wt) for 11 weeks. Oil-Red-O staining of atherosclerotic lesions in the aorta demonstrated that LGD1069 treatment significantly reduced lesion area, though LGD1069 increased plasma TG concentrations over 50%. TG was associated with the VLDL fraction and LGD1069 produced a significant reduction of plasma total cholesterol that correspondeds to a reduction in non-HDL-C (IDL and LDL cholesterol). LGD1069 significantly decreased plasma Apo B, though no change in liver Apo B mRNA was observed. Furthermore, LGD1069 significantly induced LDL receptor mRNA in liver (approximately 2-fold). LGD1069 was also shown to significantly decrease intestinal cholesterol absorption, as evidenced by reduced mRNA levels of Niemann-Pick C1-Like1 (NPC1L1) and CD13, genes recently identified as critical components of the intestinal cholesterol absorption machinery, and ABCA1 in both duodenum and jejunum. LGD1069 also significantly increased ABCA1 and ABCG1 mRNA levels in the aortic sinus. Peritoneal macrophages obtained from control Apo E2-KI mice showed significant lipid accumulation; however, LGD1069 treatment prevented lipid accumulation in vivo and significantly enhanced Apo AI- and HDL-mediated cholesterol efflux from these macrophages in vitro. Although the mechanism(s) through which LGD1069 exerts these effects remains unclear, the authors suggest that the RXR:LXR pathway may play an important role, but emphasize that LGD1069 may selectively operate through RXR:LXR in a tissue-specific and gene-specific manners. In contrast to the ZDF rat model [41], these results suggest that LGD1069-mediated hypertriglyceridemia in the Apo E2-KI mouse model is countered by a decrease in non-HDL cholesterol (IDL and HDL), a corresponding increase in hepatic LDL receptor mRNA, decreased intestinal cholesterol absorption, and increased Apo A- and HDL-dependent cholesterol efflux [49].

5.3. LG100268

Studies of the effects of LG268 on TG are difficult to interpret due to conflicting results. Studies using db/db mice showed that LG268 lowers TG [30, 32, 34, 50] while a study using

ZDF rats showed that LG268 increases TG [41]. Mukherjee demonstrated that gemfibrozil and LG268, as single agents, significantly reduced plasma TG, while coadministration produced an additive reduction. Similarly, genfibrozil and LG268 each increased plasma HDL-C levels, while coadministration produced an additive increase. The authors concluded that the RXR:PPARα heterodimer was the common target of genfibrozil and LG268 in TG-lowering and HDL-raising [50]. In a separate study, Mukherjee et al. [30] suggested that the RXR:PPARγ heterodimer was the common target of ROSI and LG269 in TG-lowering. ROSI and LG268, as single agents, significantly reduced serum TG, though coadministration produced an additive decrease [30]. In contrast, Lenhard et al. reported that although ROSI and LG268 displayed similar effects on reducing serum glucose and glycohemoglobin in db/db mice with progressed pancreatic β-cell dysfunction, LG268 lowered TG, but not as potently as ROSI [32]. This suggested that the TG-lowering effects of LG268 in these animals were not mediated by the RXR:PPARγ heterodimer [32]. To further investigate these discrepant results, Davies et al. [41] treated ZDF rats with either ROSI or LG100268 for 14 days. ROSI maximally decreased serum TG levels at day 3, while LG268 steadily increased TG levels throughout the treatment period. When a single dose of LG268 was administered to nonobese, nondiabetic Sprague Dawley rats, after a lag period of 60 minutes, serum TG rose rapidly and remained elevated for approximately 6 hours before returning to normal by 24 hours. The LG268-mediated TG increase was abolished by pretreatment with actinomycin D. Post-heparin plasma LPL activity was significantly decreased 3 hours after administration of the single LG268 dose. To further characterize the effect of LG268 on LPL activity, C2C12 differentiated mouse myocytes were stably transfected with an LPL expression vector. In vitro LPL activity was potently suppressed by LG268 and this suppression was abolished by cotreatment with actinomycin D. Furthermore, in vitro LPL activity was not suppressed by WY14,643 or ROSI, suggesting that LG268 mediates these effects via pathways distinct from PPARα and PPARγ [41].

5.4. AGN194204

The rexinoid AGN194204 significantly increased serum TG concentrations while TROG decreased TG in female Zucker diabetic fatty rats (ZFF) [39]. A time course examining the effect of AGN194204 on serum TG demonstrated that TG levels are induced by AGN194204 approximately 3-fold after only 1 day of treatment and remain elevated at that level until day 3. By day 7, serum TG levels have begun to decrease to levels less than 2-fold control. Liver TG content increases less than 2-fold with AGN194204, and those levels remain stable throughout a 7-day treatment period. Affymetrix gene chips were utilized to gain further insight into the metabolic actions of AGN194204. Mice were treated with vehicle or AGN194204 and total liver RNA hybridized to the chip. As described in detail in [39], the data were analyzed using a web-based expression analysis program. To summarize, two gene expression networks were found to be significantly altered in response to treatment with AGN194204. One network centered on the increased expression of SREBP-1c and genes such as FAS, ACO, 3-keto-CoA thiolase, and FABP; the second network consisted of genes containing G-protein subunits coupled to the glucagon receptor as well as to FoxA. Glucagon receptor mRNA levels in liver did not change in response to either AGN194204 or TROG treatment, however, FoxA2 and FoxA3 mRNA levels were significantly reduced in liver following treatment with AGN194204. Based on these findings, the authors speculated that at least one mechanism explaining AGN194204-mediated hypertriglyceridemia (in addition to the possibility of AGN194204 activating the RXR:LXR heterodimer) could be the AGN194204-mediated increase of IRS-2 and decrease of FoxA2 in liver. FoxA2 increases the expression of fatty acid oxidizing enzymes in the liver, and insulin inhibits FA oxidation in part by sequestering FoxA2 in the cytoplasm. Therefore, AGN194204 treatment would ultimately inhibit fatty acid oxidation and increase SREBP-1c and other enzymes involved in de novo TG synthesis.

5.5. LG101506

Compared with LG268, which potently induced TG in nonobese, nondiabetic Sprague Dawley rats, LG1506 had no effect on TG levels [40]. TG levels were significantly elevated 2 hours after administration of a single dose of LG268, whereas TG levels were similar to control 2 hours after treatment with LG1506. In Zucker fa/fa rats, similar results were observed in that levels that were potently induced by LG268 over a 14-day treatment period, and TG levels were increased, though not significantly, by treatment with LG1506. Interestingly, the greatest effect of LG1506 on TG levels was observed at a lower dose of 3 mg/kg, and this effect was most evident on day 7. This curious observation was further investigated in lean and obese Zucker rats treated with 1, 3, or 30 mg/kg LG1506 for 7 days. Indeed, low doses (1 and 3 mg/kg) of LG1506 induced TG by day 3 in both lean and obese animals with maximal elevations at day 7, while TG levels after treatment with higher doses of LG1506 (30 mg/kg) remained similar to control levels for the duration of the treatment period. These findings led the authors to speculate that LG1506 may affect multiple pathways that regulate TG metabolism: pathways that increase TG levels at low doses of LG1506 and pathways that oppose the TG-raising effects at higher doses of LG1506. The authors add that the complexity of the effects of LG1506 on TG requires further investigation before agents such as LG1506 can be used for the treatment of insulin resistance and type 2 diabetes, however, progress has been made in developing RXR-selective agonists that maintain glucose-lowering properties with the potential to minimize unwanted side effects.

5.6. Suppression of the thyroid hormone axis

Regulation of thyroid hormone levels is the result of a complex interaction involving the hypothalamic-pituitary-thyroid axis. Thyroid-stimulating hormone (TSH), also known

Table 3: Summary of the effects of RXR-specific ligands on hypertriglyceridemia and suppression of the thyroid hormone axis.

RXR ligand	Effects on triglycerides [references]	Effects on thyroid hormone axis [references]
LGD1069	No change in PPARα WT mice; ↑ in PPAR$\alpha-/-$ mice [48] ↓ [50] ↑ [41, 49] Human data: ↑ ([46, 47, 51]; reviewed in [29])	Human data: ↓ ([47, 51, 52]; reviewed in [29])
LG100268	No change [44] ↓ [30, 32, 34, 50] ↑ [36, 40, 41] Note that [36] shows increased TG with oral administration but not with intracerebroventricular administration	↓ [36, 40, 53, 54] Note that [36] shows thyroid hormone axis suppression with oral administration but not with intracerebroventricular administration. References [53, 54] investigate the mechanism of suppression of thyroid hormone axis by rexinoids
AGN194204	↑ [39, 42]	↓ [42]
LG101506	No change in Sprague Dawley rats; ↑ at low doses and no change at higher doses in Zucker rats, with effects most evident on day 7 of a 14-day treatment period [40]	No change in either Sprague Dawley or Zucker rats [40]

as thyrotropin, is a glycoprotein hormone that stimulates development of the thyroid gland and also its secretory activity. Thyrotropin-releasing hormone (TRH) is produced by the hypothalamus and triggers TSH release from the anterior pituitary. TSH release is inhibited by negative feedback exerted by rising blood levels of thyroid hormone acting on both the pituitary and the hypothalamus. Thyroid hormone is actually two active iodine-containing hormones, thyroxine (T4) and triiodothyronine (T3). Thyroxine is the major hormone secreted by the thyroid gland, with the majority of T3 formed at the target tissue by conversion of T4 to T3 [55].

The relationship between alterations in thyroid hormone levels and retinoid administration has been known for many years. In 1947, Simkins described the use of high doses of vitamin A (retinol) for the treatment of hyperthyroidism [56]. Through extensive conversion in vivo, retinol is modified to retinaldehyde, all-trans retinoic acid, and finally 13-cis and 9-cis retinoic acid, which are known to activate genes through the RAR and RXR pathways. Many years later, central hypothyroidism with significant suppression of serum TSH levels was noted in patients with refractory or persistent early-stage cutaneous T-cell lymphoma following treatment with the synthetic RXR-selective retinoid LG1069 [51, 52].

The mechanism for RXR-induced thyroid hormone alterations was investigated in preclinical studies using the RXR-selective ligands, LG268 and AGN194204. After a single administration of LG268 to Sprague Dawley rats, a rapid and statistically significant decrease in TSH levels was seen acutely, 0.5 to 1 hour after treatment. In contrast, total T3 and T4 levels declined more gradually reaching statistical significance 24 hours after compound administration. Further studies investigating the mechanism for TSH suppression showed that neither TSHb mRNA nor TSH protein levels were altered; however, LG100268 treatment reduced TRH-stimulated TSH secretion by 54% [53]. Similar findings were seen with another high affinity RXR-selective ligand, AGN194204. When administrated to female Zucker rats and nondiabetic littermates, AGN194204 decreased TSH levels by 70–80%, which was followed by a decrease in T3 and T4 levels. In diabetic mice, AGN194204 caused a time-dependent decrease in TSH levels after one day of treatment proceeding the fall in T4 levels that was significant at three days after the initiation of treatment [42]. More recent studies with LG268 in mice and using a thyrotrope-derived cell line showed that rexinoids directly suppress TSH secretion, TSHβ mRNA levels, and promoter activity, but no direct effect on hypothalamic TRH levels was seen. These studies also demonstrated that any of the RXR isotypes ($\alpha, \beta,$ or γ) can mediate TSH suppression by rexinoids, but the RXRγ isotype is most efficient at mediating this response [54].

5.7. Summary of the effects of rexinoids on hypertriglyceridemia and suppression of the thyroid hormone axis

The effects of RXR-specific ligands on hypertriglyceridemia and suppression of the thyroid hormone axis are summarized in Table 3. By reviewing the literature investigating the effects of rexinoids on lipids, particularly hypertriglyceridemia, it is clear that these compounds have diverse and complex effects; furthermore, the ability of rexinoids to lower or raise TG levels may depend on a number of factors, including species and strain of the animal model used and the characteristics of the individual ligand. For example, LG1069 increased TG in the ZDF rat [41], had no effect on TG levels in the C57BL/6J mouse [48], and increased TG levels significantly in the apoE2-KI mouse model [49]. Interestingly, using the apoE2-KI model, Lalloyerc et al. demonstrated that LG1609 significantly inhibited the progression of atherosclerosis despite TG levels being increased over 50% [49]. In addition to LG1069 significantly reducing atherosclerotic lesion area in the apoE2-KI model, the rexinoid also resulted in a number of additional beneficial effects on lipid metabolism: significant reduction of non-HDL

cholesterol, reduction of plasma apoB levels, induction of hepatic LDL receptors, reduced intestinal cholesterol absorption, and enhanced apoAI- and HDL-mediated cholesterol efflux. These findings prompted a rather controversial speculation by the authors: perhaps elevated TG, long thought to be an independent risk factor for cardiovascular disease, may not increase the risk for cardiovascular disease when associated with a concomitant decrease in non-HDL cholesterol. LG268 was shown to consistently lower TG in the db/db mice model and increase TG in ZDF rat [30, 32, 34, 41, 50]. Li et al. showed that AGN194204 increased TG levels in ZFF rats [39], and speculated on the mechanism of AGN194204-mediated hypertriglyceridemia by using data obtained from Affymetrix gene chip analysis. Finally, Leibowitz et al. provided data on LG1506, a selective RXR modulator that preferentially activates RXR heterodimers with PPARα, PPARγ, and PPARδ [40]. LG1506 was shown to increase TG at low doses while having no effect on TG levels at higher doses that are efficacious for the antidiabetic effects of LG1506.

Suppression of the thyroid hormone axis has been observed in patients receiving LG1609 for the treatment of cutaneous T-cell lymphoma [51, 52]. The mechanism of LG268-mediated hypothyroidism was investigated in the rat [53] and mouse [54]. Sharma et al. [54] report that LG268 exerts multiple effects on the hypothalamic-pituitary-thyroid axis, and Ogilvie et al. [36] demonstrated that hypertriglyceridemia and suppression of the HPT axis are separable from effects on body weight and food intake. Whether administered orally or ICV, LG268 reduced food intake and body weight; on the other hand, LG268 only increased TG levels and suppressed total T4 levels when administered orally. Macchia et al. [42] demonstrated that AGN194204 caused central hypothyroidism independently of TR, the main mediator of hormone-induced TSH suppression. Finally, Leibowitz et al. [40] showed that suppression of the HPT axis could be minimized with the selective RXR modulator, LG1506.

6. FUTURE DIRECTIONS

The number of individuals with obesity and type 2 diabetes is growing at epidemic rates, reaching younger populations and expanding into newly emerging industrialized nations. RXR-specific ligands have potent glucose-lowering, insulin-sensitizing, and antiobesity effects in animal models of obesity, insulin resistance, and type 2 diabetes. As the mechanisms underlying the adverse side effects of the RXR agonists become better understood, the potential to enhance the beneficial effects and minimize (or even abolish) the negative side effects of RXR ligands may be feasible. By eliminating the alterations in the thyroid hormone axis and modifying the triglyceride liabilities, the selective RXR modulator approach in the example of LG101506 is promising. More extensive studies are clearly needed, but the global epidemic of obesity and type 2 diabetes highlights the opportunity to further explore the therapeutic potential of retinoid X receptor modulators for the treatment of the metabolic syndrome.

REFERENCES

[1] B. Desvergne, L. Michalik, and W. Wahli, "Transcriptional regulation of metabolism," *Physiological Reviews*, vol. 86, no. 2, pp. 465–514, 2006.

[2] D. Li, T. Li, F. Wang, H. Tian, and H. H. Samuels, "Functional evidence for retinoid X receptor (RXR) as a nonsilent partner in the thyroid hormone receptor/RXR heterodimer," *Molecular and Cellular Biology*, vol. 22, no. 16, pp. 5782–5792, 2002.

[3] D. Li, T. Yamada, F. Wang, A. I. Vulin, and H. H. Samuels, "Novel roles of retinoid X receptor (RXR) anal RXR ligand in dynamically modulating the activity of the thyroid hormone receptor/RXR heterodimer," *Journal of Biological Chemistry*, vol. 279, no. 9, pp. 7427–7437, 2004.

[4] A. I. Castillo, R. Sánchez-Martínez, J. L. Moreno, O. A. Martínez-Iglesias, D. Palacios, and A. Aranda, "A permissive retinoid X receptor/thyroid hormone receptor heterodimer allows stimulation of prolactin gene transcription by thyroid hormone and 9-cis-retinoic acid," *Molecular and Cellular Biology*, vol. 24, no. 2, pp. 502–513, 2004.

[5] A. Kassam, B. Miao, P. R. Young, and R. Mukherjee, "Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding," *Journal of Biological Chemistry*, vol. 278, no. 12, pp. 10028–10032, 2003.

[6] C. K. Glass, "Going nuclear in metabolic and cardiovascular disease," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 556–560, 2006.

[7] A. I. Shulman and D. J. Mangelsdorf, "Retinoid X receptor heterodimers in the metabolic syndrome," *New England Journal of Medicine*, vol. 353, no. 6, pp. 604–615, 2005.

[8] G. Reaven, "The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals," *Endocrinology and Metabolism Clinics of North America*, vol. 33, no. 2, pp. 283–303, 2004.

[9] G. Reaven, F. Abbasi, and T. McLaughlin, "Obesity, insulin resistance, and cardiovascular disease," *Recent Progress in Hormone Research*, vol. 59, pp. 207–223, 2004.

[10] S. M. Grundy, "Obesity, metabolic syndrome, and cardiovascular disease," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2595–2600, 2004.

[11] R. H. Eckel, S. M. Grundy, and P. Z. Zimmet, "The metabolic syndrome," *Lancet*, vol. 365, no. 9468, pp. 1415–1428, 2005.

[12] K. Reynolds and J. He, "Epidemiology of the metabolic syndrome," *American Journal of the Medical Sciences*, vol. 330, no. 6, pp. 273–279, 2005.

[13] S. B. Wyatt, K. P. Winters, and P. M. Dubbert, "Overweight and obesity: prevalence, consequences, and causes of a growing public health problem," *American Journal of the Medical Sciences*, vol. 331, no. 4, pp. 166–174, 2006.

[14] C. L. Ogden, M. D. Carroll, L. R. Curtin, M. A. McDowell, C. J. Tabak, and K. M. Flegal, "Prevalence of overweight and obesity in the United States, 1999–2004," *Journal of the American Medical Association*, vol. 295, no. 13, pp. 1549–1555, 2006.

[15] K. G. M. M. Alberti and P. Z. Zimmet, "Definition, diagnosis and classification of diabetes mellitus and its complications—part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation," *Diabetic Medicine*, vol. 15, no. 7, pp. 539–553, 1998.

[16] B. Balkau and M. A. Charles, "Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR)," *Diabetic Medicine*, vol. 16, no. 5, pp. 442–443, 1999.

[17] National Cholesterol Education Program, "Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report," *Circulation*, vol. 106, no. 25, pp. 3143–3421, 2002.

[18] D. Einhorn, G. M. Reaven, R. H. Cobin, et al., "American college of endocrinology position statement on the insulin resistance syndrome," *Endocrine Practice*, vol. 9, no. 3, pp. 237–252, 2003.

[19] International Diabetes Federation, "The IDF consensus worldwide definition of the metabolic syndrome," http://www.idf.org/webdata/docs/IDF_Metasyndrome_definition.pdf.

[20] E. Reisin and M. A. Alpert, "Definition of the metabolic syndrome: current proposals and controversies," *American Journal of the Medical Sciences*, vol. 330, no. 6, pp. 269–272, 2005.

[21] E. S. Ford, W. H. Giles, and A. H. Mokdad, "Increasing prevalence of the metabolic syndrome among U.S. adults," *Diabetes Care*, vol. 27, no. 10, pp. 2444–2449, 2004.

[22] R. Weiss, J. Dziura, T. S. Burgert, et al., "Obesity and the metabolic syndrome in children and adolescents," *New England Journal of Medicine*, vol. 350, no. 23, pp. 2362–2374, 2004.

[23] S. Cook, M. Weitzman, P. Auinger, M. Nguyen, and W. H. Dietz, "Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third national health and nutrition examination survey, 1988–1994," *Archives of Pediatrics and Adolescent Medicine*, vol. 157, no. 8, pp. 821–827, 2003.

[24] H. S. Ahuja, A. Szanto, L. Nagy, and P. J. A. Davies, "The retinoid X receptor and its ligands: versatile regulators of metabolic function, cell differentiation and cell death," *Journal of Biological Regulators and Homeostatic Agents*, vol. 17, no. 1, pp. 29–45, 2003.

[25] A. Szanto, V. Narkar, Q. Shen, I. P. Uray, P. J. A. Davies, and L. Nagy, "Retinoid X receptors: X-ploring their (patho)physiological functions," *Cell Death and Differentiation*, vol. 11, supplement 2, pp. S126–S143, 2004.

[26] J. Bastien and C. Rochette-Egly, "Nuclear retinoid receptors and the transcription of retinoid-target genes," *Gene*, vol. 328, no. 1-2, pp. 1–16, 2004.

[27] L. G. Hamann, "An efficient, stereospecific synthesis of the dimer-selective retinoid X receptor modulator (2*E*, 4*E*, 6*Z*)-7-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2(*n*-propyloxy)naphthalen-3-yl]-3-methylocta-2,4,6-trienoic acid," *Journal of Organic Chemistry*, vol. 65, no. 10, pp. 3233–3235, 2000.

[28] M. F. Boehm, L. Zhang, B. A. Badea, et al., "Synthesis and structure-activity relationships of novel retinoid X receptor-selective retinoids," *Journal of Medicinal Chemistry*, vol. 37, no. 18, pp. 2930–2941, 1994.

[29] L. T. Farol and K. B. Hymes, "Bexarotene: a clinical review," *Expert Review of Anticancer Therapy*, vol. 4, no. 2, pp. 180–188, 2004.

[30] R. Mukherjee, P. J. A. Davies, D. L. Crombie, et al., "Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists," *Nature*, vol. 386, no. 6623, pp. 407–410, 1997.

[31] D. S. Lala, R. Mukherjee, I. G. Schulman, et al., "Activation of specific RXR heterodimers by an antagonist of RXR homodimers," *Nature*, vol. 383, no. 6599, pp. 450–453, 1996.

[32] J. M. Lenhard, M. E. Lancaster, M. A. Paulik, et al., "The RXR agonist LG100268 causes hepatomegaly, improves glycaemic control and decreases cardiovascular risk and cachexia in diabetic mice suffering from pancreatic beta-cell dysfunction," *Diabetologia*, vol. 42, no. 5, pp. 545–554, 1999.

[33] H. S. Ahuja, S. Liu, D. L. Crombie, et al., "Differential effects of rexinoids and thiazolidinediones on metabolic gene expression in diabetic rodents," *Molecular Pharmacology*, vol. 59, no. 4, pp. 765–773, 2001.

[34] Q. Shen, G. W. Cline, G. I. Shulman, M. D. Leibowitz, and P. J. A. Davies, "Effects of rexinoids on glucose transport and insulin-mediated signaling in skeletal muscles of diabetic (db/db) mice," *Journal of Biological Chemistry*, vol. 279, no. 19, pp. 19721–19731, 2004.

[35] Y.-L. Liu, M. V. Sennitt, D. C. Hislop, D. L. Crombie, R. A. Heyman, and M. A. Cawthorne, "Retinoid X receptor agonists have anti-obesity effects and improve insulin sensitivity in Zucker *fa*/*fa* rats," *International Journal of Obesity*, vol. 24, no. 8, pp. 997–1004, 2000.

[36] K. M. Ogilvie, R. Saladin, T. R. Nagy, M. S. Urcan, R. A. Heyman, and M. D. Leibowitz, "Activation of the retinoid X receptor suppresses appetite in the rat," *Endocrinology*, vol. 145, no. 2, pp. 565–573, 2004.

[37] V. Emilsson, J. O'Dowd, S. Wang, et al., "The effects of rexinoids and rosiglitazone on body weight and uncoupling protein isoform expression in the Zucker *fa*/*fa* rat," *Metabolism*, vol. 49, no. 12, pp. 1610–1615, 2000.

[38] V. Vuligonda, S. M. Thacher, and R. A. S. Chandraratna, "Enantioselective syntheses of potent retinoid X receptor ligands: differential biological activities of individual antipodes," *Journal of Medicinal Chemistry*, vol. 44, no. 14, pp. 2298–2303, 2001.

[39] X. Li, P. A. Hansen, L. Xi, R. A. S. Chandraratna, and C. F. Burant, "Distinct mechanisms of glucose lowering by specific agonists for peroxisomal proliferator activated receptor γ and retinoic acid X receptors," *Journal of Biological Chemistry*, vol. 280, no. 46, pp. 38317–38327, 2005.

[40] M. D. Leibowitz, R. J. Ardecky, M. F. Boehm, et al., "Biological characterization of a heterodimer-selective retinoid X receptor modulator: potential benefits for the treatment of type 2 diabetes," *Endocrinology*, vol. 147, no. 2, pp. 1044–1053, 2006.

[41] P. J. A. Davies, S. A. Berry, G. L. Shipley, et al., "Metabolic effects of rexinoids: tissue-specific regulation of lipoprotein lipase activity," *Molecular Pharmacology*, vol. 59, no. 2, pp. 170–176, 2001.

[42] P. E. Macchia, P. Jiang, Y.-D. Yuan, et al., "RXR receptor agonist suppression of thyroid function: central effects in the absence of thyroid hormone receptor," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 283, no. 2, pp. E326–E331, 2002.

[43] T. N. Tulenko and A. E. Sumner, "The physiology of lipoproteins," *Journal of Nuclear Cardiology*, vol. 9, no. 6, pp. 638–649, 2002.

[44] A. M. Standeven, R. L. Beard, A. T. Johnson, et al., "Retinoid-induced hypertriglyceridemia in rats is mediated by retinoic acid receptors," *Fundamental and Applied Toxicology*, vol. 33, no. 2, pp. 264–271, 1996.

[45] M. Takeuchi, T. Yano, E. Omoto, et al., "Relapsed acute promyelocytic leukemia previously treated with all-trans retinoic acid: clinical experience with a new synthetic retinoid, Am-80," *Leukemia and Lymphoma*, vol. 31, no. 5-6, pp. 441–451, 1998.

[46] V. A. Miller, F. M. Benedetti, J. R. Rigas, et al., "Initial clinical trial of a selective retinoid X receptor ligand, LGD1069," *Journal of Clinical Oncology*, vol. 15, no. 2, pp. 790–795, 1997.

[47] N. A. Rizvi, J. L. Marshall, W. Dahut, et al., "A phase I study of LGD1069 in adults with advanced cancer," *Clinical Cancer Research*, vol. 5, no. 7, pp. 1658–1664, 1999.

[48] L. Ouamrane, G. Larrieu, B. Gauthier, and T. Pineau, "RXR activators molecular signalling: involvement of a PPARα-dependent pathway in the liver and kidney, evidence for an alternative pathway in the heart," *British Journal of Pharmacology*, vol. 138, no. 5, pp. 845–854, 2003.

[49] F. Lalloyer, C. Fiévet, S. Lestavel, et al., "The RXR agonist bexarotene improves cholesterol homeostasis and inhibits atherosclerosis progression in a mouse model of mixed dyslipidemia," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 12, pp. 2731–2737, 2006.

[50] R. Mukherjee, J. Strasser, L. Jow, P. Hoener, J. R. Paterniti Jr., and R. A. Heyman, "RXR agonists activate PPARα-inducible genes, lower triglycerides, and raise HDL levels in vivo," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 18, no. 2, pp. 272–276, 1998.

[51] M. Duvic, A. G. Martin, Y. Kim, et al., "Phase 2 and 3 clinical trial of oral bexarotene (Targretin capsules) for the treatment of refractory or persistent early-stage cutaneous T-cell lymphoma," *Archives of Dermatology*, vol. 137, no. 5, pp. 581–593, 2001.

[52] S. I. Sherman, J. Gopal, B. R. Haugen, et al., "Central hypothyroidism associated with retinoid X receptor-selective ligands," *New England Journal of Medicine*, vol. 340, no. 14, pp. 1075–1079, 1999.

[53] S. Liu, K. M. Ogilvie, K. Klausing, et al., "Mechanism of selective retinoid X receptor agonist-induced hypothyroidism in the rat," *Endocrinology*, vol. 143, no. 8, pp. 2880–2885, 2002.

[54] V. Sharma, W. R. Hays, W. M. Wood, et al., "Effects of rexinoids on thyrotrope function and the hypothalamic-pituitary-thyroid axis," *Endocrinology*, vol. 147, no. 3, pp. 1438–1451, 2006.

[55] "Endocrine organs of the body: the thyroid gland," in *Human Anatomy and Physiology*, E. N. Marieb, Ed., pp. 554–558, The Benjamin/Cummings, Redwood City, Calif, USA, 2nd edition, 1992.

[56] S. Simkins, "Use of massive doses of vitamin A in the treatment of hyperthyroidism," *Journal of Clinical Endocrinology and Metabolism*, vol. 7, pp. 574–585, 1947.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 53843, 11 pages
doi:10.1155/2007/53843

Review Article

Nuclear Receptor Cofactors in PPARγ-Mediated Adipogenesis and Adipocyte Energy Metabolism

Emily Powell, Peter Kuhn, and Wei Xu

McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue, Madison, WI 53706, USA

Received 14 July 2006; Revised 17 October 2006; Accepted 17 October 2006

Recommended by Francine M. Gregoire

Transcriptional cofactors are integral to the proper function and regulation of nuclear receptors. Members of the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors are involved in the regulation of lipid and carbohydrate metabolism. They modulate gene transcription in response to a wide variety of ligands, a process that is mediated by transcriptional coactivators and corepressors. The mechanisms by which these cofactors mediate transcriptional regulation of nuclear receptor function are still being elucidated. The rapidly increasing array of cofactors has brought into focus the need for a clear understanding of how these cofactors interact in ligand- and cell-specific manners. This review highlights the differential effects of the assorted cofactors regulating the transcriptional action of PPARγ and summarizes the recent advances in understanding the physiological functions of corepressors and coactivators.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of structurally similar members of the nuclear hormone receptor superfamily [1]. However, unlike classical nuclear hormone receptors, PPARs do not bind their ligands with high affinity, but possess a relatively low binding affinity for unsaturated fatty acids and a broad range of compounds that includes eicosanoids and their metabolites (notably prostaglandin PGJ2 and leukotriene LTB4) and synthetic ligands such as fibrates (a drug for treatment of hyperlipidemia) and thiazolidinediones (TZDs, antidiabetic drugs). Thus, these receptors are considered to be nutrient sensors that regulate lipid and glucose metabolism in adipocytes and other metabolically active tissues. PPARs have also been shown to be involved in a diverse array of nonmetabolic functions including inflammation, tissue repair, atherosclerosis, and cancer [2–4].

PPARγ is the most highly characterized member of this subfamily and its regulation by nuclear receptor cofactors will be the focus of this review. Two major splice variants have been found; PPARγ1 is expressed in adipocytes, skeletal muscle, liver and heart tissue, while PPARγ2 is almost exclusively found in adipose tissue [5]. Although PPARγ2 may be more adipogenic than PPARγ1 [6, 7], both isoforms are thought to be essential regulators of adipogenesis [8–10]. A common model for adipogenesis 3T3-L1 cell differentiation into adipocytes is mediated by PPARγ2 [11]. This model has been used extensively to define the relationship between PPARγ and its cofactors. In addition to adipogenesis, PPARγ has been shown to play a role in insulin sensitivity, atherosclerosis, inflammation, and cancer [12, 13].

1.1. Overview of cofactors involved in transcriptional regulation of PPARγ

PPAR transactivation is induced by ligand-dependent and independent mechanisms. Ligand-dependent transactivation is induced by ligand binding to the C-terminal activation function (AF-2) domain [14]. The role of transcriptional cofactors in ligand-independent transactivation is poorly understood and outside of the scope of this review. PPARs form heterodimers with the retinoid X receptor (RXR) and bind to PPAR response elements (PPREs) in enhancer sites of regulated genes [15]. In the absence of ligand, nuclear receptor corepressors bind to these heterodimers and recruit histone deactylases (HDACs) to repress transcription. Ligand binding induces a conformational change in the receptor dimer which excludes corepressors from the complex [16].

Ligand binding also increases PPAR's affinity for a number of coactivators, whose binding facilitates chromatin remodeling by histone modification and nucleosome mobilization, leading to the recruitment of the basal transcription machinery to PPAR target genes [17–19]. The short motif LXXLL, where L is leucine and X is any amino acid, is necessary for many coactivators to bind to nuclear receptors [20]. This "NR box" is found in the majority of nuclear receptor coactivators and binds to a hydrophobic pocket in the nuclear receptor binding domain [21].

Cofactors that have been shown to interact directly with PPARγ to initiate its transactivation include members of the p160 family of coactivators, which includes SRC-1/NCoA1, TIF2/GRIP1/NCoA2/SRC-2, and pCIP/ACTR/AIB1/SRC-3 [22]. While having weak histone acetyltransferase (HAT) activities, the C-terminal activation domains of p160 proteins appear to primarily serve as foundations upon which coactivator complexes are assembled. The p160 family of coactivators contains functional activation domains that recruit factors such as cAMP responsive element binding protein (CREB) binding protein (CBP)/p300 via activation domain 1 (AD1). The CBP/p300 complex possesses promiscuous HAT activity, which aids in remodeling chromatin to allow transcriptional activation [23].

The prominent ATP-dependent chromatin remodeling complex SWI/SNF includes components such as BAF250, BAF57, BAF60a, and BRG1 [24]. The SWI/SNF complex is thought to be targeted to nuclear receptor target genes upon ligand induction by interaction with receptors, coactivators, or the general transcription machinery [23]. This complex has also been implicated in chromatin remodeling leading to activation of the PPARγ promoter, thus regulating its expression and adipogenesis [25, 26].

The thyroid receptor associated protein (TRAP)/vitamin D receptor interacting proteins (DRIP)/Mediator complex contains subunits which interact with a variety of transcription factors and serve as a bridge between the basal transcriptional machinery and DNA-bound nuclear receptor cofactors [27, 28]. The TRAP complex interacts with PPARγ in a ligand-dependent fashion. This complex acts more directly on the general transcription machinery, as is evident by its ability to transactivate transcription on naked DNA templates [29]. Furthermore, the TRAP complex interacts with nuclear receptors through PPAR binding protein (PBP)/TRAP220/DRIP205 [30]. Thus, TRAP220 is a critical component of this complex and is required for transcriptional activation of PPARγ [31].

The PPAR-gamma coactivator-1α(PGC-1α) is a unique PPAR coactivator, which serves as a scaffolding protein to integrate a variety of coactivator [32]. Upon docking to PPARγ, PGC-1α recruits HATs such as CBP/p300 and steroid receptor coactivator 1 (SRC-1) to remodel chromatin and initiate transcription [32, 33]. However, interaction of PGC-1α and HAT proteins is not sufficient to activate gene transcription; the C-terminal domain of PGC-1α also interacts with the TRAP complex through direct association with PBP/TRAP220 to induce transcription (Wallberg et al. [33]). PGC-1α has several RNA recognition motifs (RRM), which function in the coupling of transcription to mRNA splicing [34]. The modes of regulation of PPARγ by PGC-1α have been reviewed [35, 36].

Although much is known about the mechanisms by which PPARγ recruits coactivators to initiate transcription, considerably less has been demonstrated with regard to transcriptional repression by corepressors. Both NCoR (nuclear receptor corepressor protein) [37] and SMRT (silencing mediator of retinoid and thyroid hormone receptors) [38] directly interact with PPARγ in vitro [39–41]. It may be noted that PPARγ does not appear to be a strong repressor, however, increasing evidence suggests that NCoR and SMRT do repress PPARγ-modulated gene expression during adipogenesis [42, 43].

The exchange of cofactors may be facilitated by nuclear corepressor exchange factors (NCoEx), namely, transducin β-like 1 (TBL1) and the related protein TBLR1 [44]. TBL1 and TBLR1 are components of the NCoR corepressor complex [45]. However, they activate PPARγ-dependent transcription in response to rosiglitazone. Moreover, embryonic stem cells with a TBL1 deletion fail to differentiate into adipocytes [46] suggesting that TBL1 is necessary for PPARγ activation. The mechanism of TBL1/TBLR1 activation of PPARγ remains elusive, but is probably linked to the proteasome-dependent degradation of corepressors [46].

1.2. Physiological functions of cofactors in adipogenesis

The molecular modes of regulation of nuclear receptor signaling by cofactors have been extensively reviewed [16, 17, 23, 47–49]. Herein we focus on the recent advances in understanding the physiological functions of cofactors in PPARγ-modulated processes, in particular, adipogenesis and energy metabolism. The diversified functions of PPARγ cofactors are studied in cell-based system and/or mice models, which are summarized in Table 1.

2. COACTIVATORS

2.1. PGC-1α a master regulator of adaptive thermogenesis in brown adipose tissue

The thermogenic effect of PPARγ in brown adipose tissue (BAT) is mediated by PGC-1α, which is induced by cold and highly expressed in BAT [35, 36]. PGC-1α regulates the action of PPARγ on adaptive thermogenesis and fatty acid oxidation by interacting with the PPARγ/RXRα heterodimer. This interaction stimulates expression of uncoupling protein 1 (UCP-1), which is responsible for uncoupling β-oxidation from ATP synthesis in oxidative phosphorylation, ultimately resulting in the loss of energy as heat [32].

PGC-1α is unique in that, in addition to its ligand-dependent binding to the PPARγ ligand-binding domain (LBD), it can also bind to the DNA-binding domain (DBD) and the hinge region of nuclear receptors in a ligand-independent fashion [59]. The ligand-independent binding

Table 1: Loss-of-function studies on PPARγ cofactors in adipogenesis and energy metabolism

PPARγ cofactor	Phenotype in the absence of the cofactor	
	Cell-based studies	Mouse studies
Brg1, hBrm (SWI/SNF components)	Blocked adipogenesis (Salma et al. [25]) Reduced presence of Pol II and GTFs on the promoter (Salma et al. [25]) Decreased PPARγ transcription (Salma et al. [25])	—
TIF2	Increased lipolysis (Picard et al. [50])	Enhanced adaptive thermogenesis (Picard et al. [50]) Protection against obesity (Picard et al. [50]) Increased insulin-sensitivity (Picard et al. [50]) Improved metabolic profile. Increased lipolysis (Picard et al. [50]) Decreased presence of PPARγ
SRC-1	—	Predisposition to obesity (Picard et al. [50]) Reduced energy expenditure (Picard et al. [50]) Reduced fatty acid oxidation in brown adipose tissue (Picard et al. [50]) Decreased energy expenditure, attenuated fatty acid oxidation (Picard et al. [50])
SRC-1/pCIP double knockout	Abrogated preadipocyte differentiation (Wang et al. [51]) Reduced expression of PPARγ-target genes, including UCP-1, due to corepressor recruitment and decreased PPARγ recognition of PPREs (Wang et al. [51])	Diminished lipid storage in brown fat; increased caloric intake on both chow and high-fat diet due to increased leptin levels; resistance to diet-induced obesity; increased basal metabolic rate and energy expenditure (Wang et al.[51])
PGC-1α	Impaired induction of thermogenic genes in BAT (Uldry et al. [52]) Decreased number and impaired function of mitochondria (Uldry et al.[52])	Reduced mitochFondrial function (Lin et al. [53]) Resistance to obesity and hyperactivity (Lin et al. [53])
TRAP220/DRIP205/PBP	Defective PPARγ-stimulated adipogenesis (Ge et al. [31])	Defective vascular development similar to that seen in PPARγ-null mice (Barak et al. [54]; Zhu et al. [55])
PRIP/NRC/RAP250/TRBP	Decreased PPARγ-mediated transcriptional activation (Antonson et al. [56]; Zhu et al. [57])	—
RIP140	Upregulation of genes involved in energy dissipation (Poweka et al., 2006) Increased PGC-1α expression (Poweka et al., 2006)	Increased oxygen consumption and resistance to high-fat diet-induced obesity (Leonardsson et al. [58]) Expression of lipgenic enzymes is decreased. UCP-1 (involved in energy dissipation in BAT) expression is increased (Leonardsson et al. [58])
NCoR and SMRT	Increased adipocyte differentiation (Yu et al. [42])	—
Sirt1	Decreased NCoR levels (Picard et al. [43])	—

of PGC-1α to PPARγ is mediated by the PGC-1α N-terminal domain and results in the expression of enzymes involved in the mitochondrial respiratory chain to activate adaptive thermogenesis [32, 60]. Chromatin immunoprecipitation (ChIP) analyses revealed that the presence of PGC-1α decreases the association of corepressors on a PPRE-containing gene in the absence of exogenous ligand without altering the binding of PPARγ, and PGC-1α is sufficient to recruit SRC-1, p300, and RNA polymerase II to the PPRE-containing gene in the absence of rosiglitazone [61].

The ectopic expression of PGC-1α in white adipose tissue (WAT) in vitro causes induction of the genes associated with the brown fat phenotype, such as UCP-1 and components of the electron transport chain [62, 63]. The presence

of UCP-1 in WAT is associated with a more brown-fat like phenotype, enhanced metabolic rate and insulin sensitivity, and resistance to obesity [64–66], which could indicate a potential therapeutic role for PGC-1α and UCP-1.

The function of PGC-1α in adaptive energy metabolism is reinforced in the PGC-1α knockout mouse model [53]. PGC-1α null mice are born with no obvious defects during embryonic development but have reduced mitochondrial function. Intriguingly, null mice are lean and resistant to diet-induced obesity. The lean phenotype is largely due to hyperactivity caused by lesions in the striatal region of the brain which controls movement [53]. The closely related family member PGC-1β has been less studied, but it appears to induce mitochondrial biogenesis and fatty acid oxidation in several cell types [67–69]. Thus, PGC-1β can regulate some but not all activities of PGC-1α. The most recent PGC-1β knockdown studies in immortal preadipocyte lines derived from PGC-1α null mice reveal complementary actions of the two PGC-1 proteins [52]. Loss of PGC-1α alone severely impairs the induction of thermogenic genes but does not affect brown fat differentiation (Figure 1). Loss of either PGC-1α or PGC-1β exhibits a small decrease in the differentiation-induced mitochondrial biogenesis; however, double knockdown results in a reduced number of mitochondria and functional defects [52]. This study implicates that PGC-1β plays a role in brown fat differentiation, and is at least as important as PGC-1α in this process (Figure 1).

2.2. Effects of the p160 coregulators SRC-1, TIF2/SRC-2, and p/CIP/SRC-3 on energy metabolism and homeostasis

Members of the 160 kd protein family of coactivators are able to interact directly with the AF2 domain of PPARγ to allow nuclear receptor transactivation function in a ligand-dependent manner via an α-helical LXXLL motif on p160 protein's N-terminal domain. Furthermore, CBP/p300 interacts with p160 cofactors and directly with PPARγ, possibly providing additional stability to the complex through an increased number of contact points [70]. However, although CBP/p300 binding is required for maximal PPARγ activity in vitro, minimal data exists showing a requirement for these cofactors in adipogenesis [71].

Mice deficient in p160 family members exhibit very different phenotypes, providing insights into their physiological functions in adipogenesis and energy metabolism [50]. TIF2$^{-/-}$ mice exhibit enhanced adaptive thermogenesis and protection against obesity, whereas SRC-1$^{-/-}$ mice are predisposed to obesity with accompanying reduced energy expenditure [50]. TIF2$^{-/-}$ mice also show improved metabolic profiles and increased whole-body insulin sensitivity [50]. TIF2 seems to have a greater influence on the p300/PPARγ complex than does the SRC-1 complex, which could possibly be attributed to a weaker capacity of SRC-1 to interact with other coregulators such as p300/CBP and TRAP220, as these coregulators have been shown to have roles in adipogenesis [31, 71]. An increase in lipolysis is observed in TIF2$^{-/-}$ cells, indicating a reduced potential for the storage of fatty acids. Furthermore, a TIF2 dose-dependent attenuation of the PGC-1α/PPARγ activation complex in the presence of SRC-1 suggests that TIF2 competes with SRC-1 for the formation of PGC-1α/PPARγ complexes. However, TIF2 does not significantly enhance PPARγ transactivation mediated by PGC-1α, and an increase in PGC-1α expression level was observed in BAT of TIF2$^{-/-}$ mice [50]. Thus, TIF2 appears to be linked to WAT differentiation and fat storage by potentiating PPARγ activity (Figure 1). In contrast, SRC-1$^{-/-}$ mice displayed increased fat mass and plasma leptin levels. Moreover, the mRNA of UCP-1, PGC1α, and AOX were decreased in BAT, suggesting that the thermogenic machinery in BAT is diminished in the absence of SRC-1. Thus, SRC-1 largely contributes to brown fat differentiation and energy expenditure in brown fat (Figure 1).

A recent study involving p/CIP$^{-/-}$ SRC-1$^{-/-}$ double knockout (DKO) mice revealed that p/CIP and SRC-1 are required for induction of genes necessary for adaptive thermogenesis and lipid storage in BAT [51]. These DKO mice consume more food, both on chow and high fat diets, as a result of decreased blood leptin levels; however, the DKO mice are resistant to diet-induced obesity and remain lean when compared to single knockout and wild type littermates. Furthermore, these mice are more physically active and have increased basal metabolic rates. This phenotype appears to be the result of failed induction of PPARγ target genes, resulting in increased basal metabolism and decreased adipogenesis [51]. Although p/CIP single knockout mice do not exhibit a strong phenotype in adipogenesis, p/CIP appears to potentiate SRC-1-mediated fat storage in BAT and perhaps adaptive thermogenesis (Figure 1).

2.3. The SWI/SNF chromatin remodeling complex is required for induction of the PPARγ promoter and adipogenesis

The mammalian SWI/SNF (mating type switching/sucrose nonfermenting) family of ATP-dependent chromatin remodeling enzymes plays critical roles in the activation of PPARγ transcription for adipogenesis. The core components of the complex include either the Brg1 or Brm ATPase and several Brg1/Brm-associated factors (BAFs). Although in vitro analyses of SWI/SNF complexes containing Brg1 or Brm reveal similarities in chromatin remodeling [72], differences in their functions have been observed in vivo. Brg1 knockout mice are embryonically lethal, and heterozygotes show a predisposition for tumor development [73]. In contrast, Brm knockout mice and cells show only a slight difference in proliferation from wild type [74].

PBAF, a multisubunit complex containing Brg1 and BAF180 subunit was shown to activate PPARγ transcription in an in vitro chromatin-based system [75]. The necessity of the SWI/SNF chromatin remodeling complex is illustrated by experiments revealing that Pol II and general transcription factors are dissociated from the PPARγ promoter when cells are transfected with dominant negative components of the SWI/SNF complex [25]. This suggests that function of the SWI/SNF complex is essential to formation of the preinitiation complex (PIC) on the PPARγ2 promoter and subsequent transcription initiation. Expression of dominant

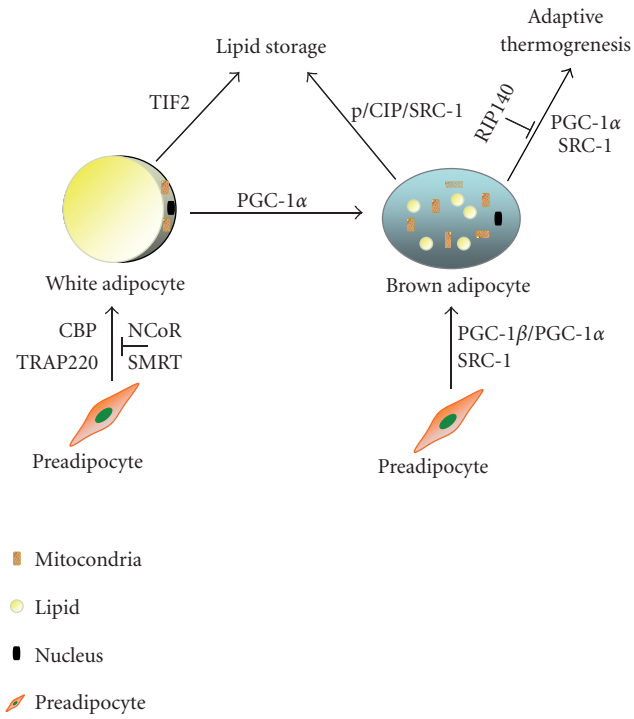


Figure 1: Putative functions of PPARγ cofactors in white adipose- and brown adipose-modulated lipid and energy metabolism. Positive regulators are highlighted in red. Preadipocytes can be differentiated into white adipocytes via transcriptional regulation of PPARγ by coactivators CBP and TRAP220, or differentiated into brown adipocytes via transactivation by PGC-1β, PGC-1α, and SRC-1. TIF2 plays roles in lipid storage from white adipocytes, while p/CIP and SRC-1 function to promote lipid storage in brown fat. PGC-1α is not only involved in adaptive thermogenesis but it also promotes the conversion of white adipocytes into brown adipocytes. SRC-1 is the only member of p160 proteins that show clear function in energy expenditure.

negative Brg1 or hBrm leads to blocked induction of the PPARγ activator and adipogenesis, which was measured both morphologically and by expression of two adipogenic marker genes, aP2 and adipsin [25]. Because Brg1 and hBrm are both crucial members of the SWI/SNF chromatin remodeling complex, this evidence suggests that the SWI/SNF enzymes are required for the activation of PPARγ and adipogenesis [25].

BAF60c, another component of the SWI/SNF complex, serves to anchor the SWI/SNF complex to PPARγ. GST pull-down experiments as well as co-IP confirmed the ability of BAF60c to interact with PPARγ. Moreover, BAF60c interacts with PPARγ in a ligand-dependent fashion to enhance the transcriptional activity of the receptor [26]. However, BAF60c was not shown to affect adipocyte differentiation in these experiments suggesting that BAF60c is not the only factor docking SWI/SNF to PPARγ [26].

2.4. TRAP220/DRIP205/PBP is required for transactivation of PPARγ2 and adipogenesis

The TRAP complex has been implicated as a general transactivator of nuclear receptors [76], apparently functioning by direct interaction with DNA-bound activators and RNA polymerase II [30]. Appreciable evidence for the TRAP complex serving as a coactivator for PPARγ is derived from an in vitro transcription assay in which purified TRAP complex significantly enhanced the transcriptional activity of PPARγ2 on a PPRE-template. GST pull-down assays confirmed the ability of the TRAP complex to bind PPARγ2 only in the presence of TRAP220 [31]. Thus, TRAP220, also known as DRIP205 and PBP [77], anchors the TRAP complex to PPARγ target promoters. A TRAP220$^{-/-}$ mutation is embryonically lethal at day 11.5, showing defects in vascular development similar to those in PPARγ$^{-/-}$ mice, indicating that TRAP220 function is nonredundant and essential for development [54, 78]. Studies using immortalized TRAP220$^{-/-}$ MEFs reveal that TRAP220 acts as a coactivator for PPARγ2 and is an essential mediator of adipogenesis [31]. TRAP220$^{-/-}$ cells exhibit defective PPARγ2-stimulated adipogenesis and expression of adipogenic marker genes. These adipogenic defects can be rescued by ectopic expression of TRAP220 [31]. These data support the model that TRAP220 acts as an anchor in TRAP complex binding, and may also play a role in binding to the CBP-associated complex.

2.5. Evidence of a megacomplex in PPAR transactivation

PPAR interacting protein PRIP/NRC/RAP250/TRBP is ubiquitously expressed in adult mice, and binds to PPARγ enhancing ligand-dependent transcription [55, 56, 79]. PRIP is also necessary for embryonic vascular development, as well

as normal cardiac and neural development, as shown by a lethal null mutation [56, 57]. Mouse embryonic fibroblasts isolated from these PRIP null mice exhibited a decreased capacity for ligand-dependent transcriptional activation of PPARγ [56, 57]. PRIP interacting protein with methyltransferase domain (PIMT) was isolated in a yeast two-hybrid screen using PRIP as bait and enhances PRIP-mediated PPARγ transactivation [80]. Interestingly, PIMT binds to CBP/p300 and TRAP220 supporting a model in which the TRAP complex anchored by TRAP220 is bound to PPAR at the same time as the CBP/p300-associated complex [81].

The isolation of PPARα-interacting cofactor (PRIC) complex which enhances the transcription of PPARα further supports the existence of megacomplex on PPAR-target gene promoters [82]. Of the 25 polypeptides comprising PRIC complex, 18 contained one or more LXXLL motifs. Recognized proteins identified in the PRIC complex include SRC-1, CBP, TRAP220, PRIP, PIMT, TRAP100, and PGC-1, suggesting that CBP-associated complex and TRAP220 bound basal transcription factors may be bound simultaneously. PRIC285, a novel member of the PRIC complex renamed PPAR DNA-binding domain interacting protein (PDIP-1), was shown to bind to the DBD of PPARγ in a yeast two-hybrid assay. Two splice variants, PDIP-1a and PDIP-1b, were identified, and both were shown to transactivate all three isotypes of PPAR and thyroid receptor, whereas PDIP-1a but not PDIP-1b transactivates estrogen receptor (ER) α and androgen receptor (AR), indicating some receptor specificity [82].

3. COREPRESSORS

3.1. Corepressor RIP140 regulates energy metabolism but not adipogenesis

RIP140 was originally identified as a corepressor of ligand-dependent ER function by binding to the AF-2 domain [83]. It was later shown to bind to PPARα in a yeast two-hybrid screen [84]. Although PPARγ and RXR ligands promote the interaction of RIP140 with rat PPARγ in solution, RIP140 interaction with PPARγ/RXR heterodimers does not occur on DNA. This cofactor downregulates the activity of several nuclear receptors specifically by attenuating transactivation mediated by SRC-1. For instance, RIP140 competes with the coactivator SRC-1 for binding to PPARγ [84]. This evidence is suggestive of a model in which RIP140 indirectly regulates the activity of PPARγ by competing with coactivators such as SRC-1. RIP140$^{-/-}$ mice exhibit upregulation of energy metabolic genes UCP-1 and carnitine O-palmitoyl transferase I (CPT-I) and increased β-oxidation in adipocytes, albeit adipogenesis is unaffected [58]. This data suggests that a highly specific set of PPARγ mediated functions is modulated by RIP140 repression while other PPARγ functions such as adipogenesis remain unaltered.

3.2. Transcriptional corepressors for PPARγ: NCoR and SMRT

NCoR and SMRT function to recruit HDAC (histone deacetylase) complexes, which covalently modify nucleosomes to compact DNA and repress transcription [47]. Binding of NCoR and SMRT to NRs is mediated by the corepressor nuclear receptor box (CoRNR) [85]. This motif is very similar to the NR box with a consensus sequence of hydrophobic residues including leucine and isoleucine [86, 87]. The α-helix that contains the CoRNR box is predicted to be longer than the helix containing the NR box in coactivators [87], presenting a possible mechanism for cofactor selection via the ligand-induced conformational change of the NR. Thus, conformational change may exclude corepressors from the AF-2 binding pocket.

Evidence exists suggesting that in the absence of ligand, PPARγ recruits the transcriptional corepressors NCoR and SMRT to downregulate PPARγ-mediated transcriptional activity. Gene silencing of NCoR or SMRT in 3T3-L1 preadipocytes has been shown to increase adipocyte differentiation, a classical PPARγ2 function [42]. Moreover, treatment with the synthetic PPARγ ligand pioglitazone decreases both PPARγ-SMRT and PPARγ-NCoR interactions, although the PPARγ-SMRT interaction decrease is much more prominent. Furthermore, in a separate study by Krogsdam et al., repression of PPARγ-mediated transcription by NCoR exists even in the presence of ligand [88]. These studies underscore the transcriptional repression of PPARγ by NCoR and SMRT in vivo.

It appears that gene-specific factors may affect the conformation of PPARγ, further complicating the ligand-receptor-repressor interaction. One example of this variability is the differential activation of glycerol kinase (GyK) and aP2 transcription. Although both contain PPREs, PPARγ recruits corepressor NCoR to the GyK gene while recruiting coactivators to the aP2 gene [89]. The addition of TZD results in the activation of GyK by recruiting PGC-1α and displacing NCoR, while TZD treatment has little effect on transcription of aP2 and does not recruit PGC-1α to the aP2 promoter [89]. These data suggest that gene-specific PPARγ receptor conformation leads to the recruitment of different cofactor complexes.

Another corepressor, Sirt1, has also been shown to effectively inhibit PPARγ-mediated transcription [90]. This NAD-dependent deacetylase binds to NCoR and SMRT, presenting a model where Sirt1 is recruited to PPARγ via interactions with NCoR and/or SMRT. This was further supported by loss of Sirt1-mediated repression when NCoR levels were decreased via RNAi [90].

3.3. Summary of coactivators and corepressors in lipid and energy metabolism

Cellular energy metabolism is maintained through a delicate balance between energy intake and energy expenditure. When energy intake exceeds energy expenditure, excess energy is stored as lipid in WAT. Although BAT also allows storage of small amount of lipids, it is mainly responsible for energy dissipation. As PPARγ plays an essential role in lipid homeostasis, it is not surprising that multiple PPAR cofactors are involved in lipid and energy metabolism; namely, processes including adipocyte differentiation, lipid storage, and adaptive thermogenesis (Figure 1). PPARγ/RXR

heterodimers are master regulators of preadipocyte differentiation into brown and white adipocytes. Multiple lines of evidence support the model that CBP/p300 and TRAP220 participate in white adipocyte differentiation, and this process is reversibly regulated by corepressors NCoR and SMRT [31, 42, 71]. On the contrary, differentiation of preadipocytes into BAT is regulated by a different set of coactivators such as PGC-1β/PGC-1α and SRC-1 [50, 52]. Conversion of white adipocyte to brown adipocyte-like cells can be at least partially catalyzed by ectopically expressed PGC-1α [62]. TIF2 plays important functions in the storage of fatty acids in WAT as evident by the fact that TIF2$^{-/-}$ mice are protected from obesity and TIF2$^{-/-}$ cells show an increase in lipolysis [50]. Brown adipocytes are enriched in mitochondria and the major function is adaptive thermogenesis in rodents. PGC-1α and SRC-1 are positive regulators of the thermogenic capacity of BAT [50, 52, 53], whereas the corepressor RIP140 appears to negatively regulate this process [58]. Lipid storage in brown adipocytes can be regulated by coactivators p/CIP and SRC-1 [51]. Figure 1 summarizes some of the major players in lipid and energy homeostasis based on current literature. It is worthy to note that some cellular processes require more stringent regulation than others, such that more than one member of the closely related proteins are simultaneously involved. For example, complementary actions of p/CIP and SRC-1 in lipid storage of brown adipocytes and two PGC-1 coactivators in brown fat differentiation are absolutely essential.

3.4. *Ligand- and promoter-specific coregulator recruitment in PPARγ transactivation*

A comparison of natural and synthetic PPARγ ligands reveals a distinct differential recruitment of transcriptional coactivators. 15d-PGJ2, an endogenous PPARγ ligand, is capable of inducing interactions between the PPARγ/RXR heterodimer and SRC-1, TIF2, p/CIP, p300, and TRAP220 [91]. However, the synthetic PPARγ ligand troglitazone did not induce interaction between the PPARγ/RXR heterodimer and any of these coactivators. Furthermore, the transactivation function of PPARγ was shown to be increased by these coactivators in the presence of 15d-PGJ2 and 9-HODE, but not troglitazone. FK614, a non-TZD synthetic PPARγ ligand, and two TZDs, rosiglitazone and pioglitazone, induce recruitment of SRC-1, CBP, and PGC-1α when bound to PPARγ. However, the level to which SRC-1 and CBP are recruited by FK614-bound PPARγ is altered in comparison to rosiglitazone- and pioglitazone-bound receptor (Fujimura, 2005) while PGC-1α showed similar levels of recruitment. These data suggest specific ligands can differentially define the coactivator complex, and that similar coactivators might have distinct in vivo functions.

4. CONCLUSIONS

The race to find new nuclear receptor coactivators and corepressors has resulted in a rapid increase in the number of known cofactors accompanied by insufficient knowledge as to their mechanisms of interaction and transcriptional mediation. Initial investigation has shown that seemingly redundant or promiscuous cofactors have a high amount of context specificity. Gene sequence- and ligand-specific nuclear receptor conformation appears to affect cofactor complex recruitment. The relative expression levels of coactivators and corepressors modulate nuclear receptor transactivation. In the case of PPARγ, there are only a few examples of these differential conditions thus far. Further investigation of these interactions may eventually allow for a better comprehension of context-specific expression profiles. Partial PPARγ agonists, such as FK614, that differentially activate PPARγ target genes may be effective in treating metabolic disease while reducing the side effects (e.g., promoting obesity) caused by current TZD-based treatments. The ability to target unique expression profiles may also lead to a more widespread ability to treat illnesses related to nuclear receptor function.

LIST OF ABBREVIATIONS

15dPGJ2:	15-deoxy-Δ 12, 14-prostaglandin J2
9-HODE:	OX-LDL, 9-hydroxy-10, 12-octadecadienoic acid
ACTR:	Activator of thyroid and retinoic acid receptor
AF:	Activation function
AIB1:	Amplified in breast cancer 1
AR:	Androgen receptor
BAF:	Brg1/Brm-associated factor
BAT:	Brown adipose tissue
CBP:	CREB-binding protein
ChIP:	Chromatin immunoprecipitation
CoRNR:	Corepressor nuclear receptor box
CPT-I:	Carnitine O-palmitoyl transferase I
CREB:	cAMP-responsive element binding protein
DBD:	DNA-binding domain
DKO:	Double knockout
DRIP:	Vitamin D-interacting protein
EMSA:	Electrophoretic mobility shift assay
ER:	Estrogen receptor
GRIP:	Glucocorticoid receptor interacting protein
GST:	Glutathione *s*-transferase
GyK:	Glycerol kinase
HAT:	Histone acetyltransferase
HDAC:	Histone deacetylase
HMT:	Histone methyltransferase
LBD:	Ligand binding domain
LTB4:	Leukotriene B4
MEF:	Mouse embryonic fibroblast
NAD:	Nicotinamide adenine dinucleotide

NCoA: Nuclear coactivator
NCoEx: Nuclear corepressor exchange factors
NCoR: Nuclear corepressor
NR: Nuclear receptor
NRC: Nuclear hormone receptor coregulator
p/CIP: p300/CBP interacting protein
PBP: PPAR binding protein
PDIP: PPAR DNA-binding domain interacting protein
PGC: PPAR-gamma coactivator
PGJ2: Prostaglandin J2
PIC: Preinitiation complex
PIMT: PRIP interacting protein with methyltransferase domain
PPAR: Peroxisome proliferator-associated receptor
PPRE: PPAR-response element
PRIC: PPARα-interacting cofactor
PRIP: PPAR interacting protein
PRMT: Protein arginine methyltransferase
RAP: Receptor-associated protein
RIP140: Receptor interacting protein 140
RRM: RNA-recognition motif
RXR: Retinoid X receptor
Sirt1: Sirtuin 1
SMRT: Silencing mediator of retinoid and thyroid receptors
SRC: Steroid receptor coactivator
SWI/SNF: Mating type switching/sucrose nonfermenting
TBL1: Transducin β-like 1
TBLR1: Transducin β-like related 1
TIF: Transcriptional intermediary factor
TRAP: Thyroid receptor-associated protein
TRBP: Thyroid receptor-binding protein
TZD: Thiazolidinedione
UCP-1: Uncoupling protein 1
WAT: White adipose tissue
Wy-14643: (4-Chloro-6-[(2, 3-dimethylphenyl)amino]-2-pyrimidinyl) thioacetic acid

ACKNOWLEDGMENTS

We thank Chih-Hao Lee and Weimin He for critical reading of the manuscript. The third author is supported by a Susan Komen Breast Cancer Foundation Grant BCTR95306 and UWCCC core grant. The second author was supported by NIH Grant T32 CA009135.

REFERENCES

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, no. 1, pp. 409–435, 2002.

[3] R. M. Evans, G. D. Barish, and Y.-X. Wang, "PPARs and the complex journey to obesity," *Nature Medicine*, vol. 10, no. 4, pp. 355–361, 2004.

[4] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, "From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions," *Progress in Lipid Research*, vol. 45, no. 2, pp. 120–159, 2006.

[5] A. J. Vidal-Puig, R. V. Considine, M. Jimenez-Liñan, et al., "Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids," *Journal of Clinical Investigation*, vol. 99, no. 10, pp. 2416–2422, 1997.

[6] D. Ren, T. N. Collingwood, E. J. Rebar, A. P. Wolffe, and H. S. Camp, "PPARγ knockdown by engineered transcription factors: exogenous PPARγ2 but not PPARγ1 reactivates adipogenesis," *Genes & Development*, vol. 16, no. 1, pp. 27–32, 2002.

[7] A. Werman, A. Hollenberg, G. Solanes, C. Bjørbæk, A. J. Vidal-Puig, and J. S. Flier, "Ligand-independent activation domain in the N terminus of peroxisome proliferator-activated receptor γ (PPARγ). Differential activity of PPARγ1 and -2 isoforms and influence of insulin," *Journal of Biological Chemistry*, vol. 272, no. 32, pp. 20230–20235, 1997.

[8] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[9] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes & Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[10] S. Yu, N. Viswakarma, S. K. Batra, M. Sambasiva Rao, and J. K. Reddy, "Identification of promethin and PGLP as two novel up-regulated genes in PPARγ1-induced adipogenic mouse liver," *Biochimie*, vol. 86, no. 11, pp. 743–761, 2004.

[11] A. Chawla, E. J. Schwarz, D. D. Dimaculangan, and M. A. Lazar, "Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation," *Endocrinology*, vol. 135, no. 2, pp. 798–800, 1994.

[12] L. Gelman, J.-C. Fruchart, and J. Auwerx, "An update on the mechanisms of action of the peroxisome proliferator-activated receptors (PPARs) and their roles in inflammation and cancer," *Cellular and Molecular Life Sciences*, vol. 55, no. 6-7, pp. 932–943, 1999.

[13] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[14] J. Torchia, C. Glass, and M. G. Rosenfeld, "Co-activators and co-repressors in the integration of transcriptional responses," *Current Opinion in Cell Biology*, vol. 10, no. 3, pp. 373–383, 1998.

[15] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[16] M. G. Rosenfeld, V. V. Lunyak, and C. K. Glass, "Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response," *Genes & Development*, vol. 20, no. 11, pp. 1405–1428, 2006.

[17] O. Hermanson, C. K. Glass, and M. G. Rosenfeld, "Nuclear receptor coregulators: multiple modes of modification," *Trends in Endocrinology and Metabolism*, vol. 13, no. 2, pp. 55–60, 2002.

[18] S. Westin, M. G. Rosenfeld, and C. K. Glass, "Nuclear receptor coactivators," *Advances in Pharmacology*, vol. 47, pp. 89–112, 2000.

[19] L. Xu, C. K. Glass, and M. G. Rosenfeld, "Coactivator and corepressor complexes in nuclear receptor function," *Current Opinion in Genetics & Development*, vol. 9, no. 2, pp. 140–147, 1999.

[20] D. M. Heery, S. Hoare, S. Hussain, M. G. Parker, and H. Sheppard, "Core LXXLL motif sequences in CREB-binding protein, SRC1, and RIP140 define affinity and selectivity for steroid and retinoid receptors," *Journal of Biological Chemistry*, vol. 276, no. 9, pp. 6695–6702, 2001.

[21] D. M. Heery, E. Kalkhoven, S. Hoare, and M. G. Parker, "A signature motif in transcriptional co-activators mediates binding to nuclear receptors," *Nature*, vol. 387, no. 6634, pp. 733–736, 1997.

[22] C. Leo and J. D. Chen, "The SRC family of nuclear receptor coactivators," *Gene*, vol. 245, no. 1, pp. 1–11, 2000.

[23] W. Xu, "Nuclear receptor coactivators: the key to unlock chromatin," *Biochemistry and Cell Biology*, vol. 83, no. 4, pp. 418–428, 2005.

[24] J. A. Martens and F. Winston, "Recent advances in understanding chromatin remodeling by Swi/Snf complexes," *Current Opinion in Genetics & Development*, vol. 13, no. 2, pp. 136–142, 2003.

[25] N. Salma, H. Xiao, E. Mueller, and A. N. Imbalzano, "Temporal recruitment of transcription factors and SWI/SNF chromatin-remodeling enzymes during adipogenic induction of the peroxisome proliferator-activated receptor γ nuclear hormone receptor," *Molecular and Cellular Biology*, vol. 24, no. 11, pp. 4651–4663, 2004.

[26] M. B. Debril, L. Gelman, E. Fayard, J. S. Annicotte, S. Rocchi, and J. Auwerx, "Transcription factors and nuclear receptors interact with the SWI/SNF complex through the BAF60c subunit," *Journal of Biological Chemistry*, vol. 279, no. 16, pp. 16677–16686, 2004.

[27] J. D. Fondell, H. Ge, and R. G. Roeder, "Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 16, pp. 8329–8333, 1996.

[28] C. Rachez, B. D. Lemon, Z. Suldan, et al., "Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex," *Nature*, vol. 398, no. 6730, pp. 824–828, 1999.

[29] R. G. Roeder, "Role of general and gene-specific cofactors in the regulation of eukaryotic transcription," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 63, pp. 201–218, 1998.

[30] S. Malik and R. G. Roeder, "Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells," *Trends in Biochemical Sciences*, vol. 25, no. 6, pp. 277–283, 2000.

[31] K. Ge, M. Guermah, C. X. Yuan, et al., "Transcription coactivator TRAP220 is required for PPAR γ 2-stimulated adipogenesis," *Nature*, vol. 417, no. 6888, pp. 563–567, 2002.

[32] P. Puigserver, G. Adelmant, Z. Wu, et al., "Activation of PPARγ coactivator-1 through transcription factor docking," *Science*, vol. 286, no. 5443, pp. 1368–1371, 1999.

[33] A. E. Wallberg, S. Yamamura, S. Malik, B. M. Spiegelman, and R. G. Roeder, "Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1α," *Molecular Cell*, vol. 12, no. 5, pp. 1137–1149, 2003.

[34] M. Monsalve, Z. Wu, G. Adelmant, P. Puigserver, M. Fan, and B. M. Spiegelman, "Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1," *Molecular Cell*, vol. 6, no. 2, pp. 307–316, 2000.

[35] P. Puigserver and B. M. Spiegelman, "Peroxisome proliferator-activated receptor-γ coactivator 1 α (PGC-1 α): transcriptional coactivator and metabolic regulator," *Endocrine Reviews*, vol. 24, no. 1, pp. 78–90, 2003.

[36] J. Lin, C. Handschin, and B. M. Spiegelman, "Metabolic control through the PGC-1 family of transcription coactivators," *Cell Metabolism*, vol. 1, no. 6, pp. 361–370, 2005.

[37] A. J. Horlein, A. M. Naar, T. Heinzel, et al., "Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor," *Nature*, vol. 377, no. 6548, pp. 397–404, 1995.

[38] J. D. Chen and R. M. Evans, "A transcriptional co-repressor that interacts with nuclear hormone receptors," *Nature*, vol. 377, no. 6548, pp. 454–457, 1995.

[39] P. Dowell, J. E. Ishmael, D. Avram, V. J. Peterson, D. J. Nevrivy, and M. Leid, "Identification of nuclear receptor corepressor as a peroxisome proliferator-activated receptor α interacting protein," *Journal of Biological Chemistry*, vol. 274, no. 22, pp. 15901–15907, 1999.

[40] T. B. Stanley, L. M. Leesnitzer, V. G. Montana, et al., "Subtype specific effects of peroxisome proliferator-activated receptor ligands on corepressor affinity," *Biochemistry*, vol. 42, no. 31, pp. 9278–9287, 2003.

[41] A-M. Krogsdam, C. A. Nielsen, S. Neve, et al., "Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor δ-mediated transactivation," *Biochemical Journal*, vol. 363, no. pt 1, pp. 157–165, 2002.

[42] C. Yu, K. Markan, K. A. Temple, D. Deplewski, M. J. Brady, and R. N. Cohen, "The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor γ transcriptional activity and repress 3T3-L1 adipogenesis," *Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13600–13605, 2005.

[43] F. Picard, M. Kurtev, N. Chung, et al., "Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ," *Nature*, vol. 429, no. 6993, pp. 771–776, 2004.

[44] V. Perissi and M. G. Rosenfeld, "Controlling nuclear receptors: the circular logic of cofactor cycles," *Nature Reviews. Molecular Cell Biology*, vol. 6, no. 7, pp. 542–554, 2005.

[45] H. G. Yoon, D. W. Chan, Z. Q. Huang, et al., "Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1," *EMBO Journal*, vol. 22, no. 6, pp. 1336–1346, 2003.

[46] V. Perissi, A. Aggarwal, C. K. Glass, D. W. Rose, and M. G. Rosenfeld, "A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors," *Cell*, vol. 116, no. 4, pp. 511–526, 2004.

[47] M. L. Privalsky, "The role of corepressors in transcriptional regulation by nuclear hormone receptors," *Annual Review of Physiology*, vol. 66, pp. 315–360, 2004.

[48] N. J. McKenna and B. W. O'Malley, "From ligand to response: generating diversity in nuclear receptor coregulator function," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 74, no. 5, pp. 351–356, 2000.

[49] J. Xu and B. W. O'Malley, "Molecular mechanisms and cellular biology of the steroid receptor coactivator (SRC) family in steroid receptor function," *Reviews in Endocrine & Metabolic Disorders*, vol. 3, no. 3, pp. 185–192, 2002.

[50] F. Picard, M. Gehin, J. Annicotte, et al., "SRC-1 and TIF2 control energy balance between white and brown adipose tissues," *Cell*, vol. 111, no. 7, pp. 931–941, 2002.

[51] Z. Wang, C. Qi, A. Krones, et al., "Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance," *Cell Metabolism*, vol. 3, no. 2, pp. 111–122, 2006.

[52] M. Uldry, W. Yang, J. St-Pierre, J. Lin, P. Seale, and B. M. Spiegelman, "Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation," *Cell Metabolism*, vol. 3, no. 5, pp. 333–341, 2006.

[53] J. Lin, P. H. Wu, P. T. Tarr, et al., "Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice," *Cell*, vol. 119, no. 1, pp. 121–135, 2004.

[54] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPAR γ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[55] Y. Zhu, L. Kan, C. Qi, et al., "Isolation and characterization of peroxisome proliferator-activated receptor (PPAR) interacting protein (PRIP) as a coactivator for PPAR," *Journal of Biological Chemistry*, vol. 275, no. 18, pp. 13510–13516, 2000.

[56] P. Antonson, G. U. Schuster, L. Wang, et al., "Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction," *Molecular and Cellular Biology*, vol. 23, no. 4, pp. 1260–1268, 2003.

[57] Y. J. Zhu, S. E. Crawford, V. Stellmach, et al., "Coactivator PRIP, the peroxisome proliferator-activated receptor-interacting protein, is a modulator of placental, cardiac, hepatic, and embryonic development," *Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1986–1990, 2003.

[58] G. Leonardsson, J. H. Steel, M. Christian, et al., "Nuclear receptor corepressor RIP140 regulates fat accumulation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 22, pp. 8437–8442, 2004.

[59] P. Puigserver, Z. Wu, C. W. Park, R. Graves, M. Wright, and B. M. Spiegelman, "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis," *Cell*, vol. 92, no. 6, pp. 829–839, 1998.

[60] Y. Wu, W. W. Chin, Y. Wang, and T. P. Burris, "Ligand and coactivator identity determines the requirement of the charge clamp for coactivation of the peroxisome proliferator-activated receptor γ," *Journal of Biological Chemistry*, vol. 278, no. 10, pp. 8637–8644, 2003.

[61] H. P. Guan, T. Ishizuka, P. C. Chui, M. Lehrke, and M. A. Lazar, "Corepressors selectively control the transcriptional activity of PPARγ in adipocytes," *Genes & Development*, vol. 19, no. 4, pp. 453–461, 2005.

[62] Z. Wu, P. Puigserver, and U. Andersson, "Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1," *Cell*, vol. 98, no. 1, pp. 115–124, 1999.

[63] C. Tiraby and D. Langin, "Conversion from white to brown adipocytes: a strategy for the control of fat mass?" *Trends in Endocrinology and Metabolism*, vol. 14, no. 10, pp. 439–441, 2003.

[64] K. Tsukiyama-Kohara, F. Poulin, M. Kohara, et al., "Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1," *Nature Medicine*, vol. 7, no. 10, pp. 1128–1132, 2001.

[65] A. Cederberg, L. M. Gronning, B. Ahren, K. Tasken, P. Carlsson, and S. Enerback, "FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance," *Cell*, vol. 106, no. 5, pp. 563–573, 2001.

[66] J. Kopecky, G. Clarke, S. Enerback, B. Spiegelman, and L. P. Kozak, "Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity," *Journal of Clinical Investigation*, vol. 96, no. 6, pp. 2914–2923, 1995.

[67] J. Lin, P. Puigserver, J. Donovan, P. Tarr, and B. M. Spiegelman, "Peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), a novel PGC-1-related transcription coactivator associated with host cell factor," *Journal of Biological Chemistry*, vol. 277, no. 3, pp. 1645–1648, 2002.

[68] J. Lin, P. T. Tarr, R. Yang, et al., "PGC-1β in the regulation of hepatic glucose and energy metabolism," *Journal of Biological Chemistry*, vol. 278, no. 33, pp. 30843–30848, 2003.

[69] J. St-Pierre, J. Lin, S. Krauss, et al., "Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1α and 1β (PGC-1α and PGC-1β) in muscle cells," *Journal of Biological Chemistry*, vol. 278, no. 29, pp. 26597–26603, 2003.

[70] L. Gelman, G. Zhou, L. Fajas, E. Raspe, J. C. Fruchart, and J. Auwerx, "p300 interacts with the N- and C-terminal part of PPARγ2 in a ligand-independent and -dependent manner, respectively," *Journal of Biological Chemistry*, vol. 274, no. 12, pp. 7681–7688, 1999.

[71] N. Takahashi, T. Kawada, T. Yamamoto, et al., "Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferator-activated receptor γ," *Journal of Biological Chemistry*, vol. 277, no. 19, pp. 16906–16912, 2002.

[72] S. Sif, A. J. Saurin, A. N. Imbalzano, and R. E. Kingston, "Purification and characterization of mSin3A-containing Brg1 and hBrm chromatin remodeling complexes," *Genes & Development*, vol. 15, no. 5, pp. 603–618, 2001.

[73] S. Bultman, T. Gebuhr, D. Yee, et al., "A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes," *Molecular Cell*, vol. 6, no. 6, pp. 1287–1295, 2000.

[74] J. C. Reyes, J. Barra, C. Muchardt, A. Camus, C. Babinet, and M. Yaniv, "Altered control of cellular proliferation in the absence of mammalian brahma (SNF2α)," *EMBO Journal*, vol. 17, no. 23, pp. 6979–6991, 1998.

[75] B. Lemon, C. Inouye, D. S. King, and R. Tjian, "Selectivity of chromatin-remodelling cofactors for ligand-activated transcription," *Nature*, vol. 414, no. 6866, pp. 924–928, 2001.

[76] C. X. Yuan, M. Ito, J. D. Fondell, Z. Y. Fu, and R. G. Roeder, "The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 14, pp. 7939–7944, 1998.

[77] Y. Zhu, C. Qi, S. Jain, M. S. Rao, and J. K. Reddy, "Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor," *Journal of Biological Chemistry*, vol. 272, no. 41, pp. 25500–25506, 1997.

[78] Y. Zhu, C. Qi, Y. Jia, J. S. Nye, M. S. Rao, and J. K. Reddy, "Deletion of PBP/PPARBP, the gene for nuclear receptor coactivator peroxisome proliferator-activated receptor-binding protein,

results in embryonic lethality," *Journal of Biological Chemistry*, vol. 275, no. 20, pp. 14779–14782, 2000.

[79] F. Caira, P. Antonson, M. Pelto-Huikko, E. Treuter, and J. A. Gustafsson, "Cloning and characterization of RAP250, a novel nuclear receptor coactivator," *Journal of Biological Chemistry*, vol. 275, no. 8, pp. 5308–5317, 2000.

[80] Y . Zhu, C. Qi, W.-Q. Cao, et al., "Cloning and characterization of PIMT, a protein with a methyltransferase domain, which interacts with and enhances nuclear receptor coactivator PRIP function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10380–10385, 2001.

[81] P. Misra, C. Qi, S. Yu, et al., "Interaction of PIMT with transcriptional coactivators CBP, p300, and PBP differential role in transcriptional regulation," *Journal of Biological Chemistry*, vol. 277, no. 22, pp. 20011–20019, 2002.

[82] S. Surapureddi, S. Yu, H. Bu, et al., "Identification of a transcriptionally active peroxisome proliferator-activated receptor α -interacting cofactor complex in rat liver and characterization of PRIC285 as a coactivator," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11836–11841, 2002.

[83] V. Cavailles, S. Dauvois, F. L'Horset, et al., "Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor," *EMBO Journal*, vol. 14, no. 15, pp. 3741–3751, 1995.

[84] E. Treuter, T. Albrektsen, L. Johansson, J. Leers, and J. A. Gustafsson, "A regulatory role for RIP140 in nuclear receptor activation," *Molecular Endocrinology*, vol. 12, no. 6, pp. 864–881, 1998.

[85] X. Hu and M. A. Lazar, "The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors," *Nature*, vol. 402, no. 6757, pp. 93–96, 1999.

[86] L. Nagy, H. Y. Kao, J. D. Love, et al., "Mechanism of corepressor binding and release from nuclear hormone receptors," *Genes & Development*, vol. 13, no. 24, pp. 3209–3216, 1999.

[87] V. Perissi, L. M. Staszewski, E. M. McInerney, et al., "Molecular determinants of nuclear receptor-corepressor interaction," *Genes & Development*, vol. 13, no. 24, pp. 3198–3208, 1999.

[88] A.-M. Krogsdam, C. A. Nielsen, S. Neve, et al., "Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor δ-mediated transactivation," *Biochemical Journal*, vol. 363, no. pt 1, pp. 157–165, 2002.

[89] H. P. Guan, T. Ishizuka, P. C. Chui, M. Lehrke, and M. A. Lazar, "Corepressors selectively control the transcriptional activity of PPARγ in adipocytes," *Genes & Development*, vol. 19, no. 4, pp. 453–461, 2005.

[90] F. Picard, M. Kurtev, N. Chung, et al., "Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ," *Nature*, vol. 429, no. 6993, pp. 771–776, 2004.

[91] Y. Kodera, K. Takeyama, A. Murayama, M. Suzawa, Y. Masuhiro, and S. Kato, "Ligand type-specific interactions of peroxisome proliferator-activated receptor γ with transcriptional coactivators," *Journal of Biological Chemistry*, vol. 275, no. 43, pp. 33201–33204, 2000.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 32696, 7 pages
doi:10.1155/2007/32696

Review Article

Selective Modulators of PPAR-γ Activity: Molecular Aspects Related to Obesity and Side-Effects

Fang Zhang, Brian E. Lavan, and Francine M. Gregoire

Department of Biology, Metabolex Inc., 3876 Bay Center Place, Hayward, CA 94545, USA

Received 3 October 2006; Revised 21 November 2006; Accepted 22 November 2006

Recommended by Sander Kersten

Peroxisome proliferator-activated receptor γ (PPAR-γ) is a key regulator of lipid metabolism and energy balance implicated in the development of insulin resistance and obesity. The identification of putative natural and synthetic ligands and activators of PPAR-γ has helped to unravel the molecular basis of its function, including molecular details regarding ligand binding, conformational changes of the receptor, and cofactor binding, leading to the emergence of the concept of selective PPAR-γ modulators (SPPARγMs). SPPARγMs bind in distinct manners to the ligand-binding pocket of PPAR-γ, leading to alternative receptor conformations, differential cofactor recruitment/displacement, differential gene expression, and ultimately differential biological responses. Based on this concept, new and improved antidiabetic agents for the treatment of diabetes are in development. This review summarizes the current knowledge on the mechanism of action and biological effects of recently characterized SPPARγMs, including metaglidasen/halofenate, PA-082, and the angiotensin receptor antagonists, recently characterized as a new class of SPPARγMs.

1. INTRODUCTION

PPAR-γ belongs to the nuclear receptor superfamily and is a member of the NR1C subgroup that includes PPAR-α and PPAR-δ. These receptors form heterodimers with the retinoid X receptor (RXR), bind to PPAR response elements (PPREs) in the regulatory region of target genes, and modulate their transcription. PPAR-γ is expressed most abundantly in adipose tissue and is a master regulator of adipogenesis. PPAR-γ activation promotes adipocyte differentiation and is associated with induction of lipogenic enzymes and glucoregulatory molecules. PPAR-γ ligands include a surprisingly diverse set of natural ligands [1], such as prostaglandin PGJ2, linolenic, eicosapentaenoic, docohexaenoic, and arachidonic acids, and synthetic ligands, such as the thiazolidinediones (TZDs), L-tyrosine-based compounds, several nonsteroidal anti-inflammatory drugs (NSAIDs), and a variety of new chemical classes.

The clinical relevance of PPAR-γ is highlighted by the currently marketed antidiabetic blockbuster drugs, rosiglitazone (Avandia), and pioglitazone (Actos). These antidiabetic drugs of the TZD class behave as potent and selective PPAR-γ full agonists [2]. In humans, they enhance insulin action, improve glycemic control with a significant reduction in the level of glycated haemoglobin (HbA_{1C}), and have variable effects on serum triglyceride levels in patients with type 2 diabetes [3, 4]. Despite their proven efficacy and widespread use, these drugs possess a number of deleterious side effects, including significant weight gain and peripheral edema [5].

The weight gain associated with the use of TZDs is due to multiple interacting factors. Because these agents promote adipocyte differentiation and lipid storage [6], increased adiposity is likely to be a major cause of the observed weight gain. Several studies have indeed shown that the weight gain with TZDs is associated with an increase in subcutaneous adipose tissue and either no change or a concomitant decrease in visceral fat (reviewed by Larsen et al.) [7]. Since about 90% of type 2 diabetics are obese, treatment with agents that exacerbate obesity is clearly suboptimal. In addition administration of TZDs is often accompanied by an increase in plasma volume [8] and therefore fluid retention is another potential cause of increased body weight.

Edema is a prominent problem in patients taking TZDs particularly those who are also taking insulin or sulfonylureas, and TZD treatment has been linked to an increased incidence of congestive heart failure [8, 9]. Diabetic macular edema has also been recently associated with glitazone use [10]. Because of these serious concerns, several PPAR

agonists have failed to progress to FDA approval. A number of glitazars have been terminated in late stage clinical trials because of serious side effects and/or carcinogenesis-related issues including Novo Nordisk's ragaglitazar, GlaxoSmithKline's farglitazar, Merck's MK-767, Takeda's TAK559, and more recently Bristol-Myers Squibb's muraglitazar (Pargluva) and AstraZeneca's tesaglitazar (Galida). Such a high attrition rate emphasizes the critical need for the discovery and characterization of alternative PPAR modulators that would retain the antidiabetic properties while avoiding the side effects.

Starting less than 10 years ago, several TZD-like and non-TZD-like partial PPAR-γ agonists that display insulin-sensitizing activity associated with lower stimulation of adipogenesis were described, leading to the emergence of the concept of selective PPAR-γ modulators or SPPARγMs. This concept is reminiscent of the SERM concept that proposes that different estrogen receptor ligands can have different agonist or antagonist properties depending on the cell context and the specific target gene in question [11, 12]. SPPARγMs bind in distinct manners to the ligand-binding pocket of the PPAR-γ receptor, leading to differential cofactor displacement and recruitment to the receptor, ultimately resulting in tissue and promoter-selective gene expression. A compound identified by the former Glaxo-Welcome, GW0072, one of the first SPPARγMs described in the literature, helped to unravel the partial agonist binding mode. All small molecule PPAR-γ full agonists share a common binding mode, in which the acidic head groups bind with 3 amino acid residues (Y473, H449, and H323) within the ligand-binding pocket. These interactions stabilize a charge clamp between the C-terminal activation function 2(AF-2) helix and a conserved lysine residue on the surface of the receptor, through which coactivator proteins are recruited to the receptor [13]. GW0072 was shown to bind to PPAR-γ in a unique manner, such that it does not directly interact with the AF-2 helix. Compared to full agonists, the differential binding mode of GW0072 resulted in a differential biological profile that included partial receptor transactivation and reduced ability to recruit specific cofactors and inhibition of adipocyte differentiation [14–16].

The ability to recruit differentially certain cofactors, that is, NR coactivators or corepressors to the PPAR receptor, appears to be the hallmark of the SPPARγMs. This likely results in a tissue-specific and promoter-selective expression of a favorable panel of target genes [14, 16–18]. Based on their in vitro and/or in vivo actions, coactivators have been grouped into "adverse" or "beneficial" regarding their proadipogenic or insulin-sensitizing effects. Adverse coactivators include DRIP205/TRAP220 and TIF2. DRIP205/TRAP220-deficient embryonic fibroblasts lack the ability to undergo adipogenesis while TIF2 knockout mice are resistant to diet-induced obesity and are more insulin-sensitive. In contrast, beneficial co-activators include SRC1, as highlighted by the phenotype of SRC1-deficient mice which have reduced energy expenditure and are prone to obesity [12, 19].

Although several PPAR-γ agonists have been classified as SPPARγMs, the majority of these synthetic ligands remain to be characterized at the molecular level or need to be evaluated in in vivo preclinical models in terms of weight gain. The published characteristics of several SPPARγMs have been recently reviewed by others [12, 16, 48, 50] and are summarized in Table 1. This review concentrates on the most recent developments in the SPPARγM arena, including metaglidasen/halofenate, PA-082, and the angiotensin receptor antagonists, recently characterized as a new class of selective PPAR-γ modulators.

2. HALOFENATE AND METAGLIDASEN: TWO SPPARγM WITH CLINICAL PROOF OF CONCEPT

Halofenate is a racemic mixture of (−)- and (+)-(2-acetoaminoethyl [4-chlorophenyl] [3-trifluoromethylphenoxy] acetate). It was tested clinically in the 1970's as a hypolipidemic and hypouricemic agent. In addition to triglyceride and uric acid lowering, significant decreases in fasting plasma glucose were observed in type 2 diabetics. A recently published study demonstrates that halofenate acts as a SPPARγM [45]. In vivo, halofenate is administered as a prodrug ester, which is rapidly and completely modified to its mature circulating free acid form, halofenic acid (HA). In vitro, HA directly binds to PPAR-γ and selectively activates PPAR-γ with partial agonism in gene reporter assays (maximal activity at ∼10–15% of the maximal activity of rosiglitazone). HA is also capable of fully antagonizing the activity of the full agonist rosiglitazone. Cofactor recruitment studies reveal that HA effectively displaces the corepressors NCoR and SMRT but is unable to efficiently recruit coactivators (p300, CBP, and DRIP205/TRAP220). HA also displays weak adipogenic activity in human adipocytes and selectively modulates PPAR-γ responsive genes in 3T3-L1 adipocytes. Compared with rosiglitazone, HA is unable to efficiently induce genes involved in fatty acid storage and transport, such as FABP4, CD36, GyK, and PEPCK. In vivo, halofenate possesses acute antidiabetic properties in diabetic *ob/ob* mice. Compared with rosiglitazone, long-term treatment of obese Zucker (*fa/fa*) rats with halofenate has comparable insulin sensitization efficacy in the absence of body weight increases. Overall, these in vitro and preclinical data support the concept of halofenate as a novel SPPARγM.

Metaglidasen (formerly MBX-102) is the (−) enantiomer of halofenate which is currently in Phase II clinical development as an oral glucose-lowering agent for the treatment of type 2 diabetes. In vitro and in vivo preclinical studies revealed that metaglidasen, like halofenate, behaves as a SPPARγM with antidiabetic and hypolipidemic activity in multiple diabetic and insulin-resistant rodent models [46]. Compared to full PPAR-γ agonists, metaglidasen acts as a partial PPAR-γ agonist/antagonist that interacts with PPAR-γ in a distinct manner. The key amino acid, Tyr473, required for the binding between full agonists to human PPAR-γ is not required for metaglidasen activity. Metaglidasen also shows the lack of ability or weak ability to recruit coactivators, including CBP, DRIP205/TRAP220, and p300. Consistently, when compared to rosiglitazone, metaglidasen

Table 1: Investigational SPPARγM ligands for the treatment of type 2 diabetes.

Compound	Transcriptional activity (% full agonist)	Adipogenesis (versus full agonists)	Body weight gain (versus full agonist)	Cofactors recruitment capacity (versus full agonists)	Development stage	Refs.
GW0072	∼20–40%	Partial	No data	Decreased (CBP, SRC1, TIF2, SCR3) Similar (PGC1-α) Lack of recruitment (NCoR, SMRT)	Preclinical	[14, 15]
FMOC-L-Leucine	∼40–100%	Partial	None in a week	Decreased (p300, PGC1-α) Lack of recruitment (CBP, TIF2, SCR3) Inconsistent data for SRC1	Preclinical	[14, 20]
nTZDpa	∼25%	Partial	Decreased	No data	Preclinical	[21]
L-764406	∼25%	Partial	No data	Decreased (CBP)	Preclinical	[22]
YM440	∼10–80% (CV-1) 100% (hepG2)	Minimal	None	Similar (p300, SRC1)	Phase II discontinued	[23–25]
DRF-2593 (balaglitazone)	∼78%	Partial	Moderate	No data	Phase II	[26–28]
MCC555 (netoglitazone)	∼50–100%	Similar	None	Decreased (CBP, SRC1) Similar (SMRT)	Phase II	[29–31]
CLX-0921	100%	Partial	None in 9 days	Recruit CBP (no data in comparison with full agonists)	Preclinical discontinued	[32]
Compound 24 (benzoyl-2-methyl indole)	21%	Partial	Minimal	No data	Preclinical	[33]
Compound 12 (N-benzyl-indole)	24%	Minimal	No data	No data	Preclinical	[34]
Compound 5 (aryl indole-2-carboxylic acid)	31%	No data	Minimal in 11 days	No data	Preclinical	[35]
FK-614	∼65%	Similar	Similar	Decreased (CBP, SRC1) Similar (PBP, PRIP, PGC1-α, NCoR, SMRT)	Preclinical	[36–39]
KR-62980	∼30%	None to partial	Decreased	Decreased (AIB-1, SRC1, TRAP220) Similar (TIF2, p300)	Preclinincal	[40]
Telmisartan (ARBs)	∼30%	Partial	Decreased	Decreased (NcoR release) Similar (DRIP205) Lack of recruitment (TIF2)	Marketed	[41–44]
PA-082	∼40%	Partial	No data	Decreased (SRC1, TIF2, SCR3) Similar (PGC1-α)	Preclinical	[14]
Halofenate/ metaglidasen	∼10–15%	Partial	Decreased	Decreased (CBP, P300, TRAP220) Similar (NCoR, SMRT)	Phase II (metaglidasen)	[45–47]
AMG-131 T-131	Cell type dependent	Minimal	No data	Decreased (DRIP205) Increased association (NCoR)	Phase II discontinued	[48, 49]

shows moderate ability to promote adipogenesis and displays largely attenuated induction of PPAR-γ target genes involved in fatty acid uptake, synthesis, and storage in primary human adipocytes and mouse 3T3-L1 adipocytes. In vivo, metaglidasen lowers plasma glucose levels in multiple diabetic rodent models (*db/db* mice and ZDF rats) to comparable levels seen with full agonists without causing significant body weight gain, heart weight [46, 47], or plasma volume expansion (unpublished data), a parameter believed to contribute to edema. These observations further support the SPPARγM concept, confirming the feasibility to separate efficacy and side effects such as edema and weight gain. With respect to edema, thiazolidinediones have been recently reported to expand body fluid volume through PPAR-γ stimulation of ENaC-mediated renal salt absorption [51, 52]. Determining if metaglidasen lacks the ability to stimulate increased amiloride-sensitive $Na^{(+)}$ absorption would therefore be important. Consistent with the preclinical findings reported above, in insulin-treated type 2 diabetic patients, metaglidasen appears to have comparable efficacy to the marketed TZDs Actos (pioglitazone) and Avandia (rosiglitazone) while avoiding the limiting side effects of weight gain and edema [53]. These results position metaglidasen as an optimized SPPARγM with an improved safety profile in comparison to these TZDs.

3. A NOVEL PROMISING CLASS OF SPPARγM: PA-082, A KEY TO UNDERSTANDING THE DISSOCIATION BETWEEN WEIGHT GAIN AND INSULIN SENSITIZATION?

Researchers from Roche have recently described an isoquinoline derivative PA-082 that behaves as a novel partial agonist of the PPAR-γ receptor [14]. In cell-based reporter assays, PA-082 was capable of transactivating PPAR-γ to about 40% of the level achieved with rosiglitazone. Interestingly this partial agonism was mirrored in its ability to cause partial recruitment of some but not all coactivators to PPAR-γ. Using a FRET-based in vitro system, the authors demonstrated that PA-082 elicited a partial recruitment of an LXXLL peptide derived from SRC1, TIF2, and SRC3 to the PPAR-γ ligand-binding domain but full recruitment of the LXXLL peptide derived from PGC1-α. Importantly this selective recruitment of PGC1-α was also observed with the structurally unrelated partial agonists GW0072 and FMOC-L-Leu but not with full agonists that recruited all peptides equally. Preferential recruitment of PGC1-α might therefore be a universal hallmark of partial agonists. When compared to the full agonist rosiglitazone, PA-082 prevented triglyceride accumulation during de novo adipogenesis of C3H10T1/2 cells and was also able to antagonize rosiglitazone-induced lipid accumulation. In spite of the partial PPAR-γ agonism, PA-082 enhanced insulin-stimulated glucose uptake in adipocytes as well as rosiglitazone suggesting that PA-082 may act to improve whole body glucose disposal without increasing adipose mass. An interesting difference between rosiglitazone and PA-082 was revealed by the observation that in adipocytes PA-082 was more effective than rosiglitazone in preventing insulin resistance induced by TNFα. The crystal structure of PA-082 bound to PPAR LBD complexed with LXXLL peptide from SRC1 was also solved. Not surprisingly for a partial agonist, PA-082 did not interact with helix 12, its binding occurring in a part of the binding pocket formed by helices 3, 5, and 7, a site almost identical to that occupied by GW0072 [15]. No preclinical in vivo data are currently available for this compound.

4. ANGIOTENSIN RECEPTOR ANTAGONISTS: A NOVEL APPROACH TO ADDRESS THE MULTIFACTORIAL COMPONENTS OF THE METABOLIC SYNDROME?

Recently, angiotensin receptor blockers (ARBs) were reported to have selective PPAR-γ modulating activity [41–43, 54, 55]. Among the commercially available ARBs, structurally unique telmisartan appears to be the most potent in terms of PPAR-γ activation when tested at concentrations typically achieved in plasma with conventional oral dosing. A growing body of data indicates that telmisartan is a SPPARγM. In cell-based gene reporter assays, telmisartan behaves as a partial agonist of PPAR-γ, giving $\sim$30% of the maximal PPAR-γ activation by full agonist rosiglitazone [41]. Molecular modeling of telmisartan in the PPAR-γ ligand-binding domain reveals a different binding mode between telmisartan and rosiglitazone. Specifically, the superimposition of telmisartan on the cocrystal structure of rosiglitazone and PPAR-γ showed that telmisartan, like other partial agonists including GW0072 and nTZDpa [15, 21], does not appear to make direct contact with the activation function helix (AF-2). Interaction with the AF-2 helix has been shown to be responsible for receptor stabilization and activation by full agonists of PPAR-γ [13]. The lack of interaction of telmisartan with the AF-2 helix likely explains its inability to fully activate the receptor. This differential binding of telmisartan to PPAR-γ produced a distinct conformational change compared with rosiglitazone as assessed using a protease protection assay [42]. This in turn results in selective cofactor binding, with the absence of TIF2 recruitment and an attenuated release of the nuclear receptor corepressor NCoR compared with rosiglitazone as assessed by GST pulldown and FRET assays. Differential gene expression profiles by telmisartan versus rosiglitazone were also seen in adipocytes. Compared with rosiglitazone, telmisartan treatment resulted in attenuated induction of genes involved in FA transport and TG storage, including GyK and CD36. Although telmisartan was able to induce adipocyte differentiation [41, 56], the induction was relatively modest compared with full agonists. This is consistent with previous reports showing that other partial agonists of PPAR-γ are relatively weak stimulators, or even inhibitors, of adipogenesis [15, 21, 29]. These in vitro data suggest that telmisartan has the potential to lead to less weight gain than the full agonists. This was recently confirmed in vivo. Experiments using diet-induced obese mouse models showed that telmisartan improved insulin sensitivity without causing weight gain [42, 44]. In one study, 10 weeks of telmisartan treatment significantly reduced fasting plasma insulin and glucose levels

and improved glucose tolerance and insulin sensitivity. In terms of body weight gain and body fat content, compared with mice treated with vehicle or pioglitazone, mice treated with telmisartan had significantly less weight gain and decreased body fat content in absence of change in food intake [42]. Similar results were reported in a second study of telmisartan treatment for 14 days in diet-induced obese mice. While improving the hyperglycemia, hyperinsulinemia and hypertriglyceridemia, telmisartan treatment attenuated the diet-induced weight gain and decreased the weight of visceral adipose tissue without affecting food intake. Furthermore, telmisartan treatment was also accompanied with increased adiponectin mRNA in visceral white adipose tissue and the serum adiponectin level, reduced the serum level of resistin, increased UCP1 mRNA in brown adipose tissue, and increased oxygen consumption [44]. This suggests that telmisartan treatment may prevent the development of obesity and related metabolic disorders by altering the levels of adiponectin, resistin, and uncoupling protein 1 in these mice. Telmisartan represents a new class of SPPARγM with in vivo preclinical evidence of maintaining insulin sensitization efficacy while lacking of or preventing weight gain. Although it is unclear yet how much of the efficacy seen is contributed by the attenuated body weight gain and decreased fat mass, preclinical results indicate that telmisartan may be used for treatment of metabolic syndrome and prevention of obesity including visceral obesity.

Whether telmisartan has clinical efficacy in terms of insulin sensitization remains an open question. Several recent studies support the view that telmisartan exerts beneficial effects on lipid and glucose metabolism that involves more than its ability to block the angiotensin II receptor. In an open label post-marketing surveillance study, telmisartan treatment of patients with diabetes (40–80 mg/day in 3642 patients for 6 months) reduced serum glucose and TG compared with baseline [57]. In a randomized, parallel-group study with 40 patients, telmisartan treatment (80 mg/day for 3 months) reduced fasting plasma glucose, insulin resistance (HOMA-IR), and glycated hemoglobin compared with baseline, whereas losartan treatment had no significant effect on any of these parameters [58]. Others angiotensinogen receptor antagonists including losartan, eprosartan, valsartan, and candesartan have also been investigated. In a randomized double-blind, placebo-controlled study with 119 patients, telmisartan treatment (40 mg/day for 12 months), but not eprosartan treatment, reduced plasma total cholesterol, LDL cholesterol, and TG compared with placebo. No change in BMI or glucose metabolism was observed in any group [59]. In a recent study in which valsartan or candesartan were replaced with telmisartan in hypertensive patient with diabetes, the switch to telmisartan was associated with significant reductions in plasma insulin, serum TG, serum CRP levels, as well as increases in serum adiponectin [60]. Telmisartan also reduced serum insulin levels and improved insulin sensitivity as assessed by the homeostasis model in hypertensive nondiabetic patients [61]. Overall, compared with full PPAR-γ agonists, the magnitude of the sensitizing effect observed with telmisartan appeared weaker. In terms of adverse effects, no peripheral edema or fluid retention was observed. So far no comprehensive clinical study has evaluated the effects of telmisartan on body weight or adiposity and therefore this remains to be clarified.

5. SUMMARY AND FUTURE DIRECTIONS

The in vitro/in vivo data originating from several newly described SPPARγMs validate the SPPARγM concept in term of differential receptor binding, selective cofactor recruitment, and subsequent selective gene expression regulation. The inability to recruit adipogenic cofactors (such as TIF2), the attenuated adipogenic gene expression profile, and the attenuated adipocyte differentiation activity of these SPPARγMs are consistent with their lack of weight gain in preclinical models. It is still unclear if the ability to recruit energy expenditure prone cofactor PGC1-α is a common characteristic of SPPARγMs. Nevertheless, the recruitment of PGC1-α may provide a partial explanation in term of their ability to increase UCP1 levels, energy expenditure, and for their antiobesity effects. At this point, the predictive value of interactions between PPAR-γ and other coactivators remains uncertain and additional studies using various SPPARγMs are needed to further our understanding of these complex interactions. The key questions are does the optimal SPPARγM already exist? If not, what would the ideal profile of such an optimal SPPARγM be? And can we rationally design preclinical strategies to identify it? There is no doubt that comparison of the differential cofactor recruitment and selective gene expression regulation by various SPPARγMs will generate a wealth of information that will further our mechanistic understanding of SPPARγM biology. Recent clinical data obtained with metaglidasen confirm that SPPARγMs can maintain efficacy while lacking the typical side effects such as edema and weight gain, supporting the concept that the SPPARγM represents the next generation of insulin sensitizers.

REFERENCES

[1] S. A. Kliewer, S. S. Sundseth, S. A. Jones, et al., "Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 9, pp. 4318–4323, 1997.

[2] T. M. Willson, J. E. Cobb, D. J. Cowan, et al., "The structure-activity relationship between peroxisome proliferator-activated receptor γ agonism and the antihyperglycemic activity of thiazolidinediones," *Journal of Medicinal Chemistry*, vol. 39, no. 3, pp. 665–668, 1996.

[3] M. Gurnell, D. B. Savage, V. K. K. Chatterjee, and S. O'Rahilly, "The metabolic syndrome: peroxisome proliferator-activated receptor γ and its therapeutic modulation," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 6, pp. 2412–2421, 2003.

[4] F. Picard and J. Auwerx, "PPARγ and glucose homeostasis," *Annual Review of Nutrition*, vol. 22, pp. 167–197, 2002.

[5] R. W. Nesto, D. Bell, R. O. Bonow, et al., "Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association," *Circulation*, vol. 108, no. 23, pp. 2941–2948, 2003.

[6] F. Zhang, B. E. Lavan, and F. M. Gregoire, "Peroxisome proliferator-activated receptors as attractive antiobesity targets," *Drug News and Perspectives*, vol. 17, no. 10, pp. 661–669, 2004.

[7] T. M. Larsen, S. Toubro, and A. Astrup, "PPARγ agonists in the treatment of type II diabetes: is increased fatness commensurate with long-term efficacy?" *International Journal of Obesity*, vol. 27, no. 2, pp. 147–161, 2003.

[8] R. W. Nesto, D. Bell, R. O. Bonow, et al., "Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association," *Diabetes Care*, vol. 27, no. 1, pp. 256–263, 2004.

[9] Z. Hussein, J. M. Wentworth, A. J. Nankervis, J. Proietto, and P. G. Colman, "Effectiveness and side effects of thiazolidinediones for type 2 diabetes: real-life experience from a tertiary hospital," *Medical Journal of Australia*, vol. 181, no. 10, pp. 536–539, 2004.

[10] E. H. Ryan Jr., D. P. Han, R. C. Ramsay, et al., "Diabetic macular edema associated with glitazone use," *Retina*, vol. 26, no. 5, pp. 562–570, 2006.

[11] J. M. Olefsky, "Treatment of insulin resistance with peroxisome proliferator-activated receptor γ agonists," *Journal of Clinical Investigation*, vol. 106, no. 4, pp. 467–472, 2000.

[12] C. Knouff and J. Auwerx, "Peroxisome proliferator-activated receptor-γ calls for activation in moderation: lessons from genetics and pharmacology," *Endocrine Reviews*, vol. 25, no. 6, pp. 899–918, 2004.

[13] R. T. Nolte, G. B. Wisely, S. Westin, et al., "Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ," *Nature*, vol. 395, no. 6698, pp. 137–143, 1998.

[14] E. Burgermeister, A. Schnoebelen, A. Flament, et al., "A novel partial agonist of peroxisome proliferator-activated receptor-γ (PPARγ) recruits PPARγ-coactivator-1α, prevents triglyceride accumulation, and potentiates insulin signaling in vitro," *Molecular Endocrinology*, vol. 20, no. 4, pp. 809–830, 2006.

[15] J. L. Oberfield, J. L. Collins, C. P. Holmes, et al., "A peroxisome proliferator-activated receptor γ ligand inhibits adipocyte differentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 11, pp. 6102–6106, 1999.

[16] A. R. Miller and G. J. Etgen, "Novel peroxisome proliferator-activated receptor ligands for type 2 diabetes and the metabolic syndrome," *Expert Opinion on Investigational Drugs*, vol. 12, no. 9, pp. 1489–1500, 2003.

[17] T.-A. Cock, S. M. Houten, and J. Auwerx, "Peroxisome proliferator-activated receptor-γ: too much of a good thing causes harm," *EMBO Reports*, vol. 5, no. 2, pp. 142–147, 2004.

[18] S. M. Rangwala and M. A. Lazar, "The dawn of the SPPARMs?" *Science's Signal Transduction Knowledge Environment*, vol. 2002, no. 121, p. Pe9, 2002.

[19] F. Picard, M. Géhin, J.-S. Annicotte, et al., "SRC-1 and TIF2 control energy balance between white and brown adipose tissues," *Cell*, vol. 111, no. 7, pp. 931–941, 2002.

[20] S. Rocchi, F. Picard, J. Vamecq, et al., "A unique PPARγ ligand with potent insulin-sensitizing yet weak adipogenic activity," *Molecular Cell*, vol. 8, no. 4, pp. 737–747, 2001.

[21] J. P. Berger, A. E. Petro, K. L. Macnaul, et al., "Distinct properties and advantages of a novel peroxisome proliferator-activated protein γ selective modulator," *Molecular Endocrinology*, vol. 17, no. 4, pp. 662–676, 2003.

[22] A. Elbrecht, Y. Chen, A. Adams, et al., "L-764406 is a partial agonist of human peroxisome proliferator-activated receptor γ. The role of Cys313 in ligand binding," *Journal of Biological Chemistry*, vol. 274, no. 12, pp. 7913–7922, 1999.

[23] E. Kurosaki, R. Nakano, K. Momose, et al., "Hypoglycemic agent YM440 suppresses hepatic glucose output via gluconeogenesis by reducing glucose-6-phosphatase activity in obese Zucker rats," *European Journal of Pharmacology*, vol. 468, no. 2, pp. 151–158, 2003.

[24] A. Shimaya, E. Kurosaki, R. Nakano, R. Hirayama, M. Shibasaki, and H. Shikama, "The novel hypoglycemic agent YM440 normalizes hyperglycemia without changing body fat weight in diabetic db/db mice," *Metabolism*, vol. 49, no. 3, pp. 411–417, 2000.

[25] R. Nakano, E. Kurosaki, A. Shimaya, and M. Shibasaki, "The novel hypoglycemic agent YM440 improves hepatic insulin resistance in obese Zucker fatty rats," *Journal of Pharmacological Sciences*, vol. 101, no. 4, pp. 311–317, 2006.

[26] R. Chakrabarti, R. K. Vikramadithyan, P. Misra, J. Suresh, and R. Rajagopalan, "Balaglitazone, a quinazalone analogue of thiazolidione shows excellent antidiabetic and hypolipidemic potential with less adipogenic activity," *Diabetes*, vol. 52, supplement 1, p. 601-P, 2003.

[27] D. Koev, C. Ionescu-Trgoviste, A. Rosenthal, et al., "Balaglitazone, a new thiazolidinedione, improves glucose and lipid control in patients with type 2 diabetes," *Diabetes*, vol. 52, supplement 1, p. 530-P, 2003.

[28] E. M. Wulff, K. E. Pedersen, and P. Sauerberg, "Balaglitazone a new partial PPAR γ agonist has a better cardio vascular safety profile and glycemic control compared with the full PPAR γ agonist rosiglitazone," *Diabetes*, vol. 52, supplement 1, p. 594P, 2003.

[29] M. J. Reginato, S. T. Bailey, S. L. Krakow, et al., "A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor γ-activating properties," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32679–32684, 1998.

[30] L. S. Liu, H. Tanaka, S. Ishii, and J. Eckel, "The new antidiabetic drug MCC-555 acutely sensitizes insulin signaling in isolated cardiomyocytes," *Endocrinology*, vol. 139, no. 11, pp. 4531–4539, 1998.

[31] R. Upton, P. S. Widdowson, S. Ishii, H. Tanaka, and G. Williams, "Improved metabolic status and insulin sensitivity in obese fatty (fa/fa) Zucker rats and Zucker Diabetic Fatty (ZDF) rats treated with the thiazolidinedione, MCC-555," *British Journal of Pharmacology*, vol. 125, no. 8, pp. 1708–1714, 1998.

[32] D. Dey, S. Medicherla, P. Neogi, et al., "A novel peroxisome proliferator-activated gamma (PPARγ) agonist, CLX-0921, has potent antihyperglycemic activity with low adipogenic potential," *Metabolism*, vol. 52, no. 8, pp. 1012–1018, 2003.

[33] J. J. Acton III, R. M. Black, A. B. Jones, et al., "Benzoyl 2-methyl indoles as selective PPARγ modulators," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 2, pp. 357–362, 2005.

[34] K. Liu, R. M. Black, J. J. Acton III, et al., "Selective PPARγ modulators with improved pharmacological profiles," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 10, pp. 2437–2440, 2005.

[35] J. F. Dropinski, T. Akiyama, M. Einstein, et al., "Synthesis and biological activities of novel aryl indole-2-carboxylic acid

analogs as PPARγ partial agonists," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 22, pp. 5035–5038, 2005.

[36] T. Fujimura, C. Kimura, T. Oe, et al., "A selective peroxisome proliferator-activated receptor γ modulator with distinct fat cell regulation properties," *Journal of Pharmacology and Experimental Therapeutics*, vol. 318, no. 2, pp. 863–871, 2006.

[37] T. Fujimura, H. Sakuma, S. Konishi, et al., "FK614, a novel peroxisome proliferator-activated receptor γ modulator, induces differential transactivation through a unique ligand-specific interaction with transcriptional coactivators," *Journal of Pharmacological Sciences*, vol. 99, no. 4, pp. 342–352, 2005.

[38] H. Minoura, S. Takeshita, T. Yamamoto, et al., "Ameliorating effect of FK614, a novel nonthiazolidinedione peroxisome proliferator-activated receptor γ agonist, on insulin resistance in Zucker fatty rat," *European Journal of Pharmacology*, vol. 519, no. 1-2, pp. 182–190, 2005.

[39] H. Minoura, S. Takeshita, M. Ita, et al., "Pharmacological characteristics of a novel nonthiazolidinedione insulin sensitizer, FK614," *European Journal of Pharmacology*, vol. 494, no. 2-3, pp. 273–281, 2004.

[40] K. R. Kim, J. H. Lee, S. J. Kim, et al., "KR-62980: a novel peroxisome proliferator-activated receptor γ agonist with weak adipogenic effects," *Biochemical Pharmacology*, vol. 72, no. 4, pp. 446–454, 2006.

[41] S. C. Benson, H. A. Pershadsingh, C. I. Ho, et al., "Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARγ-modulating activity," *Hypertension*, vol. 43, no. 5, pp. 993–1002, 2004.

[42] M. Schupp, M. Clemenz, R. Gineste, et al., "Molecular characterization of new selective peroxisome proliferator-activated receptor γ modulators with angiotensin receptor blocking activity," *Diabetes*, vol. 54, no. 12, pp. 3442–3452, 2005.

[43] M. Schupp, L. D. Lee, N. Frost, et al., "Regulation of peroxisome proliferator-activated receptor γ activity by losartan metabolites," *Hypertension*, vol. 47, no. 3, pp. 586–589, 2006.

[44] K. Araki, T. Masaki, I. Katsuragi, K. Tanaka, T. Kakuma, and H. Yoshimatsu, "Telmisartan prevents obesity and increases the expression of uncoupling protein 1 in diet-induced obese mice," *Hypertension*, vol. 48, no. 1, pp. 51–57, 2006.

[45] T. Allen, F. Zhang, S. A. Moodie, et al., "Halofenate is a selective peroxisome proliferator-activated receptor γ modulator with antidiabetic activity," *Diabetes*, vol. 55, no. 9, pp. 2523–2533, 2006.

[46] F. Zhang, E. Clemens, F. M. Gregoire, et al., "Metaglidasen, a selective PPARγ modulator (SPPARγM) with anti-diabetic and hypo-lipidemic activity in multiple diabetic and insulin-resistant rat models," in *Conference on Diabetes Mellitus and the Control of Cellular Energy Metabolism*, Keystone Symposia, Vancouver, BC, Canada, January 2006, abstract no. 342a.

[47] F. Zhang, E. L. Clemens, F. M. Gregoire, et al., "Metaglidasen, a novel selective peroxisome proliferator-activated receptor-γ modulator, preserves pancreatic islet structure and function in db/db mice," *Diabetes*, vol. 55, p. 1396-P, 2006.

[48] R. S. Savkur and A. R. Miller, "Investigational PPAR-γ agonists for the treatment of type 2 diabetes," *Expert Opinion on Investigational Drugs*, vol. 15, no. 7, pp. 763–778, 2006.

[49] Y. Li, Z. Wang, A. Motani, et al., "T0903131 (T131): A selective modulator of PPARγ," *Diabetes*, vol. 53, supplement 2, p. A157, 2004.

[50] B. L. Balint and L. Nagy, "Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases," *Endocrine, Metabolic and Immune Disorders - Drug Targets*, vol. 6, no. 1, pp. 33–43, 2006.

[51] Y. Guan, C. Hao, D. R. Cha, et al., "Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption," *Nature Medicine*, vol. 11, no. 8, pp. 861–866, 2005.

[52] H. Zhang, A. Zhang, D. E. Kohan, R. D. Nelson, F. J. Gonzalez, and T. Yang, "Collecting duct-specific deletion of peroxisome proliferator-activated receptor γ blocks thiazolidinedione-induced fluid retention," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 26, pp. 9406–9411, 2005.

[53] J. Rosenstock, F. Flores-Lozano, S. Schartz, G. Gonzalez-Galvez, and D. Karpf, "MBX-102: a novel non-TZD insulin sensitizer that improves glycemic control without causing edema or weight gain in patients with type 2 diabetes (T2DM) on concomitant insulin therapy," *Diabetes*, vol. 54, supplement 1, p. A11, 2005.

[54] H. A. Pershadsingh, "Treating the metabolic syndrome using angiotensin receptor antagonists that selectively modulate peroxisome proliferator-activated receptor-γ," *International Journal of Biochemistry and Cell Biology*, vol. 38, no. 5-6, pp. 766–781, 2006.

[55] C. Di Filippo, E. Lampa, E. Tufariello, et al., "Effects of irbesartan on the growth and differentiation of adipocytes in obese Zucker rats," *Obesity Research*, vol. 13, no. 11, pp. 1909–1914, 2005.

[56] J. Janke, M. Schupp, S. Engeli, et al., "Angiotensin type 1 receptor antagonists induce human in-vitro adipogenesis through peroxisome proliferator-activated receptor-γ activation," *Journal of Hypertension*, vol. 24, no. 9, pp. 1809–1816, 2006.

[57] M. C. Michel, H. Bohner, J. Köster, R. Schäfers, and U. Heemann, "Safety of telmisartan in patients with arterial hypertension: an open-label observational study," *Drug Safety*, vol. 27, no. 5, pp. 335–344, 2004.

[58] C. Vitale, G. Mercuro, C. Castiglioni, et al., "Metabolic effect of telmisartan and losartan in hypertensive patients with metabolic syndrome," *Cardiovascular Diabetology*, vol. 4, Article ID 6, 2005.

[59] G. Derosa, P. D. Ragonesi, A. Mugellini, L. Ciccarelli, and R. Fogari, "Effects of telmisartan compared with eprosartan on blood pressure control, glucose metabolism and lipid profile in hypertensive, type 2 diabetic patients: a randomized, double-blind, placebo-controlled 12-month study," *Hypertension Research*, vol. 27, no. 7, pp. 457–464, 2004.

[60] Y. Miura, N. Yamamoto, S. Tsunekawa, et al., "Replacement of valsartan and candesartan by telmisartan in hypertensive patients with type 2 diabetes: metabolic and antiatherogenic consequences," *Diabetes Care*, vol. 28, no. 3, pp. 757–758, 2005.

[61] R. A. Benndorf, T. Rudolph, D. Appel, et al., "Telmisartan improves insulin sensitivity in nondiabetic patients with essential hypertension," *Metabolism*, vol. 55, no. 9, pp. 1159–1164, 2006.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 97125, 13 pages
doi:10.1155/2007/97125

Research Article

The Effect of PPARα, PPARδ, PPARγ, and PPARpan Agonists on Body Weight, Body Mass, and Serum Lipid Profiles in Diet-Induced Obese AKR/J Mice

W. Wallace Harrington, Christy S. Britt, Joan G. Wilson, Naphtali O. Milliken, Jane G. Binz, David C. Lobe, William R. Oliver, Michael C. Lewis, and Diane M. Ignar

Department of Metabolic Diseases, GlaxoSmithKline Research, Research Triangle Park, NC 27709, USA

Received 18 November 2006; Revised 8 February 2007; Accepted 3 March 2007

Recommended by Francine M. Gregoire

Activation of peroxisome proliferator-activated receptor (PPAR) α, δ, and γ subtypes increases expression of genes involved in fatty acid transport and oxidation and alters adiposity in animal models of obesity and type-2 diabetes. PPARpan agonists which activate all three receptor subtypes have antidiabetic activity in animal models without the weight gain associated with selective PPARγ agonists. Herein we report the effects of selective PPAR agonists (GW9578, a PPARα agonist, GW0742, a PPARδ agonist, GW7845, a PPARγ agonist), combination of PPARα and δ agonists, and PPARpan (PPAR$\alpha/\gamma/\delta$) activators (GW4148 or GW9135) on body weight (BW), body composition, food consumption, fatty acid oxidation, and serum chemistry of diet-induced obese AKR/J mice. PPARα or PPARδ agonist treatment induced a slight decrease in fat mass (FM) while a PPARγ agonist increased BW and FM commensurate with increased food consumption. The reduction in BW and food intake after cotreatment with PPARα and δ agonists appeared to be synergistic. GW4148, a PPARpan agonist, induced a significant and sustained reduction in BW and FM similar to an efficacious dose of rimonabant, an antiobesity compound. GW9135, a PPARpan agonist with weak activity at PPARδ, induced weight loss initially followed by rebound weight gain reaching vehicle control levels by the end of the experiment. We conclude that PPARα and PPARδ activations are critical to effective weight loss induction. These results suggest that the PPARpan compounds may be expected to maintain the beneficial insulin sensitization effects of a PPARγ agonist while either maintaining weight or producing weight loss.

1. INTRODUCTION

Obesity has risen to epidemic proportions world wide and is one the most visible, yet often neglected,of public health issues. It is now prevalent in virtually all age and socioeconomic groups in both developed and developing nations [1]. Obesity is a complex, multifactorial condition produced by genetic, social, and psychological factors,the most significant being high-fat diet and sedentary life style. The health consequences of obesity range from increased risk of premature death to serious chronic conditions such as type 2 diabetes, dyslipidemia, atherosclerosis, hypertension, cardiovascular diseases, stroke, and certain forms of cancer [2–5]. Agents that reduce obesity through reductions in food intake or increased energy expenditure could serve as therapeutic options for the prevention of obesity and its comorbidities [6–8].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the superfamily of nuclear receptors [9]. Three subtypes, designated PPARα (NR1C1), PPARδ (NR1C2), and PPARγ (NR1C3) have been identified whose endogenous ligands include fatty acids and fatty acid metabolites. PPARs form heterodimers with retinoid X receptors (RXRs) and bind to the hexanucleotidic PPAR responsive element (PPRE), thereby regulating the expression of target genes involved in lipid and carbohydrate metabolism.

PPARs are found in species ranging from Xenopus to humans [9] with each receptor having a distinct tissue expression profile. PPARα is expressed mainly in the liver, heart, and muscle. The discovery that fibrates are hypolipidemic agents which activate PPARα suggested that this receptor may play a role in lipid metabolism [9, 10]. Indeed, activation of PPARα has been shown to upregulate genes involved in

hepatic lipid and lipoprotein metabolism and fatty acid oxidation in skeletal muscle. In addition, these agents decrease adiposity in animal models of obesity and type-2 diabetes mellitus (T2DM). For example, fenofibrate has been shown to reduce food intake, body weight, and adiposity in several mouse models and obesity-prone rats [11, 12]. PPARδ has a broad pattern of distribution and is expressed in many tissues, including muscle and kidney [13]. Recent work has suggested that PPARδ is involved in overall energy regulation and fatty acid oxidation in the muscle. Activation of PPARδ has also been shown to increase high-density lipoprotein cholesterol (HDL-c) in diabetic db/db mice and obese rhesus monkeys [14]. Studies by Wang et al. [15] suggest that overexpression of PPARδ in adipose tissue protects against diet-induced obesity in mice and treatment with a PPARδ selective agonist reduces weight gain without effects on food intake in fat-fed mice [16].

The discovery that glitazones activate PPARγ receptor has elucidated the role of this receptor in lipid transport and storage and carbohydrate metabolism [17]. PPARγ is expressed predominantly in white and brown adipose tissue and is important in the regulation and control of adipocyte development and function [18]. Treatment with PPARγ agonists enhances the action of insulin and reduces serum glucose in subjects with T2DM, however, substantial body weight gain also occurs that is comprised of both fat mass and fluid volume [19–22]

PPARpan agonists can activate all three PPAR receptor subtypes and exert a variety of effects on multiple tissues simultaneously. This class of compounds has been shown to have antidiabetic efficacy in several animal models of T2DM [23]. These compounds also affect lipoprotein composition and reduce atherosclerotic plaque formation without the weight gain associated with PPARγ agonists suggesting their utility in treatment of metabolic syndrome [24, 25].

A number of studies have described the effect of individual PPAR agonists in a variety of animal models or experimental paradigms [14, 26–28]. This study provides a systematic four-week evaluation of potent and selective agonists of the three PPAR isoforms, the combination of PPARα and δ agonists and PPARpan agonists in a single chronic model of diet-induced obesity. We report the effects of these agents on body weight, body composition, fatty acid oxidation, and clinical chemistry in obesity-prone AKR/J mice.

2. METHODS

2.1. In vitro potency and selectivity

2.1.1. Assessment of PPAR activation using GAL4 transient transfection assay

The functional potency of selected ligands was evaluated using a transient transfection assay in CV-1 cells. The ligand binding domains for murine PPARα, PPARδ, and PPARγ were fused to the yeast transcription factor GAL4 DNA binding domain as a chimera. CV-1 cells were propagated and transiently transfected with expression vectors for the respective PPAR chimera as previously described [29, 30]. Test compounds were compared to reference comparators that give maximum responses in this assay. Compounds which produced an activation of at 70% or greater, compared to a positive control, were considered full agonists.

2.1.2. Ex vivo quantification of PPAR-induced fatty acid oxidation

Fatty acid oxidation (FAO) was determined by ^{14}C-labeled CO_2 capture from tissue homogenates using a method modified from Dohm et al. [31]. Following treatment with either vehicle or a PPAR agonist, livers from fed mice were surgically removed and a section excised from the same lobe. The tissue was immediately weighed, minced with scissors and placed in tubes (Falcon #2063) on ice. Cold SET buffer (250 mM Sucrose, 1 mM EDTA, 10 mM Tris, pH 7.4) was added at a ratio of 10 mL SET:1 gram of tissue and the tissue homogenized on ice for 15 sec using a hand-held homogenizer (Polytron PT1200; Kinematica AG). The homogenates remained on ice until assayed.

The labeled reaction buffer was prepared by first drying ^{14}C-oleic acid (0.5 μCi/reaction; PerkinElmer #NEC-317) under nitrogen. The dried fraction is re-suspended in unlabeled oleic acid such that the final concentration of oleic acid in the reaction buffer was 0.2 mM. BSA was added slowly while mixing to a final concentration of 0.5% and the mixture was incubated at 37°C for 15 minutes. The labeled cocktail was then added to the reaction buffer to give a produce concentration of 100 mM sucrose, 10 mM Tris pH 7.4, 4 mM ATP, 0.05 mM Coenzyme A, 0.1 mM malic acid, 1 mM magnesium chloride, 80 mM potassium chloride, 5 mM potassium phosphate, 0.2 mM EDTA, and 2 mM L-carnitine, as described previously [32, 33].

Oxidation reactions were performed in tubes (Falcon #352059) fitted with a stopper top (KONTES Glass Co., #882310-0000), center well (KONTES # 882320-0000), and filter (Socorex #322.02) soaked with 175 μL of 1N NaOH. 100 μL of homogenate was dispensed into each tube and the reactions initiated by adding 400 μL of reaction buffer. The tubes were quickly capped and incubated with gentle shaking for 60 minutes in a 37°C water bath. After incubation, the filters were removed, from the tubes, placed in 7 mL of scintillant, and counted for 2 minutes (PerkinElmer Tri-Carb 3100TR). The oxidative activity of each compound was calculated as nmole CO_2 captured/gram tissue/hour and reported as fold change relative to vehicle control.

2.2. In vivo animal studies

All procedures were performed in compliance with the Animal Welfare Act, USDA regulations and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee. Animals were housed at 72°F and 50% relative humidity with a 12-hour light and dark cycle.

2.2.1. Compounds

All compounds evaluated were synthesized by the Medicinal Chemistry Department at GlaxoSmithKline, Inc., and were

determined to be >90% pure by HPLC and/or NMR analysis [34]. Dosing solutions of GW7845, GW0742, GW9578, GW4148, and GW9135 were prepared as a suspension in a vehicle of 0.5% methylcellulose and 0.1% Tween 80 and dosed at 10 mL/kg. Doses of each PPAR ligand were chosen from results of previous in-house efficacy studies.

2.2.2. Effect of PPAR agonists on body weight, body mass, and food consumption

The effects of monotherapy with selective PPAR agonists, combination therapy with PPARα and PPARδ, and treatment with PPARpan agonists were evaluated in four experiments in diet-induced obese (DIO) AKR/J mouse. The AKR/J mouse is a polyoma-susceptible strain originally utilized to study accelerated tumor development [35]. This strain becomes obese and hyperinsulemic when fed a high fat diet [36–39]. Age-matched, male AKR/J mice were allowed ad libitum access to Research Diet D12331 (Research Diet, Brunswick, NJ) at the Jackson Laboratories (Bar Harbor, ME) beginning at 6 weeks of age. The diet has an energy density of 5.56 kcal/g (58% kcal from fat; 26% kcal from carbohydrates, and 16% kcal from protein). The animals were allowed to become obese, achieving BW >40 grams before shipping to GlaxoSmithKline laboratory animal facility at 13 weeks of age. The mice were housed 4 per cage in standard shoebox cages and were fed the high fat diet until they reached approximately 50 grams. Age-matched lean control animals obtained from Jackson Laboratories were fed a diet of normal chow (3.04 kcal/g energy density, 12% kcal from fat, LabDiet 5001, St. Louis, MO) and used for comparison.

At the beginning of each study, the animals were weighed and body composition obtained using an EchoMRI-100 quantitative magnetic resonance (qMR, EchoMRI, Houston, TX) whole body composition analyzer [40, 41]. Mice were sorted into groups (n =8–10/group) such that BW and body mass (% lean and fat mass) were not significantly different at the beginning of the study. 16 lean control mice on standard chow were used as reference. All mice were dosed orally with vehicle (0.5% methylcellulose and 0.1% Tween 80, 10 mL/kg) for six days prior to the beginning of dosing for acclimation to handling and treatment before drug treatment was initiated.

In each experiment, BW of each animal was measured and recorded three times weekly throughout the treatment period. Body mass was obtained weekly on days 0, 7, 13, 20, and 27 of treatment. The effects of selective PPARα, δ, and γ agonists on food consumption were also assessed. Food consumption is expressed as total energy consumed (kcal) over a 24-period and as cumulative consumption over the course of the experiment.

The fat content of Research Diets D12331 chow results in pellets that crumbles making it difficult to quantify food consumption thus, Research Diets D12451 chow (4.7 kcal/g (45% kcal from fat, 35% kcal from carbohydrates and 20% kcal from protein)) was used in studies where food consumption was determined as these pellets are more solid. The animals were transitioned two weeks before compound dosing from Research Diets D12331 chow to Research Diets D12451 chow. Previous experiments (data not presented) have shown that animals fed this diet maintain the same BW and fat mass level as observed at the time of transition.

On the final day of each experiment, a terminal blood sample (800–1000 μL) was obtained via cardiac puncture under isoflurane anesthesia. Whole blood was placed in a Terumo Capiject blood collection tube (Terumo Medical Corp., Elkton, Md, USA), allowed to sit at room temperature for 20 minutes then centrifuged to obtain serum. Serum levels of glucose, triglycerides, glycerol, nonesterified fatty acids, total cholesterol, the high-density lipoprotein cholesterol, and β-hydroxybutyrate were determined in all mice using an Olympus AU640 clinical chemistry immuno-analyzer (Olympus America Inc., Melville, NY, USA). In addition, liver weights were obtained following the terminal blood sample on the final day of the study and samples were used to determine liver fatty acid oxidation activity.

3. EXPERIMENTAL DESIGN

Experiment 1 was designed to study the effects of a selective PPARα agonist and PPARδ agonist as mono and combination therapy. 48 mice were sorted into 6 groups and blocked such that initial BW and body composition were not different between groups. Three groups of animals (n = 8) were dosed with Vehicle, the PPARα agonist (GW9578, 1 mg/kg), or the PPARδ agonist (GW0742, 30 mg/kg) for 4 weeks. Two additional groups of mice were dosed for the first 14 days with either a PPARα agonist or a PPARδ agonist alone. At day 15, the PPARδ agonist was added to the treatment regimen of animals dosed with PPARα, and the PPARα agonist was added to the dosing material of animals previously dosed with PPARδ alone. The sixth group was dosed with both the PPARα and PPARδ agonists for the entire 28-day period. BW and food consumption were assessed 3 times per week and body composition was measured weekly.

In Experiment 2, 32 mice were sorted into 4 groups (n = 8/group) and dosed with vehicle and a selective PPARγ agonist (GW7845, 3 mg/kg) for 28 days. Rimonabant (RIM, 10 or 30mg/kg, q.d.), a CB1 receptor antagonist, was used as a positive control for weight loss. As in Experiment 1, BW and food consumption were determined 3 times per week and body composition was measured weekly.

In Experiment 3, three groups of mice (n = 9) were dosed for 28 days with vehicle or GW4148 (3 or 10 mg/kg), a PPARpan agonist that potently activates all three receptor subtypes. In Experiment 4, five groups of mice (n = 8) were dosed for 28 days with vehicle or GW9135 (3 or 10 mg/kg), a PPARpan agonist that has a different profile of PPARα, δ, and γ activation from GW4148.

4. DATA ANALYSIS

All data are expressed as mean ± standard error of the mean. Weight loss experiments were analyzed using Analysis of Covariance (ANCOVA) with repeated measures followed by Dunnett's post hoc test. Comparison of serum chemistry

Table 1: Activation of murine PPAR receptors by PPAR agonists in cell-based transactivation assays. Compounds were assayed for agonist activity using the PPAR-GAL4 transactivation assay using an SPAP reporter transiently transfected in CV-1 cells as described in [25]. Data are mean ± SE of four or more independent experiments. The EC_{50} value was defined as the concentration of test compound that produced 50 ± 10% of the maximal reporter activity.

	Murine receptor activation (nM)					
	mPPARα	%Max	mPPARδ	%Max	mPPARγ	%Max
GW9578	8.1	95	2344.2	76	2818.4	96
GW0742	8810.5	55	28.2	73	10000.0	67
GW7845	10770.9	30	10000.0	12	1.2	247
GW4148	41.8	114	9.4	134	37.3	88
GW9135	13.4	240	676.2	99	96.8	160

values, food consumption and fat and lean mass changes between start and end of studies was analyzed by two-way analysis of variance with repeated measures model (ANOVA) followed by Dunnett's post hoc test. Values were considered to be significant when a value of $P < .05$ was achieved.

5. RESULTS

5.1. Assessment of PPAR activation using GAL4 transient transfection assay

Each compound evaluated in vivo was characterized with regard to activation of the three PPAR subtypes [36] as shown in Table 1. These compounds are full agonists of their respective receptors. GW9578 is a potent agonist of murine PPARα receptors with an $EC_{50}$of 8 nM and more than a 250-fold selectivity over PPARγ and PPARδ [34]. GW0742 is a potent and selective PPARδ agonist, (EC_{50} = 28 nM) having a 300-fold selectivity over PPARα and PPARγ [26]. GW7845 is a potent and selective PPARγ agonist with an EC_{50} of 1.2 nM and >1000-fold selectivity over the other murine PPAR subtypes [27]. Both PPARpan agonists used in this study activate all of the PPAR subtypes, however, GW4148 and GW9135 have different potency profiles. GW4148 is nearly equipotent at murine PPAR$\alpha/\delta/\gamma$ (EC_{50} < 100 nM), while GW9135 is most potent at the PPARα receptor with significant activity on PPARγ and weak potency at PPARδ.

5.2. In vivo studies

Experiment 1. Effect of mono- and combination therapy of PPARα and PPARδ Agonists in Obese AKR/J Mice

The first experiment was designed to compare the effects of selective PPARα (GW9578, 1 mg/kg) and PPARδ (GW0742, 30 mg/kg) agonists, and the combination of the two agents, on BW, fat mass (FM), lean mass (LM), and food consumption. Data are shown in Figure 1, Figure 2, and Table 2. Vehicle-treated mice weighed approximately 50 grams at initiation of the study and BW did not change during the study. While there was an initial weight loss trend, neither compound induced a sustained decrease in BW after 28 days of dosing (see Figure 1(a)).

On day 0, FM and LM (see Table 2) comprised 21.8 ± 1.6% (7.2 ± 0.6 grams) and 63.1 ± 1.3% (20.8 ± 0.4 grams) of total body weight, respectively, in lean mice. The remaining mass of each animal is composed of bone, free water (as cellular, interstitial and, blood volumes), and the contents of the gastrointestinal tract and bladder. In the DIO vehicle group, FM was nearly twice that of the lean mice (16.1 ± 1.4 grams; 39.5% of BW), but LM was similar (21.2 ± 0.2 grams; 53.1% of BW). FM and LM did not change in the DIO or lean vehicle groups in any of the experiments.

In spite of the fact that neither agent produced a significant decrease in BW, there was a slight decrease in FM after treatment with either the PPARα or PPARδ agonist while LM was unaffected (see Table 2, Experiment 1). Both agents produced a statistically significant increase in liver weight of nearly 1 gram that appears to have counterbalanced the change in fat mass resulting in unaltered BW.

Both the PPARα and PPARδ agonists affected food consumption. Compared to vehicle-treated animals, the PPARα agonist reduced food consumption while the PPARδ agonist produced a small but statistically significant increase in feeding (see Figure 2(a)). The effect of PPARα activation on feeding did not occur until day 10, the same point when weight loss had reached a plateau and subsequently began to rebound.

A second goal of Experiment 1 was to examine the effects of PPARα and PPARδ in combination on BW, body mass, and food consumption. Minimal BW changes were observed with the PPARα or PPARδ agonists alone similar to Figure 1(a). At Day 14, the PPARα agonist was added to the group dosed with PPARδ alone or vice versa for an additional 14 days. Both conditions resulted in weight loss (see Figure 1(b)) greater than observed with either agent alone. The overall weight loss from either combination was approximately 15% which was commensurate to the decrease in FM. A third group of mice was dosed with a combination of the PPARα and PPARδ agonists for the entire 28- day period. This treatment resulted in a 22% reduction in BW that occurred by 14 days. Both final BW and FM were similar to that of lean controls.

Addition of the PPARδ agonist to the PPARα agonist dosing regimen at 14 days did not have a significant effect on food consumption (see Figure 2(b)). However, adding PPARα to the dosing regimen of mice receiving the PPARδ agonist reduced food consumption to the level seen with PPARα agonist alone. Interestingly, simultaneous dosing

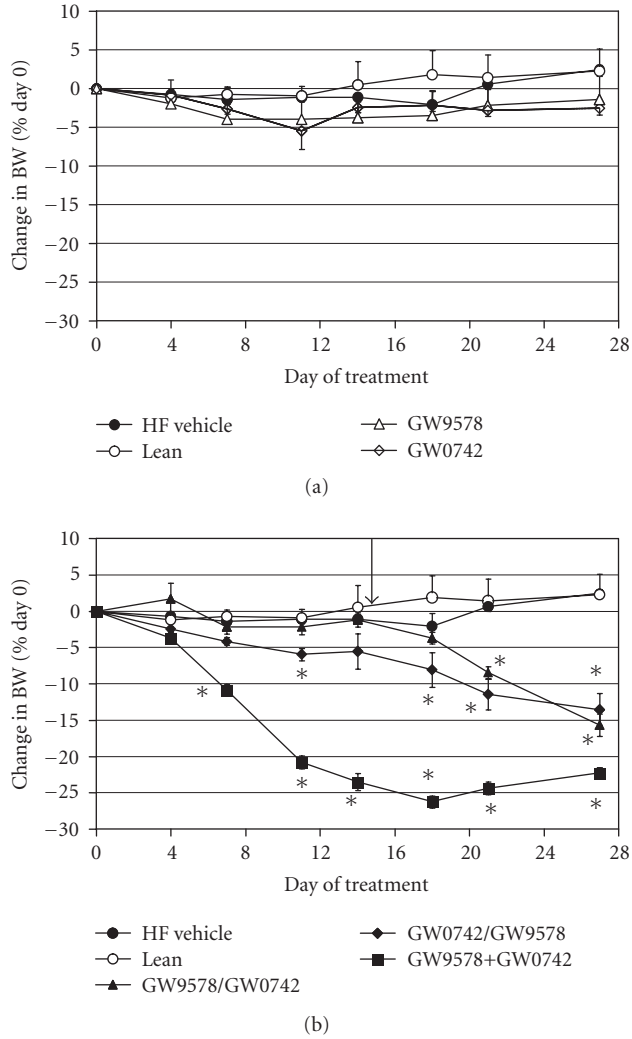


Figure 1: Effect of treatment with selective PPARα and PPARδ agonists on BW in lean and DIO AKR/J mice. (a) GW9578, a PPARα agonist (1 mg/kg), GW0742, a PPARδ agonist (30 mg/kg). (b) Filled triangle: GW9578 dosed for 14 days then combined with GW0742; filled diamond: GW0742 dosed for 14 days then combined with GW9578; filled square: GW9578 and GW0742 dosed together for 28 days. The arrow indicates the point at which the sequential combination of PPARα and PPARδ began. Data were analyzed by ANCOVA with repeated measures followed by Dunnett's post hoc test. Values were considered to be significant (*) when a value of $P < .05$ was achieved. $N = 8$–10 animals/group.

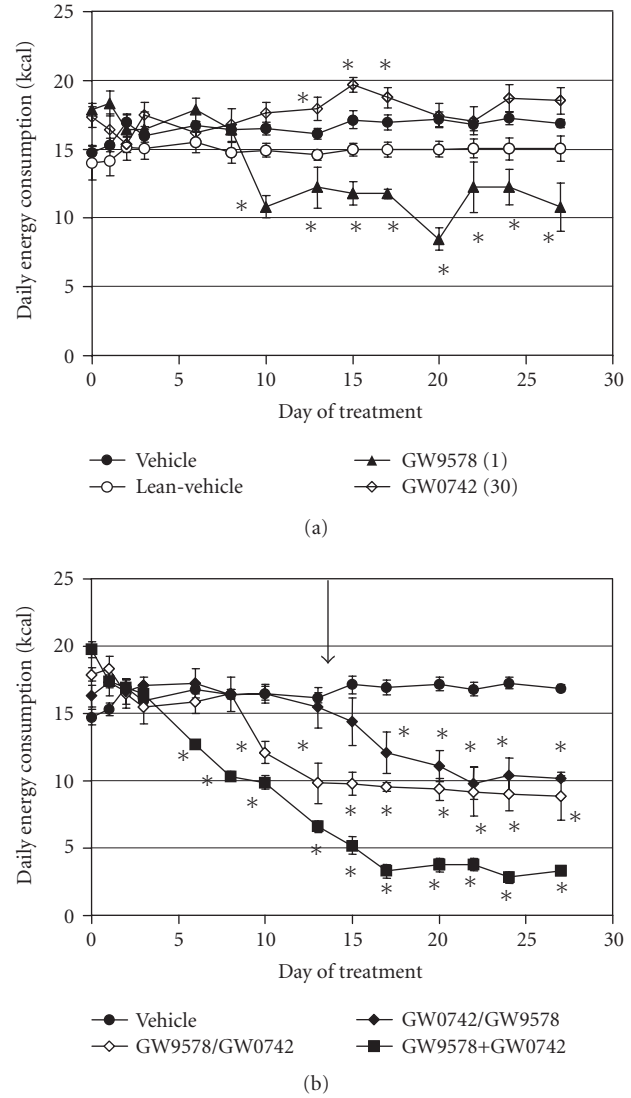


Figure 2: Effect of treatment with selective PPARα and PPARδ agonists on food consumption (kcal) in lean and DIO AKR/J mice. (a) GW9578, a PPARα agonist (1 mg/kg), GW0742, a PPARδ agonist (30 mg/kg). (b) Filled triangle: GW9578 dosed for 14 days then combined with GW0742; filled diamond: GW0742 dosed for 14 days then combined with GW9578; filled square: GW9578 and GW0742 dosed together for 28 days. The arrow indicates the point at which the sequential combination of PPARα and PPARδ began. Data were analyzed by ANCOVA with repeated measures followed by Dunnett's post hoc test. Values were considered to be significant (*) when a value of $P < .05$ was achieved. $N = 8$–10 animals/group.

from study outset with both the PPARα and PPARδ agonists reduced feeding to a greater extent then the sequential addition of the agents.

Experiment 2. Effect of a PPARγ agonist and rimonabant in obese AKR/J mice

Where Experiment 1 focused on the effects of selective PPARα and PPARδ agonists, Experiment 2 was designed to examine the effect of GW7845, a selective PPARγ agonist dosed at 3 mg/kg on BW, FM, LM, and food consumption. RIM, a CB-1R antagonist was used as a positive control for weight loss.

Treatment with RIM at doses of 10 and 30 mg/kg produced significant, dose-related decreases of BW. At the highest dose, RIM reduced BW by 17% within the first 10 days of treatment (see Figure 3(a)) and the effect was maintained over the remainder of the study. RIM also decreased FM in a dose-dependent manner (see Table 2, Experiment 2). In contrast, the PPARγ agonist produced a steady and

Table 2: Effect of treatment with PPAR agonists on body weight (BW), body composition (FM and LM), and liver weight (LW). Shown in the table are body weight (g) and fat and lean mass (g) values of each group. FM and LM were determined using qMR at the final day of the study (day 28). LW was obtained from terminal collection at the end of the experiment. $N = 8$ mice/group. Data are expressed as mean +/− SEM. Doses are in mg/kg. Data were analyzed by two-way ANOVA with repeated measures followed by post hoc t-test. Data achieved significance when $P < .05$(*).

	Treatment	BW day 0 (grams)	BW day 28 (grams)	Fat mass day 28 (grams)	Lean mass day 28 (grams)	Liver weight (grams)
Experiment 1	Lean vehicle	36.6 ± 0.8	37.0 ± 1.0	8.4 ± 1.1	23.2 ± 0.4	1.8 ± 0.1
	DIO vehicle	50.4 ± 1.3	52.1 ± 1.8	20.8 ± 1.3	29.3 ± 0.7	2.0 ± 0.2
	GW9578 (1)	52.1 ± 1.1	51.3 ± 1.6	18.4 ± 0.9	30.9 ± 0.8	2.6 ± 0.1 *
	GW0742 (30)	51.6 ± 1.1	50.2 ± 1.2	18.4 ± 0.7 *	29.7 ± 0.7	3.2 ± 0.1 *
	GW9578 + GW4148 (after week 2)	49.0 ± 0.6	41.3 ± 1.2 *	10.2 ± 0.9 *	29.1 ± 0.5	4.3 ± 0.2 *
	GW4148 + GW9578 (after week 2)	49.5 ± 1.0	42.7 ± 0.8 *	11.0 ± 0.8 *	29.5 ± 0.9	4.3 ± 0.1 *
	GW9578 and GW4148 4 weeks	50.5 ± 0.9	39.3 ± 0.9 *	9.1 ± 0.4 *	28.4 ± 0.7	4.9 ± 0.1 *
Experiment 2	DIO vehicle	50.8 ± 0.4	49.2 ± 0.5	20.7 ± 0.8	25.4 ± 1.0	1.9 ± 0.1
	RIM (10)	50.6 ± 0.8	44.1 ± 1.3 *	15.0 ± 0.9	25.2 ± 0.8	2.0 ± 0.1
	RIM (30)	51.0 ± 0.7	41.8 ± 0.5 *	11.6 ± 0.3	26.5 ± 0.5	2.0 ± 0.1
	GW7845 (3)	50.9 ± 1.2	54.6 ± 1.7 *	23.5 ± 1.3	28.7 ± 0.6	2.0 ± 0.1
Experiment 3	DIO vehicle	40.8 ± 1.3	44.9 ± 1.6	17.6 ± 1.6	23.5 ± 0.5	1.9 ± 0.1
	GW4148 (3)	40.8 ± 1.2	42.0 ± 0.9	13.5 ± 0.9 *	23.7 ± 0.3	2.9 ± 0.1 *
	GW4148 (10)	40.7 ± 1.5	36.6 ± 0.9 *	10.3 ± 0.6 *	22.3 ± 0.5	3.4 ± 0.1 *
Experiment 4	Lean vehicle	33.7 ± 0.7	34.4 ± 0.8	8.4 ± 0.8	22.0 ± 0.6	1.7 ± 0.1
	DIO vehicle	40.6 ± 1.9	43.1 ± 1.6	16.5 ± 1.1	23.4 ± 0.6	1.9 ± 0.1
	GW9135 (3)	41.1 ± 1.8	42.5 ± 1.9	14.9 ± 1.5	24.1 ± 0.3	3.0 ± 0.2 *
	GW9135 (10)	41.2 ± 1.5	40.3 ± 1.2	12.3 ± 0.8 *	23.0 ± 0.4	3.3 ± 0.1 *

consistent increase in BW over the course of the experiment (see Figure 3(b)). After 28 days, the weight of these animals had increased by almost 4 grams (8.6 ± 1.4% BW) and the mice were continuing to gain weight at 4 weeks. The PPARγ agonist produced a significant increase in FM over the 28 days of the study accounting for much of the weight gain in these animals.

RIM induced dose-related decreases in food consumption with the greatest suppression observed on day 3 (see Figure 4(a)). After day 3, food consumption suppression began to wane, eventually returning to control levels by day 10 and remained at that level for the duration of the study. In contrast to the effect of RIM, food consumption of animals dosed with the PPARγ agonist increased 46% after only one day and remained elevated by more than 20% over the remaining treatment period (see Figure 4(b)).

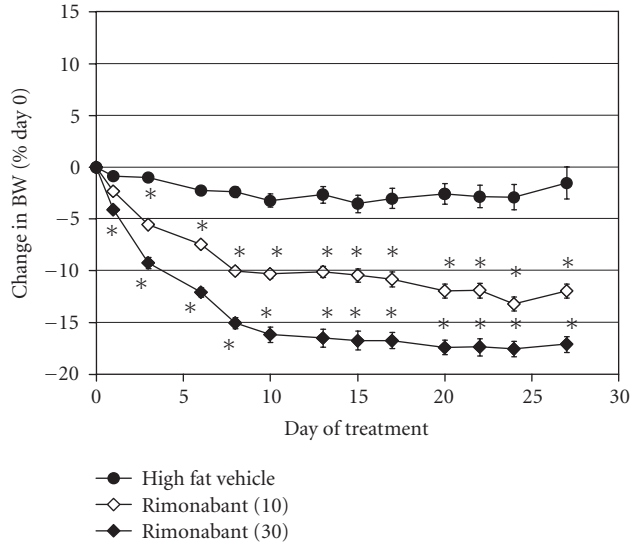
Experiments 3 and 4. Effect of PPARpan agonists in obese AKR/J mice

Experiments 3 and 4 explore the effects of two PPARpan agonists with different selectivity profiles (see Figure 5, Table 1). GW4148, a potent activator of all three PPAR receptor subtypes, was used in Experiment 3. Dosed at 3 mg/kg, GW4148 did not induce weight loss. In contrast, a dose of 10 mg/kg significantly decreased BW by 18% after 19 days of dosing (see Figure 5(a)). This change mirrored the effects seen when PPARα and PPARδ were coadministered in Experiment 1. GW4148 also produced a significant decrease in FM that was commensurate with the reduction in BW.

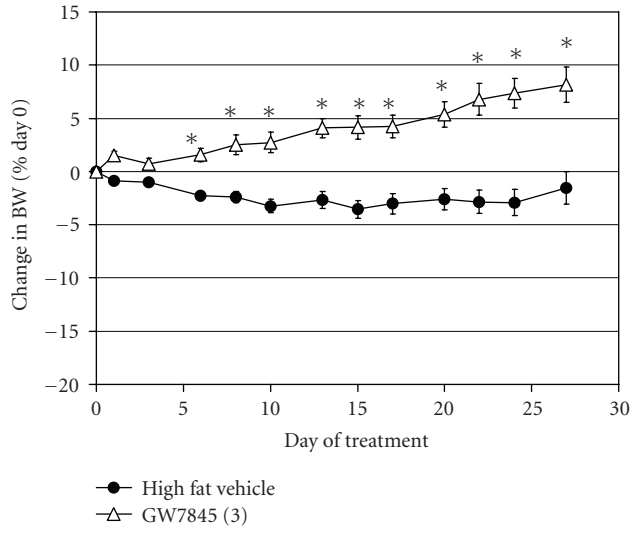
GW9135 is a PPARpan compound with a different pattern of activation than GW9148, being very potent at PPARα and PPARγ and weaker at PPARδ. Dosing GW9135 at 3 mg/kg had no effect on BW (see Figure 5(b)). Treatment with 10 mg/kg GW9135 reduced body weight 10% by day 8, however, the mice regained weight after that time and final BW was not significantly different from vehicle-treated animals at day 27 (see Figure 5(b)). This dose of GW9135 significantly reduced FM by 4 grams. Both GW4148 and GW9135 treatments increased liver weights by approximately 2.5 grams (see Table 2) which counterbalanced the final BW to some extent.

Effect of PPAR agonists on serum chemistry

Serum chemistry results are shown as group means in Table 3. None of the PPAR agonists tested in these experiments had a significant effect on blood glucose levels. The

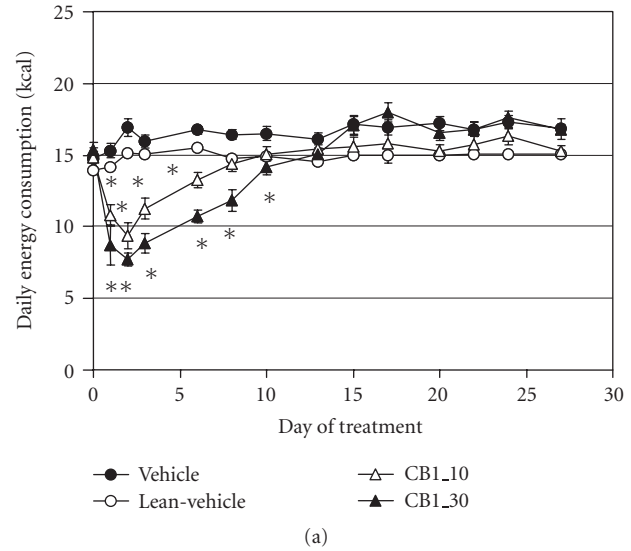


(a)

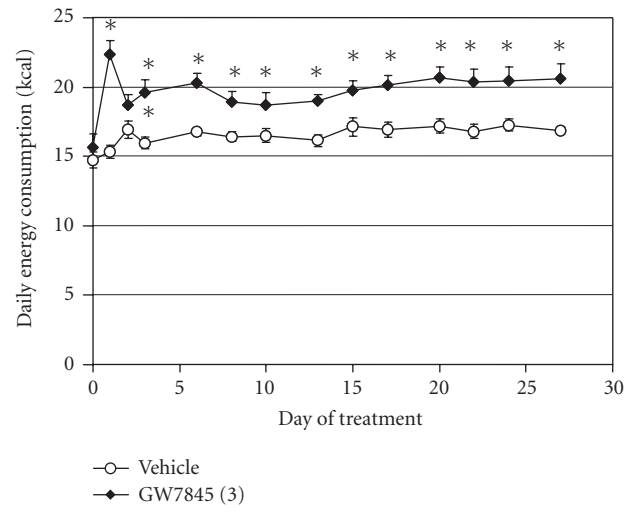


(b)

Figure 3: Effect of treatment with rimonabant or selective PPARγ agonist on BW. (a) RIM (10 and 30 mg/kg). (b) GW7845, a selective PPARγ agonist (3 mg/kg). Data were analyzed by ANCOVA with repeated measures followed by Dunnett's post hoc test. Values were considered to be significant (*) when the value of $P < .05$ was achieved. $N = 8$–10 animals/group.



(a)



(b)

Figure 4: Effect of treatment with rimonabant or selective PPARγ agonist on food consumption (kcal). (a) RIM (10 and 30 mg/kg). (b) GW7845, a selective PPARγ agonist (3 mg/kg). Data were analyzed by ANCOVA with repeated measures followed by Dunnett's post hoc test. Values were considered to be significant (*) when the value of $P < .05$ was achieved. $N = 8$–10 animals/group.

PPARα and PPARδ agonists alone significantly reduced circulating insulin (INS) levels. The combination of the two agents not only reduced insulin but also significantly reduced triglyceride (TG) and nonesterified fatty acids (NEFAs) and elevated total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-c), and β-Hydroxybutyric acid (βHBA). The selective PPARγ agonist produced a significant reduction in circulating INS, TG, and NEFA levels. Both PPARpan agonists significantly reduced fed glucose, INS, NEFAs, and TG and increased total CHOL, HDL-c, and βHBA.

Effect of PPAR agonists on ex vivo fatty acid oxidation

Changes in drug-induced fatty acid oxidation (FAO) were evaluated in mouse liver extracts from animals treated with compound for 28 days (see Figure 6). Activation of the PPARδ agonist produced a 1.9-fold increase in FAO while the PPARγ agonist and PPARα agonist were not different from vehicle. The PPARpan agonists elicited responses similar to the PPARδ agonist and this response most likely reflects the activity of PPARpan agonists at the PPARδ receptor.

Table 3: Group means of clinical chemistry results of DIO-AKR mice. Terminal blood samples were obtained at the end of treatment. Serum levels of analytes were determined using an Olympus AU640 clinical chemistry analyzer and analyzed by a two-way analysis of variance with repeated measures model (ANOVA) followed by Dunnett's post hoc test. Values were considered to be significant (*) when the value of $P < .05$ was achieved.

	Treatment	Glucose (mg/dL)	Insulin (ng/mL)	Triglyceride (mg/dL)	NEFA (mEq/L)	Cholesterol (mg/dL)	HDL-C (mg/dL)	bHBA (mg/dL)
Experiment 1	Lean vehicle	211.3 ± 7.8	1.7 ± 1.4	194.7 ± 0.7	0.7 ± 0.03	89.5 ± 1.6	57.2 ± 1.1	2.1 ± 0.2
	DIO vehicle	241.2 ± 7.4	11.2 ± 2.1	154.5 ± 8.1	0.8 ± 0.02	188.7 ± 12.5	131.8 ± 4.5	2.0 ± 0.2
	GW9578 (1)	248.0 ± 16.6	3.1 ± 1.6 *	168.3 ± 15.4	0.9 ± 0.04	117.1 ± 4.1	86.6 ± 2.7	3.2 ± 1.1
	GW0742 (30)	253.3 ± 8.9	2.2 ± 1.2 *	174.9 ± 10.3	0.9 ± 0.02	171.9 ± 2.9	118.4 ± 1.6	3.8 ± 0.3 *
	GW9578 + GW0742 after week 2	211.5 ± 14.2	1.9 ± 0.9 *	97.5 ± 5.8 *	0.6 ± 0.02 *	207.5 ± 4.5 *	131.8 ± 4.5	7.0 ± 0.5 *
	GW4148 + GW0742 after week 2	157.4 ± 2.4 *	2.9 ± 1.1 *	100.0 ± 8.9 *	0.6 ± 0.04 *	210.1 ± 8.5 *	132.5 ± 4.7	7.0 ± 1.4 *
	GW9578 and GW0742 4 weeks	199.1 ± 1.6 *	2.6 ± 0.8 *	128.1 ± 9.0 *	0.8 ± 0.04	234.4 ± 5.5 *	141.8 ± 2.3	6.9 ± 0.8 *
Experiment 2	DIO vehicle	225.4 ± 11.7	10.2 ± 0.5	152.3 ± 0.8	0.7 ± 0.8	114.9 ± 2.9	81.8 ± 1.6	1.6 ± 0.2
	RIM (10)	230.1 ± 16.6	7.2 ± 2.5 *	181.4 ± 36.1	0.8 ± 0.05	147.3 ± 9.1 *	95.1 ± 4.6 *	2.5 ± 0.2 *
	RIM (30)	215.3 ± 13.0	3.4 ± 0.7 *	159.7 ± 16.1	0.9 ± 0.02	143.4 ± 4.0 *	116.9 ± 2.6 *	1.8 ± 0.2
	GW7845 (3)	222.3 ± 9.4	2.1 ± 0.4 *	112.3 ± 5.7 *	0.6 ± 0.03 *	106.1 ± 4.2	65.0 ± 0.9 *	1.5 ± 0.1
Experiment 3	DIO vehicle	234.0 ± 9.4	6.7 ± 1.9	163.5 ± 16.2	0.8 ± 0.07	116.1 ± 5.9	91.1 ± 3.7	2.9 ± 0.2
	GW4148 (3)	233.3 ± 14.6	1.8 ± 0.3 *	62.6 ± 4.1 *	0.5 ± 0.03 *	178.9 ± 3.7 *	52.9 ± 0.1 *	5.1 ± 0.9 *
	GW4148 (10)	214.0 ± 10.9 *	1.3 ± 0.3 *	48.4 ± 3.6 *	0.5 ± 0.04 *	192.6 ± 6.8 *	131.1 ± 3.6 *	6.5 ± 1.1 *
Experiment 4	Lean vehicle	189.5 ± 8.6	1.2 ± 0.2	220.5 ± 14.0	0.7 ± 0.04	70.0 ± 1.9	51.7 ± 1.4	1.6 ± 0.1
	DIO Vehicle	196.0 ± 11.1	11.2 ± 2.1	277.6 ± 25.2	1.5 ± 0.10	122.8 ± 4.7	106.0 ± 2.8	3.7 ± 0.6
	GW9135 (3)	215.0 ± 14.1	2.9 ± 0.5 *	113.8 ± 9.9 *	0.8 ± 0.04 *	188.9 ± 4.9 *	149.4 ± 2.4 *	4.4 ± 0.6 *
	GW9135 (10)	196.1 ± 5.6	1.4 ± 0.4 *	58.8 ± 2.4 *	0.6 ± 0.02 *	183.8 ± 5.2 *	141.9 ± 2.8 *	4.3 ± 0.4 *

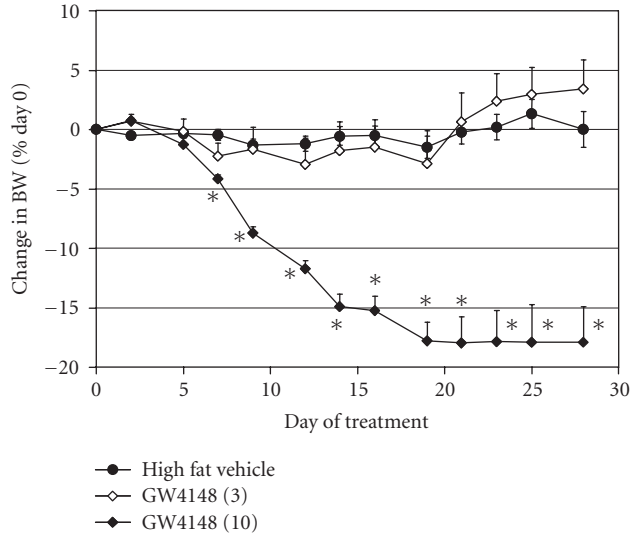
6. DISCUSSION

There is a critical medical need to develop effective strategies for long-term weight loss and weight maintenance although it is unlikely that any single therapy will yield maximal efficacy. Currently, the few therapies actually shown to be effective for weight loss include lifestyle modifications (diet and exercise), bariatric surgery, and pharmacological targets that modulate central pathways that regulate food intake [41]. PPARs are known to modulate enzymes involved in lipid metabolism and are expressed in many, if not all, metabolically active tissues including liver, heart, kidney, skeletal muscle, intestine, pancreas, and adipose tissue [42, 43]. This suggests that PPARs play a key role in energy metabolism and homeostasis that may ultimately affect body weight and body mass. In this report, we present data showing that potent and selective agonists of all three PPAR isoforms serve to modulate food intake and energy balance in DIO AKR/J mice.

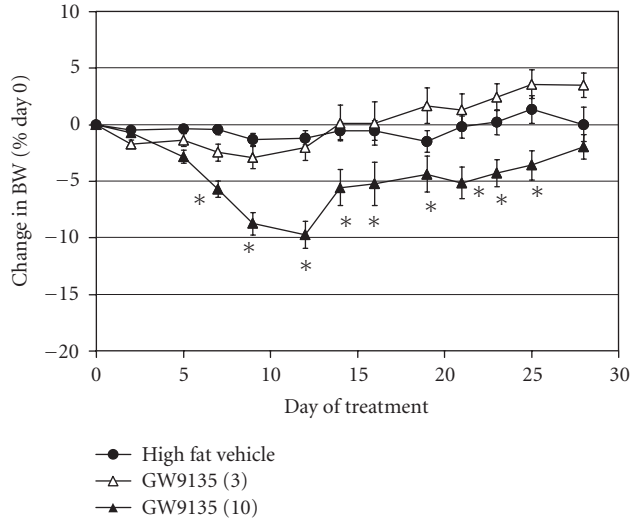
Selective activators of PPARγ, such as glitazones, have been successfully used to treat T2DM for nearly a decade. Treatment with rosiglitazone and pioglitazone induce body weight gain in mice [45, 46, 49], rats [44, 47–50], nonhuman primates [51, 52], and humans [53–55]. Weight gain is manifested as increased adiposity, total body water and plasma volume. In this report, mice treated with a potent and selective PPARγ activator gained more weight than obese vehicle controls and the weight gain could be completely accounted for by increased fat mass which was equivalent to the increase in caloric intake. In addition to stimulation of food consumption, activation of PPARγ promotes triglyceride accumulation by increasing expression of genes modulating adipogenesis [56–58], lipid transport [58, 59], storage [46, 60], and glucose homeostasis [61]. We also observed that GW7845 had no effect on FAO in mouse liver. In summary, PPARγ agonism induces food consumption and energy storage without an effect on energy utilization resulting in net weight gain.

A number of studies have suggested that PPARδ agonists regulate food intake, body weight, insulin sensitivity, and adiposity [8, 62–68]. Transgenic mice in which constitutively active PPARδ is expressed in muscle are highly resistant to high-fat, diet-induced obesity [15]. Administration of GW501516, a selective PPARδ agonist, promotes FAO and utilization, depleting lipid accumulation in adipocytes, skeletal muscle, and liver in DIO, ob/ob [68], and db/db mice [67].

Similarly, there are numerous studies that suggest that PPARα can regulate food intake, body weight, and adiposity



(a)



(b)

Figure 5: Effect of treatment with PPARpan agonists on BW. (a) GW4148 dosed at 3 and 10 mg/kg. (b) GW9135 dosed at 3 and 10 mg/kg. Data are expressed as mean ± SEM and were analyzed by ANCOVA with repeated measures followed by Dunnett's post hoc test. Values were considered to be significant (*) when the value of $P < .05$ was achieved. $N = 8$ animals/group.

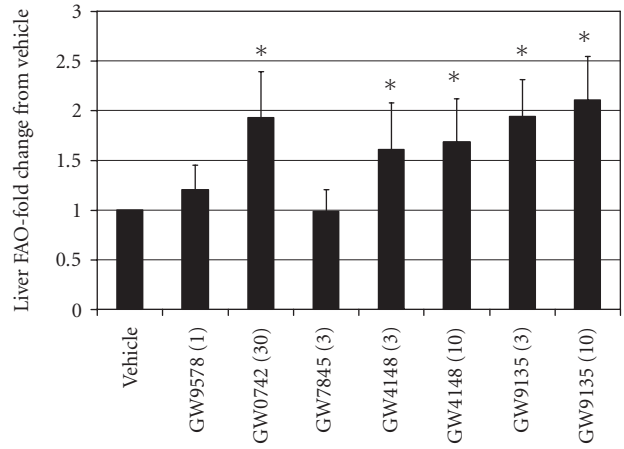


Figure 6: Effect of PPAR agonist treatment on fatty acid oxidation (FAO) in liver was assessed using a ^{14}C capture method modified from Dohm et al. [28]. Data are expressed as fold change from vehicle control (mean ± SEM). $N = 6$ determinations/compound.

in rodents [69–74]. PPARα has been shown to modulate target genes involved in uptake, activation, and degradation of fatty acids maintaining lipid homeostasis in liver, heart, and oxidative muscles [33, 75, 76]. It is possible that the combination of these mechanisms could result in reduction of body weight. Djouadi et al. [76] and Muoio et al. [33] have shown that the body weight of PPARα-KO mice was greater than WT littermates, and that they became obese when fed a high fat diet, confirming the role of PPARα receptors in modulating energy utilization and BW in rodents. In humans, fibrate treatment has not been associated with body weight loss (73), thus, the role of PPARα agonism in human body weight regulation is unclear.

Neither PPARα nor PPARδ agonists had a sustained effect on body weight. While the increase in liver weights observed with both treatments counterbalanced the initial weight loss induced by these compounds, this change did not completely explain the rebound.

GW0742, the PPARδ agonist, had a transient stimulatory effect on food intake from days 12–17 and it was during this time that the rebound increase in weight occurred. There was a significant increase in liver FAO induced by GW0742 after chronic dosing. The increase in food intake may have occurred in response to elevated energy expenditure, thus, an agent that only modulates energy expenditure did not induce significant weight loss in this model.

After 10 days of treatment with GW9578, the PPARα agonist, a significant suppression of food intake was observed that persisted throughout the rest of the study. The timing of this effect coincided with the timing of the rebound in weight gain. Currently, we do not have an explanation for this phenomenon, yet it appears that chronic PPARα agonism induces a metabolic compensation resulting in weight regain and the food intake suppression could be a counteracting mechanism. The effect on food consumption could be regulated centrally as PPARα is expressed in low but detectable levels in mouse hypothalamus, a major center of appetite and satiety regulation. PPARα could also modulate peripheral mechanisms that affect appetite or central response to lipid levels resulting from changes in FAO [12, 75]. While several reports have shown that PPARα increased FAO, the measurement of this parameter at the end of the study indicated that there was only a modest alteration. We did observe weight loss during the first 10 days of the study without a change in food intake thus it is possible that there could have been induction of FAO during this time.

A combination study of PPARα and PPARδ agonists was performed to determine if greater weight loss could be achieved together than with either compound alone. After 2 weeks of dosing with either single agent, addition of the second agent further reduced body weight and fat mass, suggesting a synergistic effect of the two agents. Combination dosing of both agents for the entire 4 weeks of the study produced even greater reduction in body weight and fat mass. Interestingly, the suppression of food intake after addition of GW9578 to GW0742 and with the straight combination dosing occurred immediately as opposed to the 10-day delay observed with GW9578 alone. The immediate effect on food intake through PPARα, increase in liver FAO from PPARδ, and the initial induction of weight loss by PPARα through a nonfood intake mechanism all account for the greater efficacy observed with the combination dosing from day 1 of treatment.

PPARpan agonists are a class of compounds that activate all three PPAR receptor subtypes and are currently being evaluated as antidiabetic agents. Compared to selective PPAR agonists, PPARpan ligands are expected to display unique characteristics as a result of ligand-activation profiles combining features of all three PPAR receptor subtypes, however, the effects are not simply the sum of the activities, but reflect a careful balance of lipid handling and energy. Both compounds used in this study are potent activators of all three isoforms but the potency ratio across the isoforms is different. GW4148 is an extremely potent agonist of murine PPARδ (9 nM) and is 4-fold selective over PPARα or PPARγ receptors. In contrast, GW9135 is a potent agonist of murine PPARα (13 nM) and is 18-fold and 50-fold selective over PPARγ and PPARδ, respectively. Other factors such as cofactor affinities contribute to the physiological behavior of each molecule.

GW9135 had little effect on overall weight loss, a pattern not different from PPARα agonist treatment alone, where there was an initial decrease in weight followed by regain. This effect can be explained by the greater potency of the molecule at PPARα and its weaker potency on PPARδ . In contrast, GW4148, which is most potent at the PPARα and PPARδ receptors, behaved similarly to combination dosing of GW9578 and GW0742 producing significant weight loss at 10/mg/kg.

Contrary to the differential effects on body weight, both PPARpan agonists produced similar metabolic effects. Each compound reduced TG, NEFA, and circulating insulin levels, and elevated HDL-c and bHBA. A similar pattern was noted with the combination of GW9578 and GW0742, however, these two agents alone did not have significant effects on any parameter except insulin. The combination of PPARα and PPARδ activation results in a synergistic effect on serum chemistry parameters.

In summary, these studies demonstrate that PPARs are integrally involved in energy maintenance. The PPARα and PPARδ receptors are responsible for induction of weight loss in AKR/J mice through suppression of food intake and increased energy expenditure. Activation of PPARα and PPARδ receptors by PPARpan compounds may be expected to induce weight loss or provide weight maintenance while combining the beneficial insulin sensitization effects of a PPARγ agonist.

ABBREVIATIONS

PPAR: Peroxisome proliferator-activated receptor
RIM: Rimonabant
CB1-R: Cannabinoid 1-receptor
d: Day
BW: Body weight
FM: Fat mass
LM: Lean mass
LW: Liver weight
qMR: Quantitative magnetic resonance
FAO: Fatty acid oxidation
GLU: Glucose
INS: Insulin
TG: Triglyceride
NEFA: Nonesterified fatty acid
CHOL: Cholesterol
HDLc: High-density lipoprotein cholesterol
βHBA: β-hydroxybutyric acid

ACKNOWLEDGMENTS

Portions of this study were presented at the 2004 American Diabetes Association meeting. The authors wish to acknowledge the technical skill of Tracy Brainard in helping to perform these experiments and analyze serum insulin samples, Deborah Winegar and Kathleen K. Brown for critical reading of the manuscript, and Dixie Harrington for editing the final document.

REFERENCES

[1] WHO, "Obesity: preventing and managing the global epidemic: report of WHO consultation," Tech. Rep. 884, WHO Consultation on Obesity, Geneva, Switzerland, 1999.

[2] S. Nammi, S. Koka, K. M. Chinnala, and K. M. Boini, "Obesity: an overview on its current perspectives and treatment options," *Nutrition Journal*, vol. 3, p. 3, 2004.

[3] A. von Eckardstein and G. Assmann, "Prevention of coronary heart disease by raising high-density lipoprotein cholesterol?" *Current Opinion in Lipidology*, vol. 11, no. 6, pp. 627–637, 2000.

[4] P. T. James, N. Rigby, and R. Leach, "The obesity epidemic, metabolic syndrome and future prevention strategies," *European Journal of Cardiovascular Prevention and Rehabilitation*, vol. 11, no. 1, pp. 3–8, 2004.

[5] E. N. Liberopoulos, D. P. Mikhailidis, and M. S. Elisaf, "Diagnosis and management of the metabolic syndrome in obesity," *Obesity Reviews*, vol. 6, no. 4, pp. 283–296, 2005.

[6] J. Korner and L. J. Aronne, "Pharmacological approaches to weight reduction: therapeutic targets," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2616–2621, 2004.

[7] D. Cota and S. C. Woods, "The role of the endocannabinoid system in the regulation of energy homeostasis," *Current Opinion in Endocrinology and Diabetes*, vol. 12, no. 5, pp. 338–351, 2005.

[8] R. M. Evans, G. D. Barish, and Y.-X. Wang, "PPARs and the complex journey to obesity," *Nature Medicine*, vol. 10, no. 4, pp. 355–361, 2004.

[9] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[10] H. E. Xu, M. H. Lambert, V. G. Montana, et al., "Molecular recognition of fatty acids by peroxisome proliferator-activated receptors," *Molecular Cell*, vol. 3, no. 3, pp. 397–403, 1999.

[11] M. Guerre-Millo, P. Gervois, E. Raspé, et al., "Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity," *Journal of Biological Chemistry*, vol. 275, no. 22, pp. 16638–16642, 2000.

[12] E. Erol, L. S. Kumar, G. W. Cline, G. I. Shulman, D. P. Kelly, and B. Binas, "Liver fatty acid binding protein is required for high rates of hepatic fatty acid oxidation but not for the action of PPARα in fasting mice," *The FASEB Journal*, vol. 18, no. 2, pp. 347–349, 2004.

[13] O. Braissant, F. Foufelle, C. Scotto, M. Dauca, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[14] W. R. Oliver, J. L. Shenk, M. R. Snaith, et al., "A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 9, pp. 5306–5311, 2001.

[15] Y.-X. Wang, C.-H. Lee, S. Tiep, et al., "Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity," *Cell*, vol. 113, no. 2, pp. 159–170, 2003.

[16] Y. X. Wang, C. L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[17] B. M. Spiegelman, "PPAR-γ: adipogenic regulator and thiazolidinedione receptor," *Diabetes*, vol. 47, no. 4, pp. 507–514, 1998.

[18] L. Fajas, J.-C. Fruchart, and J. Auwerx, "Transcriptional control of adipogenesis," *Current Opinion in Cell Biology*, vol. 10, no. 2, pp. 165–173, 1998.

[19] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[20] J. E. Cecil, P. Watt, C. N. Palmer, and M. Hetherington, "Energy balance and food intake: the role of PPARγ gene polymorphisms," *Physiology and Behavior*, vol. 88, no. 3, pp. 227–233, 2006.

[21] S. Mudaliar, A. R. Chang, and R. R. Henry, "Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications," *Endocrine Practice*, vol. 9, no. 5, pp. 406–416, 2003.

[22] Y. Guan, C. Hao, D. R. Cha, et al., "Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption," *Nature Medicine*, vol. 11, no. 8, pp. 861–866, 2005.

[23] M. C. Lewis, J. G. Wilson, D. M. Ignar, and W. R. Oliver, "The effects of PPARα, δ and PPARpan agonist on diet-induced obesity in fat-fed AKR mice," *Diabetes*, vol. 53, supplement 2, p. A134, 2004.

[24] B. Pourcet, J.-C. Fruchart, B. Staels, and C. Glineur, "Selective PPAR modulators, dual and pan PPAR agonists: multimodal drugs for the treatment of type 2 diabetes and atherosclerosis," *Expert Opinion on Emerging Drugs*, vol. 11, no. 3, pp. 379–401, 2006.

[25] U. Ramachandran, R. Kumar, and A. Mittal, "Fine tuning of PPAR ligands for type 2 diabetes and metabolic syndrome," *Mini-Reviews in Medicinal Chemistry*, vol. 6, no. 5, pp. 563–573, 2006.

[26] P. J. Brown, D. A. Winegar, K. D. Plunket, et al., "A ureidothioisobutyric acid (GW9578) is a subtype-selective PPARα agonist with potent lipid-lowering activity," *Journal of Medicinal Chemistry*, vol. 42, no. 19, pp. 3785–3788, 1999.

[27] B. Yang, K. K. Brown, L. Chen, et al., "Serum adiponectin as a biomarker for in vivo PPARγ activation and PPARγ agonist-induced efficacy on insulin sensitization/lipid lowering in rats," *BMC Pharmacology*, vol. 4, no. 1, p. 23, 2004.

[28] B. R. Henke, S. G. Blanchard, M. F. Brackeen, et al., "*N*-(2-benzoylphenyl)-L-tyrosine PPARγ agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents," *Journal of Medicinal Chemistry*, vol. 41, no. 25, pp. 5020–5036, 1998.

[29] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[30] J. S. Nichols, D. J. Parks, T. G. Consler, and S. G. Blanchard, "Development of a scintillation proximity assay for peroxisome proliferator-activated receptor γ ligand binding domain," *Analytical Biochemistry*, vol. 257, no. 2, pp. 112–119, 1998.

[31] G. L. Dohm, R. L. Huston, E. W. Askew, and P. C. Weiser, "Effects of exercise on activity of heart and muscle mitochondria," *The American Journal of Physiology*, vol. 223, no. 4, pp. 783–787, 1972.

[32] H. R. Scholte, Y. Yu, J. D. Ross, I. I. Oosterkamp, A. M. C. Boonman, and H. F. M. Busch, "Rapid isolation of muscle and heart mitochondria, the lability of oxidative phosphorylation and attempts to stabilize the process in vitro by taurine, carnitine and other compounds," *Molecular and Cellular Biochemistry*, vol. 174, no. 1-2, pp. 61–66, 1997.

[33] D. M. Muoio, P. S. MacLean, D. B. Lang, et al., "Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice: evidence for compensatory regulation by PPARδ," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26089–26097, 2002.

[34] P. J. Brown, T. A. Smith-Oliver, P. S. Charifson, et al., "Identification of peroxisome proliferator-activated receptor ligands from a biased chemical library," *Chemistry and Biology*, vol. 4, no. 12, pp. 909–918, 1997.

[35] D. C. Dey, R. P. Bronson, J. Dahl, J. P. Carroll, and T. L. Benjamin, "Accelerated development of polyoma tumors and embryonic lethality: different effects of p53 loss on related mouse backgrounds," *Cell Growth & Differentiation*, vol. 11, no. 5, pp. 231–237, 2000.

[36] M. L. Sznaidman, C. D. Haffner, P. R. Maloney, et al., "Novel selective small molecule agonists for peroxisome proliferator-activated receptor δ (PPARδ)—synthesis and biological activity," *Bioorganic and Medicinal Chemistry Letters*, vol. 13, no. 9, pp. 1517–1521, 2003.

[37] D. B. West, C. N. Boozer, D. L. Moody, and R. L. Atkinson, "Dietary obesity in nine inbred mouse strains," *American Journal of Physiology*, vol. 262, no. 6, part 2, pp. R1025–R1032, 1992.

[38] B. K. Smith, D. B. West, and D. A. York, "Carbohydrate versus fat intake: differing patterns of macronutrient selection in two inbred mouse strains," *The American Journal of Physiology*, vol. 272, no. 1, part 2, pp. R357–R362, 1997.

[39] V. Prpic, P. M. Watson, I. C. Frampton, M. A. Sabol, G. E. Jezek, and T. W. Gettys, "Adaptive changes in adipocyte gene expression differ in AKR/J and SWR/J mice during diet-induced obesity," *Journal of Nutrition*, vol. 132, no. 11, pp. 3325–3332, 2002.

[40] B. Künnecke, P. Verry, A. Bénardeau, and M. von Kienlin, "Quantitative body composition analysis in awake mice and rats by magnetic resonance relaxometry," *Obesity Research*, vol. 12, no. 10, pp. 1604–1615, 2004.

[41] E. E. Jobst, P. J. Enriori, P. Sinnayah, and M. A. Cowley, "Hypothalamic regulatory pathways and potential obesity treatment targets," *Endocrine*, vol. 29, no. 1, pp. 33–48, 2006.

[42] P. Escher and W. Wahli, "Peroxisome proliferator-activated receptors: insight into multiple cellular functions," *Mutation Research*, vol. 448, no. 2, pp. 121–138, 2000.

[43] D. E. Moller and J. P. Berger, "Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation," *International Journal of Obesity*, vol. 27, supplement 3, pp. S17–S21, 2003.

[44] F. Törüner, E. Akbay, N. Çakir, et al., "Effects of PPARγ and PPARα agonists on serum leptin levels in diet-induced obese rats," *Hormone and Metabolic Research*, vol. 36, no. 4, pp. 226–230, 2004.

[45] Y. Iwamoto, K. Kosaka, T. Kuzuya, Y. Akanuma, Y. Shigeta, and T. Kaneko, "Effects of troglitazone: a new hypoglycemic agent in patients with NIDDM poorly controlled by diet therapy," *Diabetes Care*, vol. 19, no. 2, pp. 151–156, 1996.

[46] M. Narce and J.-P. Poisson, "Novel PPARγ-dependent and independent effects for thiazolidinediones," *Current Opinion in Lipidology*, vol. 14, no. 6, pp. 651–652, 2003.

[47] M. Rossmeisl, J. S. Rim, R. A. Koza, and L. P. Kozak, "Variation in type 2 diabetes—related traits in mouse strains susceptible to diet-induced obesity," *Diabetes*, vol. 52, no. 8, pp. 1958–1966, 2003.

[48] L. C. Pickavance, C. L. Brand, K. Wassermann, and J. P. H. Wilding, "The dual PPARα/γ agonist, ragaglitazar, improves insulin sensitivity and metabolic profile equally with pioglitazone in diabetic and dietary obese ZDF rats," *British Journal of Pharmacology*, vol. 144, no. 3, pp. 308–316, 2005.

[49] R. Chakrabarti, R. K. Vikramadithyan, P. Misra, et al., "Ragaglitazar: a novel PPARα & PPARγ agonist with potent lipid-lowering and insulin-sensitizing efficacy in animal models," *British Journal of Pharmacology*, vol. 140, no. 3, pp. 527–537, 2003.

[50] E. H. Koh, M.-S. Kim, J.-Y. Park, et al., "Peroxisome proliferator-activated receptor (PPAR)-α activation prevents diabetes in OLETF rats: comparison with PPAR-γ activation," *Diabetes*, vol. 52, no. 9, pp. 2331–2337, 2003.

[51] M. K. Gee, L. Zhang, S. E. Rankin, J. N. Collins, R. F. Kauffman, and J. D. Wagner, "Rosiglitazone treatment improves insulin regulation and dyslipidemia in type 2 diabetic cynomolgus monkeys," *Metabolism*, vol. 53, no. 9, pp. 1121–1125, 2004.

[52] I. W. Campbell, "The clinical significance of PPAR γ agonism," *Current Molecular Medicine*, vol. 5, no. 3, pp. 349–363, 2005.

[53] C. G. Gegick and M. D. Altheimer, "Thiazolidinediones: comparison of long-term effects on glycemic control and cardiovascular risk factors," *Current Medical Research and Opinion*, vol. 20, no. 6, pp. 919–930, 2004.

[54] M. W. Stolar and R. J. Chilton, "Type 2 diabetes, cardiovascular risk, and the link to insulin resistance," *Clinical Therapeutics*, vol. 25, supplement 2, pp. B4–B31, 2003.

[55] H. Duez, J.-C. Fruchart, and B. Staels, "PPARs in inflammation, atherosclerosis and thrombosis," *Journal of Cardiovascular Risk*, vol. 8, no. 4, pp. 187–194, 2001.

[56] J. M. Way, W. W. Harrington, K. K. Brown, et al., "Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor γ activation has coordinate effects on gene expression in multiple insulin-sensitive tissues," *Endocrinology*, vol. 142, no. 3, pp. 1269–1277, 2001.

[57] S. Hummasti, B. A. Laffitte, M. A. Watson, et al., "Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target," *Journal of Lipid Research*, vol. 45, no. 4, pp. 616–625, 2004.

[58] G. Martin, K. Schoonjans, B. Staels, and J. Auwerx, "PPARγ activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes," *Atherosclerosis*, vol. 137, supplement 1, pp. S75–S80, 1998.

[59] V. Bocher, I. Pineda-Torra, J.-C. Fruchart, and B. Staels, "PPARS: transcription factors controlling lipid and lipoprotein metabolism," *Annals of the New York Academy of Sciences*, vol. 967, pp. 7–18, 2002.

[60] S. Rocchi and J. Auwerx, "Peroxisome proliferator-activated receptor-γ: a versatile metabolic regulator," *Annals of Medicine*, vol. 31, no. 5, pp. 342–351, 1999.

[61] F. Picard and J. Auwerx, "PPARγ and glucose homeostasis," *Annual Review of Nutrition*, vol. 22, pp. 167–197, 2002.

[62] F. Zhang, B. Lavan, and F. M. Gregoire, "Peroxisome proliferator-activated receptors as attractive antiobesity targets," *Drug News and Perspectives*, vol. 17, no. 10, pp. 661–669, 2004.

[63] A. Fredenrich and P. A. Grimaldi, "PPAR δ: an uncompletely known nuclear receptor," *Diabetes and Metabolism*, vol. 31, no. 1, pp. 23–27, 2005.

[64] S. Luquet, J. Lopez-Soriano, D. Holst, et al., "Roles of peroxisome proliferator-activated receptor δ (PPARδ) in the control of fatty acid catabolism. A new target for the treatment of metabolic syndrome," *Biochimie*, vol. 86, no. 11, pp. 833–837, 2004.

[65] H. D. Shin, B. L. Park, L. H. Kim, et al., "Genetic polymorphisms in peroxisome proliferator-activated receptor δ associated with obesity," *Diabetes*, vol. 53, no. 3, pp. 847–851, 2004.

[66] M. Muurling, R. P. Mensink, H. Pijl, J. A. Romijn, L. M. Havekes, and P. J. Voshol, "Rosiglitazone improves muscle insulin sensitivity, irrespective of increased triglyceride content, in ob/ob mice," *Metabolism*, vol. 52, no. 8, pp. 1078–1083, 2003.

[67] M. D. Leibowitz, C. Fiévet, N. Hennuyer, et al., "Activation of PPARδ alters lipid metabolism in db/db mice," *FEBS Letters*, vol. 473, no. 3, pp. 333–336, 2000.

[68] C.-H. Lee, P. Olson, A. Hevener, et al., "PPARδ regulates glucose metabolism and insulin sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 9, pp. 3444–3449, 2006.

[69] Y. Fukui, S.-I. Masui, S. Osada, K. Umesono, and K. Motojima, "A new thiazolidinedione, NC-2100, which is a weak PPAR-γ activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAy obese mice," *Diabetes*, vol. 49, no. 5, pp. 759–767, 2000.

[70] N. L. Bodkin, J. Pill, K. Meyer, and B. C. Hansen, "The effects of K-111, a new insulin-sensitizer, on metabolic syndrome in obese prediabetic rhesus monkeys," *Hormone and Metabolic Research*, vol. 35, no. 10, pp. 617–624, 2003.

[71] E. H. Koh, M.-S. Kim, J.-Y. Park, et al., "Peroxisome proliferator-activated receptor (PPAR)-α activation prevents diabetes in OLETF rats: comparison with PPAR-γ activation," *Diabetes*, vol. 52, no. 9, pp. 2331–2337, 2003.

[72] P. J. Larsen, P. B. Jensen, R. V. Sørensen, et al., "Differential influences of peroxisome proliferator-activated receptors γ and -α on food intake and energy homeostasis," *Diabetes*, vol. 52, no. 9, pp. 2249–2259, 2003.

[73] J. C. Corton, U. Apte, S. P. Anderson, et al., "Mimetics of caloric restriction include agonists of lipid-activated nuclear receptors," *Journal of Biological Chemistry*, vol. 279, no. 44, pp. 46204–46212, 2004.

[74] P. Ferré, "The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity," *Diabetes*, vol. 53, supplemnet 1, pp. S43–S50, 2004.

[75] V. Navarro, A. Fernández-Quintela, I. Churruca, and M. P. Portillo, "The body fat-lowering effect conjugated linoleic acid: a comparison between animal and human studies," *Journal of Physiology and Biochemistry*, vol. 62, no. 2, pp. 137–147, 2006.

[76] F. Djouadi, J. M. Brandt, C. J. Weinheimer, T. C. Leone, F. J. Gonzalez, and D. P. Kelly, "The role of the peroxisome proliferator-activated receptor α (PPARα) in the control of cardiac lipid metabolism," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 60, no. 5-6, pp. 339–343, 1999.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 28436, 7 pages
doi:10.1155/2007/28436

Review Article

PPARs in Calorie Restricted and Genetically Long-Lived Mice

Michal M. Masternak[1] and Andrzej Bartke[1,2]

[1] *Departments of Internal Medicine, Geriatrics Research, School of Medicine, Southern Illinois University, Springfield, IL 62794, USA*
[2] *Departments of Physiology, Geriatrics Research, School of Medicine, Southern Illinois University, Springfield, IL 62794, USA*

Received 15 August 2006; Revised 18 October 2006; Accepted 18 October 2006

Recommended for Publication by Francine M. Gregoire

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptors superfamily. The three subtypes, PPARα, PPARγ, and PPARβ/δ, are expressed in multiple organs. These transcription factors regulate different physiological functions such as energy metabolism (including lipid and carbohydrate metabolism), insulin action, and immunity and inflammation, and apparently also act as important mediators of longevity and aging. Calorie restriction (CR) is the most effective intervention known to delay aging and increase lifespan. Calorie restriction affects the same physiological functions as PPARs. This review summarizes recent findings on the effects of CR and aging on the expression of PPARγ, α, and β/δ in mice and discusses possible involvement of PPARs in mediating the effects of murine longevity genes. The levels of PPARs change with age and CR appears to prevent these alterations which make "PPARs-CR-AGING" dependence of considerable interest.

THE PPAR FAMILY

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that are ligand-dependent transcription factors. The activation of PPARs requires forming heterodimers with retinoid X receptors (RXRs), which allow binding to their specific peroxisome proliferator response elements (PPREs) [1]. By binding to specific PPREs in enhancer sites of targeted genes, PPAR/RXR heterodimers regulate their expression. PPAR genes are known to be expressed in different organs, including reproductive organs, major insulin target organs (liver, white adipose tissue, skeletal muscle), cardiac tissue, and other. PPARs have a wide spectrum of actions which include adipocyte differentiation, lipid metabolism, insulin sensitization, tissue injury and wound repair, inflammation, and immunity.

There are three known subtypes in the PPAR superfamily, each encoded by separate genes: PPARα, PPARβ/δ (also known as PPARβ or PPARδ), and PPARγ. The most explored gene of this superfamily and the most adipose-specific is PPARγ. There are two recognized isoforms of PPARγ: PPARγ1 and PPARγ2. These isoforms are generated by alternative splicing and alternate translation initiation [2–4]. Although PPARγ is the most recently cloned gene from PPARs, it quickly drew attention as a target receptor for thiazolidinediones (TZDs), the drugs used as insulin sensitizers in type 2 diabetic patients (5–8).

As its name implies, PPARα was the first gene cloned from this family. PPARα is mainly expressed in the liver, skeletal muscle, heart, and kidney. In these organs, it regulates a wide variety of target genes involved in cellular lipid catabolism. PPARα alters the expression of genes encoding enzymes involved in the fatty acid metabolic pathway, which activate the regulation of fatty acids β and ω-oxidation. These effects are mediated by the presence of PPREs that are under transcriptional control of PPARα in the promoter regions of genes coding for the enzymes involved in this metabolic pathway [5]. The activation of PPARα in the heart induces accumulation of myocardial lipids that leads to other features of diabetic cardiomyopathy [6]. PPARα-deficient mice have increased levels of total and HDL cholesterol [7].

The function of the third PPAR nuclear receptor, PPARβ/δ, is still somewhat unclear. There are some indications that PPARβ/δ is involved in lipid metabolism [8], and studies have shown that it plays an important role in epidermal maturation and skin wound healing [9, 10].

CALORIE RESTRICTION AND PPARs

Calorie restriction (CR) is of wide interest in the study of aging. There are numerous studies showing that CR can improve the health of individuals and help protect them from disease. CR is also recognized as the most effective intervention known to delay aging and increase lifespan [11]. The precise mechanisms of CR action on aging and longevity are still not well established, but CR is known to reduce body weight and the levels of plasma insulin, IGF-1, GH, glucose, and thyroid hormone.

Calorie restriction is also known to alter expression of large number of genes involved in lipid metabolism and insulin signaling. Expression of many of the same genes is regulated by PPARs acting as transcription factors. This suggests a possibility that PPARs mediate the effects of CR or that CR and PPAR/RXRs heterodimers activate the same signaling pathways.

ACTION OF PPARs ON INSULIN SIGNALING

The agonists for PPARα and PPARγ are widely used in diabetes. The study of rats fed a high-fat diet (HFD) indicated that PPARα and PPARγ agonists, WY14643 and pioglitazone, respectively, decreased glucose and leptin levels in plasma. The plasma levels of insulin and triglyceride were also reduced in rats treated with PPARs agonists in comparison to control animals; however, pioglitazone caused significantly greater reduction in comparison to PPARα-agonist-treated and control rats [12]. However, activation of PPARγ caused significant increase of body weight, which is opposite to CR action. PPARα agonist did not alter body weight and more importantly caused significant decrease of visceral fat weight in comparison to control and pioglitazone-treated rats [12]. This indicates that pioglitazone improves insulin sensitivity more effectively than WY14643. However, weight gain caused by PPARγ agonist is detrimental to the well-being of diabetic rats or humans.

DIET

The functions and characterization of PPARs suggest that these nuclear receptors are strongly connected with the diet. There is considerable evidence that various diets can affect PPARs action in different organs and that the responses to diets can be mediated by their effects on PPARs expression.

High-fat diet

High-fat diet is known to induce insulin resistance and promote type 2 diabetes in laboratory animals. In rats and mice HFD causes obesity and increases plasma insulin, glucose, and leptin levels. In HFD-fed rodents, PPARs agonists improve insulin sensitivity, presumably via activation of this nuclear receptor.

PPARα and high-fat diet

Studies of PPARα-null mice indicated that the deficiency of this nuclear receptor can protect from insulin resistance induced by HFD [13]. In this study the authors showed that HFD increases body weight and plasma insulin level but only in normal animals, with no alteration in PPARα-null mice. Moreover, insulin tolerance test (ITT), glucose tolerance test (GTT), and the calculated insulin resistance index indicated that HFD caused insulin resistance in normal animals, with no alteration of insulin signaling in PPARα deficient mice [13]. However, studies of PPARα-null mice subjected to fasting indicated that PPARα deficiency can cause severe hypoglycemia [14, 15]. Moreover, most of PPARα target genes were not altered in the liver and heart of fasted PPARα-null mice in comparison to normal controls [15]. The authors also reported that in PPARα deficient animals fasting caused hyperketonemia, hypothermia, and increase in plasma levels of free fatty acids, which reflects inhibition of fatty acid uptake and oxidation [14]. Concluding, PPARα participates in glucose homeostasis which may be important to prevent hypoglycemia under fasting condition or during exercise. However, long-term metabolic stress such as HFD could become negative for health by developing insulin resistance.

PPARγ and high-fat diet

Similarly to PPARα deficiency, PPARγ deficiency in adipose tissue (PPARγ-adiposeKO) was reported to protect from obesity and insulin resistance caused by HFD [16]. Under HFD, this tissue-specific PPARγ deficiency increased glucose tolerance in comparison to control animals on HFD. Moreover, the levels of insulin and leptin were significantly decreased in HFD-treated, PPARγ-adiposeKO mice in comparison to normal animals subjected to the same diet. Interestingly, the deficiency of PPARγ in adipose tissue resulted in increased PPARγ mRNA levels in the liver when compared to normal controls [16]. As stated by the authors, this model suggests that improved insulin sensitivity under HFD in PPARγ-adiposeKO mice can coexist with increased expression of PPARγ in the liver.

Calorie restriction diet

Calorie restriction is known to improve insulin sensitivity, lipid metabolism, health, and longevity. Calorie restriction is known to act on PPARs [17]; however, the effects are strikingly organ dependent. Depending on the organ, we observed a lack of changes, decrease or increase of PPARs expression in response to CR (Figure 1).

PPARs, CR, and the liver

Data reported by our laboratory indicated that 30% CR did not alter mRNA or protein levels of hepatic PPARγ in mice. This finding suggested that improvement of insulin sensitivity in mice by CR is not mediated by PPARγ in the liver [18, 19]. However, hepatic PPARα mRNA and protein levels

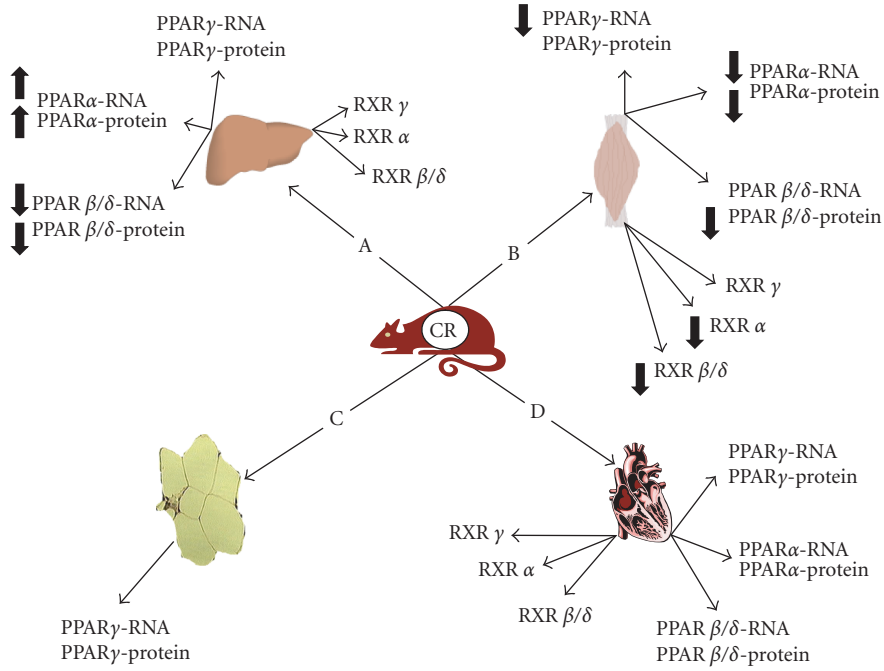


FIGURE 1: Effects of calorie restriction (CR) on the expression of PPARs family genes in mouse: (A) liver, (B) skeletal muscles, (C) white adipose tissue, and (D) heart. Arrows pointing up or down indicate statistically significant increases or decreases ($P < .05$). Lack of arrows means no alteration.

were significantly increased by CR in comparison to mice fed with unlimited (*ad libitum*; AL) access to food. This finding appears counterintuitive in view of the evidence that PPARα deficiency prevents insulin resistance in mice subjected to HFD [13]. However, the suggested involvement of PPARα in glucose homeostasis could imply that the increase of PPARα in mice subjected to CR is a mechanism protecting these animals from hypoglycemia [14, 15]. Perhaps under conditions of HFD the decrease of PPARα is adaptive, but when the animals are subjected to CR, PPARα increases to facilitate maintenance of normal glucose levels during the periods when food is not available. Additionally, a recent study conducted by Corton et al indicated that 19% of hepatic genes involved in lipid metabolism, inflammation, and cell growth which were altered by CR were dependent on PPARα. Interestingly, some of these genes were altered by CR only in normal mice but not in PPARα deficient animals. Results obtained in animals treated with a PPARα agonist indicated overlap of genes influenced by CR and by a compound activating PPARα [20]. These important findings indicated that PPARα plays an important role in mediating the action of CR [13, 20]. Corton et al also suggested that drugs activating the PPARα-RXR-LXR axis can be potential CR mimetics [20].

The expression of the remaining member of the PPAR family, PPARβ/δ, in the liver was significantly decreased by CR at both mRNA and protein levels [19]. Thus, the hepatic expression of three genes from the PPAR family is differentially altered by CR. However, CR did not alter hepatic RXRα, RXRγ, and RXRβ/δ mRNA (Figure 1A) [19].

PPARs, CR, and skeletal muscle

Similarly to the liver, the skeletal muscle is a major insulin target organ. In this tissue, the expression of PPARs and RXRs is altered differently by CR than in the liver [19, 21]. It was reported that 30% calorie restriction in mouse skeletal muscle decreased the level of PPARγ mRNA and the PPARγ protein level appeared to also be decreased [21]. We could speculate that the decrease of PPARγ in the muscle as seen in the adipose specific knockout for PPARγ is beneficial for insulin sensitivity [16]. However, muscle-specific knockout of PPARγ caused whole-body insulin resistance [22]. Interestingly, treating these knockout mice with TZD improved insulin sensitivity [22], suggesting the effect was due to PPARγ agonism in other tissues. This suggests that CR can increase insulin sensitivity through effects on PPARγ expression in tissues other than the muscle, and speculating further we could suggest that under the conditions of CR, a decreased rather than elevated PPARγ expression is beneficial.

PPARα mRNA and proteins were decreased by CR in skeletal muscle, an effect opposite to that observed in the liver [19, 21]. It was speculated that a decrease of PPARα in the muscle under CR slowed fatty acid oxidation, thus increasing the reliance on carbohydrates as the energy source. More importantly, consequences of reduced PPARα expression could prevent the muscle from using all of the FFA immediately after food intake and thus maintain a balance between energy availability and energy usage during the fasting period. The protein level of PPARβ/δ was also decreased in the

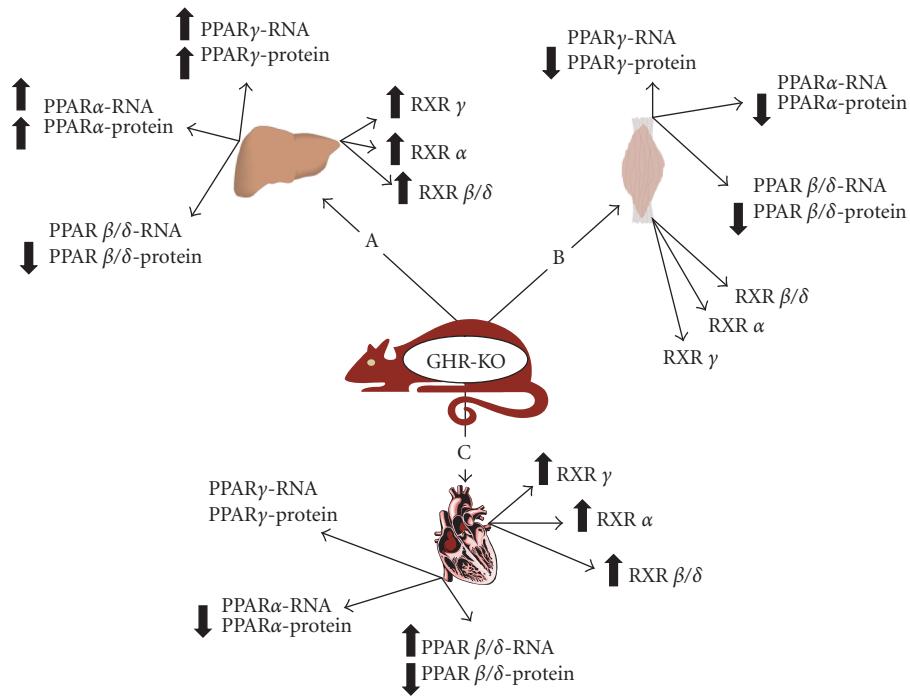


FIGURE 2: Effects of growth hormone receptor/binding protein knockout (GHR-KO) on the expression of PPARs family genes in mouse: (A) liver, (B) skeletal muscle, and (C) heart. Arrows pointing up or down indicate statistically significant increases or decreases ($P < .05$). Lack of arrow means no alteration.

skeletal muscle of CR mice [21]. It is well known that CR promotes fat depletion and prevents obesity. Studies in PPARδ-deficient mice on HFD revealed reduced energy uncoupling and obesity [21]. This would predict that reduced levels of PPARδ in the muscles of CR mice may lead to increased lipid accumulation and promote obesity. However, reduced dietary fat intake in CR animals may alter these relationships. It was suggested that CR down-regulates the pathway of lipid metabolism and accommodates it to the circumstances of restricted food intake [21]. This may serve to prevent disruption of fatty acid homeostasis in CR animals. In addition to its effects on PPARs expression, CR reduced mRNA levels of RXRα and RXRβ/δ [21]. Altered expression of these genes important to PPARs activation correlated with the changes in the expression of the corresponding members of the PPAR family (Figure 1B) [21].

PPARs, CR, and the white adipose tissue

PPARγ is mainly expressed in white adipose tissue (WAT). As mentioned previously, the deficiency of PPARγ in adipose tissue is protective against obesity and insulin resistance caused by HFD [16, 23]. However, CR increases insulin sensitivity in mice, without altering PPARγ mRNA levels in WAT (Figure 1C) [24]. It can be speculated that under conditions of reduced calorie intake diminished PPARγ would not be beneficial, or that limited fat storage does not allow increased PPARγ activation in this tissue. At the time of this writing no data are available on the effects of CR on PPARα and PPARβ/δ in WAT.

PPARs IN GENETICALLY LONG-LIVED AND SHORT-LIVED MICE

Growth hormone receptor/binding protein knockout (GHR-KO) mice

GHR-KO (Laron dwarf) mice have their GH receptor/binding protein gene disrupted and thus are deficient of GHR. Consequently these mice are GH resistant or insensitive and have greatly reduced plasma IGF-1 and insulin levels, and low glucose level [25, 26]. GHR-KO mice are also characterized by markedly extended lifespan in comparison to normal controls [27, 28].

In comparison to normal animals, GHR-KO mice also have significantly elevated PPARγ mRNA and protein level in the liver (Figure 2A) [19]. We speculated that increased level of PPARγ in the liver of those long-lived animals may be responsible for or contribute to their exceptionally high insulin sensitivity. This correlates with findings in PPARγ-adiposeKO mice, which indicated that PPARγ deficiency in WAT is compensated for by increased expression of this nuclear receptor in the liver to promote insulin sensitivity [16, 19]. The findings in the muscle also suggest that in GHR-KO mice PPARγ in the liver contributes to high insulin sensitivity, because the level of PPARγ mRNA in skeletal muscle of KO mice was not altered, while the PPARγ protein level was decreased in comparison to normal controls (Figure 2B) [21].

The increased level of PPARα measured in the liver of KO mice [19] could be suspect of exerting to negative

effect on insulin action and obesity. However, the higher level of PPARα suggests an increased usage of fatty acids, which could be beneficial for insulin sensitivity. Increased PPARα levels could also be correlated with decreased total cholesterol level in GHR-KO animals [21]. It is interesting that the level of PPARα in GHR-KO mice fed AL is maintained at the same level as in the normal animals subjected to CR. However, similarly to PPARγ, expression of PPARα was not altered in the muscle at the PPARα mRNA level, while the PPARα protein level was decreased in KO animals, again resembling the findings in normal mice under a CR regimen [21].

PPARβ/δ proteins were down-regulated in the liver and skeletal muscle of GHR-KO mice, which in both cases mimics the alterations of the expression of this gene caused by CR in the liver and muscle of normal mice [21].

These data indicate that CR alters PPARα and PPARβ/δ proteins and/or mRNA levels in the liver and skeletal muscle to the levels maintained in GHR-KO animals. Since GHR-KO mice are long-lived and CR increases longevity, it can be suggested that PPARα and PPARβ/δ play an important role in mediating the effects of both GHR-KO and/or CR on longevity. The RXRγ, RXRα, and RXRβ/δ mRNA were increased in the liver and not changed in the muscle of GHR-KO mice which corresponds to alterations of PPARγ and PPARα in these two organs (Figure 2A, B) [19, 21].

Dwarf mice

Snell dwarf, Ames dwarf, and "Little" mice live markedly longer than their normal siblings. Snell dwarf mice carry a mutation in the Pit1 gene ($Pit1^{dw}$) and Ames dwarf mice are homozygous for recessive loss-of-function mutation at the Prop1 locus ($Prop1^{df}$). These dwarf mice are deficient with GH, prolactin, and tyrotropin. Little mice have severely reduced GH levels caused by the mutation of growth hormone-releasing hormone receptor (Ghrhr). Studies of Snell dwarf mice indicated increased hepatic PPARα mRNA and protein levels in comparison to heterozygous controls [29]. The expression of PPARα mRNA and protein in the liver of Ames dwarf mice was not altered in comparison to their normal controls [18]. However, gene array studies indicated that the genes regulated by PPARα were either up-regulated in Snell dwarf, Ames dwarf, and Little mice or their expression increased in response to PPARα-agonist treatment, which was interpreted as evidence that GH action is involved in the regulation of PPARα-dependent gene products [29].

Phosphoenolpyruvate carboxykinase bovine-GH transgenic mice

PEPCK-bGH transgenic (TG) mice over-expressing the bGH gene fused to control sequences of the rat phosphoenolpyruvate carboxykinase (PEPCK) are characterized by markedly shortened lifespan in comparison to their normal siblings [30]. The findings in the liver did not indicate any alteration of PPARγ mRNA in these TG mice. However, hepatic PPARα mRNA was down-regulated in the liver of these short-living giant mice in comparison to their normal siblings [30]. This finding is very important in elucidating the potential role of PPARα in the control of longevity. As mentioned above, PPARα is increased in the liver of GHR-KO and CR mice. This contrasts with the decreased PPARα level in the liver of short-lived b-GH transgenic mice. These findings are consistent with our suggestion that PPARα can be an important mediator of genetic and dietary effects on longevity.

AGING AND CALORIE RESTRICTION

As mentioned above, the study of PPARα-null mice indicated that the deficiency of this nuclear receptor can protect from insulin resistance induced by HFD [13]. Furthermore, we speculated that PPARα can be influential in the control of longevity, and we suggested that an elevated level of this nuclear receptor is beneficial and promotes longer life. Although PPARα deficiency was useful in controlling glucose levels in HFD-fed mice, it was reported that these mice had age-dependent defects in heart, liver, and kidney, which correlated with decreased longevity in comparison to wild-type controls [31, 32]. PPARα expression is also known to decrease with age in the liver, kidney, and heart. The study of GHR-KO and normal mice fed AL and subjected to CR indicated that PPARα mRNA level in the heart is not affected by phenotype and CR (Figures 1D and 2C) [33]. CR increases the mRNA and protein level of PPARα only in GHR-KO mice [33]. Interestingly, protein level of PPARα was decreased in the heart of long-lived GHR-KO animals (Figure 2C) [33]. Moreover, earlier study [34] indicated that CR increased the cardiac level of PPARα mRNA in mice, which would support antiaging action. Analysis of gene expression in mouse heart by Lee et al indicated that CR preserved fatty acid metabolism [35]. Additionally, the study of GHR-KO mice indicated that at the age of 3 months PPARα was elevated in KO in comparison to the normal mice. When the animals reached 21 months of age this difference was no longer present [33]. The short-lived b-GH TG mice showed down regulation of this nuclear receptor in the heart at 9 months of age [33].

Sung et al reported that in rats the levels of PPARγ and PPARα in the kidney decreased with age when comparing 13- and 25-month-old animals [36]. Calorie restriction prevented these aging effects and maintained the levels of these nuclear receptors in 25-month-old rats at the same levels as in the 13-month-old animals [36]. To investigate the possible role of PPARα and PPARγ in inflammation, these authors also performed lipopolysaccharide (LPS) tests in young and old rats. Treatment with LPS decreased the level of these PPARs, to greater extent in old than in middle-age rats [36]. The authors concluded that down-regulation of PPARs in the rat's kidneys might be correlated with age-related oxidative stress, which could be reversed by antioxidative action of CR [36].

SUMMARY

Different members of the PPARs family are expressed in many tissues. Various dietary regimens such as HFD and calorie restriction can affect expression of PPARs. However,

the presence and direction of these changes depend on the organ being analyzed. Additionally, the effects of the diet on the animal depend on the actions of PPARs. For example, PPARα-null or PPARγ-adiposeKO mice are protected form insulin resistance and obesity caused by HFD. Studies in genetically long- or short-lived mice together with the studies involving CR suggest that PPARs play an important role in insulin action, lipid metabolism, immunity, and inflammation as well as regulation of aging and longevity.

ACKNOWLEDGMENTS

This paper is supported by the NIA (AG 19899 and U19 AG023122), The Ellison Medical Foundation, SIU Geriatrics Medicine and Research Initiative, and Central Research Committee of Southern Illinois University School of Medicine. We would also like to thank Steve Sandstrom for helping with the editing of the manuscript.

REFERENCES

[1] Moller DE, Berger JP. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *International Journal of Obesity.* 2003;27(suppl 3):S17–S21.

[2] Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPARγ2: tissue-specific regulator of an adipocyte enhancer. *Genes and Development.* 1994;8(10):1224–1234.

[3] Zhu Y, Qi C, Korenberg JR, et al. Structural organization of mouse peroxisome proliferator-activated receptor γ (mPPARγ) gene: alternative promoter use and different splicing yield two mPPARγ isoforms. *Proceedings of the National Academy of Sciences of the United States of America.* 1995; 92(17):7921–7925.

[4] Xue J-C, Schwarz EJ, Chawla A, Lazar MA. Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARγ. *Molecular and Cellular Biology.* 1996;16(4):1567–1575.

[5] Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Current Opinion in Lipidology.* 1997;8(3):159–166.

[6] Kelly DP. PPARs of the heart: three is a crowd. *Circulation Research.* 2003;92(5):482–484.

[7] Peters JM, Hennuyer N, Staels B, et al. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor α-deficient mice. *Journal of Biological Chemistry.* 1997;272(43):27307–27312.

[8] Wang Y-X, Lee C-H, Tiep S, et al. Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell.* 2003;113(2):159–170.

[9] Michalik L, Desvergne B, Tan NS, et al. Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)α and PPARβ mutant mice. *The Journal of Cell Biology.* 2001;154(4):799–814.

[10] Tan NS, Michalik L, Noy N, et al. Critical roles of PPARβ/δ in keratinocyte response to inflammation. *Genes and Development.* 2001;15(24):3263–3277.

[11] Weindruch R, Sohal RS. Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. *New England Journal of Medicine.* 1997;337(14):986–994.

[12] Ye J-M, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome proliferator-activated receptor (PPAR)-α activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats. Comparison with PPAR-γ activation. *Diabetes.* 2001;50(2):411–417.

[13] Guerre-Millo M, Rouault C, Poulain P, et al. PPAR-α-null mice are protected from high-fat diet-induced insulin resistance. *Diabetes.* 2001;50(12):2809–2814.

[14] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *Journal of Clinical Investigation.* 1999;103(11):1489–1498.

[15] Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: the PPARα-null mouse as a model of fatty acid oxidation disorders. *Proceedings of the National Academy of Sciences of the United States of America.* 1999;96(13):7473–7478.

[16] Jones JR, Barrick C, Kim K-A, et al. Deletion of PPARγ in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(17):6207–6212.

[17] Corton JC, Brown-Borg HM. Peroxisome proliferator-activated receptor γ coactivator 1 in caloric restriction and other models of longevity. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2005;60(12):1494–1509.

[18] Masternak MM, Al-Regaiey K, Bonkowski MS, et al. Divergent effects of caloric restriction on gene expression in normal and long-lived mice. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2004;59(8):784–788.

[19] Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, et al. Effects of caloric restriction and growth hormone resistance on the expression level of peroxisome proliferator-activated receptors superfamily in liver of normal and long-lived growth hormone receptor/binding protein knockout mice. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2005;60(11):1394–1398.

[20] Corton JC, Apte U, Anderson SP, et al. Mimetics of caloric restriction include agonists of lipid-activated nuclear receptors. *Journal of Biological Chemistry.* 2004;279(44):46204–46212.

[21] Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, et al. Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2005;60(10):1238–1245.

[22] Norris AW, Chen L, Fisher SJ, et al. Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *Journal of Clinical Investigation.* 2003;112(4):608–618.

[23] Wang Y-X, Lee C-H, Tiep S, et al. Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell.* 2003;113(2):159–170.

[24] Wang Z, Al-Regaiey KA, Masternak MM, Bartke A. Adipocytokines and lipid levels in Ames dwarf and calorie-restricted mice. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2006;61(4):323–331.

[25] Zhou Y, Xu BC, Maheshwari HG, et al. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proceedings of the National Academy of Sciences of the United States of America.* 1997;94(24):13215–13220.

[26] Liu J-L, Coschigano KT, Robertson K, et al. Disruption of growth hormone receptor gene causes diminished pancreatic islet size and increased insulin sensitivity in mice.

American Journal of Physiology - Endocrinology and Metabolism. 2004;287:E405–E413.

[27] Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ. Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Hormone and IGF Research.* 2004;14(4):309–318.

[28] Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology.* 2000;141(7):2608–2613.

[29] Stauber AJ, Brown-Borg H, Liu J, et al. Constitutive expression of peroxisome proliferator-activated receptor α-regulated genes in dwarf mice. *Molecular Pharmacology.* 2005;67(3):681–694.

[30] Masternak MM, Panici J, Wilson M, et al. The effect of calorie restriction and Metformin on insulin pathway genes in growth hormone (GH) transgenic mice. In: Proceedings of 34th American Aging Association Annual Meeting; June 2005; Oakland, Calif.

[31] Watanabe K, Fujii H, Takahashi T, et al. Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor α associated with age-dependent cardiac toxicity. *Journal of Biological Chemistry.* 2000;275(29):22293–22299.

[32] Howroyd P, Swanson C, Dunn C, Cattley RC, Corton JC. Decreased longevity and enhancement of age-dependent lesions in mice lacking the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). *Toxicologic Pathology.* 2004;32(5):591–599.

[33] Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, et al. Caloric restriction and growth hormone receptor knockout: effects on expression of genes involved in insulin action in the heart. *Experimental Gerontology.* 2006;41(4):417–429.

[34] Dhahbi JM, Tsuchiya T, Kim H-J, Mote PL, Spindler SR. Gene expression and physiologic responses of the heart to the initiation and withdrawal of caloric restriction. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2006;61(3):218–231.

[35] Lee C-K, Allison DB, Brand J, Weindruch R, Prolla TA. Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proceedings of the National Academy of Sciences of the United States of America.* 2002;99(23):14988–14993.

[36] Sung B, Park S, Yu BP, Chung HY. Modulation of PPAR in aging, inflammation, and calorie restriction. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences.* 2004; 59(10):997–1006.

Hindawi Publishing Corporation
PPAR Research
Volume 2007, Article ID 95974, 10 pages
doi:10.1155/2007/95974

Review Article

PPARs, Obesity, and Inflammation

Rinke Stienstra, Caroline Duval, Michael Müller, and Sander Kersten

Nutrition, Metabolism and Genomics Group and Nutrigenomics Consortium, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Received 15 September 2006; Revised 13 November 2006; Accepted 13 November 2006

Recommended by Francine M. Gregoire

The worldwide prevalence of obesity and related metabolic disorders is rising rapidly, increasing the burden on our healthcare system. Obesity is often accompanied by excess fat storage in tissues other than adipose tissue, including liver and skeletal muscle, which may lead to local insulin resistance and may stimulate inflammation, as in steatohepatitis. In addition, obesity changes the morphology and composition of adipose tissue, leading to changes in protein production and secretion. Some of these secreted proteins, including several proinflammatory mediators, may be produced by macrophages resident in the adipose tissue. The changes in inflammatory status of adipose tissue and liver with obesity feed a growing recognition that obesity represents a state of chronic low-level inflammation. Various molecular mechanisms have been implicated in obesity-induced inflammation, some of which are modulated by the peroxisome proliferator-activated receptors (PPARs). PPARs are ligand-activated transcription factors involved in the regulation of numerous biological processes, including lipid and glucose metabolism, and overall energy homeostasis. Importantly, PPARs also modulate the inflammatory response, which makes them an interesting therapeutic target to mitigate obesity-induced inflammation and its consequences. This review will address the role of PPARs in obesity-induced inflammation specifically in adipose tissue, liver, and the vascular wall.

1. INTRODUCTION

The prevalence of obesity worldwide has progressively increased over the past decades. In 2000, it was estimated that more than half of US adults were overweight, while the frequency of obesity, which is defined by a body mass index (BMI) $\geq$ 30 kg/m^2, was 20%, reflecting an increase of 61% within 10 years [1]. Not only have more and more adults become obese, obesity is also striking at a much younger age leading to a high number of obese children and adolescents [2]. Unless drastic action is taken, many countries will face a decline in life expectancy in the 21st century due to the obesity epidemic.

Obesity is the direct result of an imbalance between energy intake and energy expenditure. The excess energy is primarily stored in adipose tissue in the form of triglycerides. Although adipocytes are specifically designed to store energy and easily fill up with fat, the morphological changes associated with adipose tissue growth are not without consequences for the organism as a whole [3]. Evidence has accumulated suggesting that in response to adipocyte hypertrophy during development of obesity, adipose tissue function is compromised.

Obesity also provokes structural and metabolic alterations in other organs, including skeletal muscle and liver. Indeed, obesity is closely linked to fat storage in liver and is nowadays considered as a major risk factor for the development of fatty liver diseases. The incidence of nonalcoholic fatty liver disorders (NAFLDs) and obesity are therefore intimately linked. It has been estimated that about 75% of obese subjects have NAFLD while 20% develop nonalcoholic steatohepatitis (NASH), which is defined as fatty liver disease with inflammation [4]. The amount of fat stored in liver is determined by the balance between fatty acid uptake, endogenous fatty acid synthesis, triglyceride synthesis, fatty acid oxidation, and triglyceride export. Changes in any of these parameters can affect the amount of fat stored in liver.

The excessive fat accumulation in adipose tissue, liver, and other organs strongly predisposes to the development of metabolic changes that increase overall morbidity risk. The metabolic abnormalities that often accompany obesity include hypertension, impaired glucose tolerance, insulin resistance leading to hyperinsulinemia, and dyslipidemia. Collectively, these abnormalities have been clustered into the metabolic syndrome or Syndrome X [5]. Individuals that are diagnosed with metabolic syndrome have a significantly

increased risk of developing cardiovascular disease (CVD) and type II diabetes. Inasmuch as CVD is the major cause of death in industrialized countries, effective strategies to curtail the number of individuals with metabolic syndrome are badly needed. Visceral obesity, which is characterized by excess fat storage in and around the abdomen, is the prime cause of the metabolic abnormalities, and therefore represents an important target in the treatment of metabolic syndrome [6].

In recent years, it has become clear that obesity also gives rise to a heightened state of inflammation. The link between obesity and inflammation was first established by Hotamisligil et al. who showed a positive correlation between adipose mass and expression of the proinflammatory gene tumor necrosis factor-α (TNFα) [7]. The link between obesity and inflammation has been further illustrated by the increased plasma levels of several proinflammatory markers including cytokines and acute phase proteins like C-reactive protein (CRP) in obese individuals [8, 9]. Nowadays, CRP is considered as an independent biomarker for the development of CVD [10] which emphasizes the connection between inflammation, obesity, and CVD. Many of the inflammatory markers found in plasma of obese individuals appear to originate from adipose tissue [8]. These observations have led to the view that obesity is a state of chronic low-grade inflammation that is initiated by morphological changes in the adipose tissue.

One consequence of the elevated inflammatory status is insulin resistance. Proinflammatory cytokines originating from fat have been shown to directly interfere with insulin signaling pathways [11]. For example, TNFα causes insulin resistance by inhibiting tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) [12]. Other mechanisms of inhibition of IRS-1 phosphorylation by inflammatory mediators include chronic activation of JNK, PKC, and IKK [13–15].

Besides TNFα, adipose tissue produces a host of other adipokines with well-described effects on metabolism and inflammation. Resistin, adiponectin, leptin, and monocyte chemoattractant protein-1 (MCP-1) are among a group of secreted proteins from adipose tissue with immunemodulating functions [16]. The production and secretion of these adipokines are altered during obesity, resulting in a more proinflammatory or atherogenic secretion profile. Indeed, whereas secretion of MCP-1, resistin, and other proinflammatory cytokines is increased by obesity, the adipose secretion of the anti-inflammatory protein adiponectin is decreased [17].

Although increased visceral fat depots [6] and adipocyte hypertrophy [3] had been linked to a higher degree of adipose inflammation, until recently the exact pathways leading to a proinflammatory state of adipose tissue in obese individuals remained unidentified. However, recently much attention has been diverted to the role of macrophages. In 2003, two papers published back to back showed that diet-induced obesity is associated with infiltration of macrophages into white adipose tissue [18, 19]. Infiltrated macrophages, which are part of the stromal vascular fraction of adipose tissue, are subsequently responsible for the production of a wide variety of proinflammatory proteins including MCP-1, TNFα, and interleukin-6 (IL-6). The development of insulin resistance in adipocytes was closely linked to the infiltration of macrophages. However, if and how entry of macrophages into white adipose tissue (WAT) leads to systemic insulin resistance remains unclear, although it is increasingly believed that altered secretion of adipokines by WAT during obesity may represent an important piece of the puzzle.

One of the other tissues that is affected by the enlargement and proinflammatory secretion profile of adipose tissue is the liver. Chronic activation of the master regulator of inflammation nuclear factor-κB (NF-κB) by cytokines has been directly linked to the development of insulin resistance in liver [20, 21]. It has also been shown that adipose-specific overexpression of MCP-1 increases hepatic triyglyceride content [22]. Although steatosis is a common occurrence in obese individuals, the role of inflamed adipose tissue in development of steatosis needs further exploration.

Initially characterized by excess fat storage, steatosis can progress to steatohepatitis and finally leads to cirrhosis and structural alterations of the liver [23]. The molecular mechanisms underlying the development of steatosis and progression to steatohepatitis remain poorly understood. Whereas some patients only develop steatosis, others develop steatohepatitis and fibrosis. Lipid peroxidation, cytokines, and other proinflammatory compounds are believed to play a vital role in the transition [4]. In addition, the role of the expanding adipose tissue might also prove relevant to the development of steatohepatitis.

Recently, the elevated inflammatory status of adipose tissue and concurrent increased production of adipose tissue-derived cytokines have also been connected with atherosclerosis. Initially defined as a pathological lipid deposition, the atherosclerotic process is nowadays considered as an ongoing inflammatory process in which numerous cytokines, chemokines, and inflammatory cells participate [24]. Independent of its connection to the metabolic syndrome, obesity itself is a known risk factor for the development of atherosclerosis and CVD [25].

In summary, obesity represents a major health threat, and effective therapies to minimize obesity-related comorbidities are sorely needed. By targeting the inflammatory component, the progression of obesity towards insulin resistance and CVD might be slowed down.

The ligand-activated transcription factors belonging to the peroxisome proliferators- activated receptor (PPAR) family are involved in the regulation of inflammation and energy homestasis and represent important targets for obesity, obesity-induced inflammation, and metabolic syndrome in general. These receptors share a common mode of action that involves heterodimerization with the nuclear receptor RXR and subsequent binding to specific DNA-response elements in the promoter of target genes. Binding of ligands to PPARs leads to recruitment of coactivators and chromatin remodeling, resulting in initiation of DNA transcription [26, 27]. Currently, synthetic PPAR agonists are widely used for the treatment of insulin resistance and dyslipidemia. This review will explore the role of PPARs in governing chronic

inflammation with special emphasis on the connection with metabolic syndrome. The link with obesity and inflammation will be discussed separately for the three PPAR isoforms: PPARα, PPARβ/δ, and PPARγ.

2. PPARα

PPARα is well expressed in metabolically active tissues including liver, brown adipose tissue, muscle, and heart. In addition, PPARα is expressed in cells involved in immune responses including monocytes, macrophages, and lymphocytes [28]. Activation of PPARα occurs through a variety of natural agonists, including unsaturated fatty acids and eicosanoids, whereas fibrate drugs act as synthetic agonists. In liver, PPARα plays a pivotal role in fatty acid catabolism by upregulating the expression of numerous genes involved in mitochondrial fatty acid oxidation, peroxisomal fatty acid oxidation, and numerous other aspects of fatty acid metabolism in the cell [28]. As a consequence, activation of PPARα can prevent and decrease hepatic fat storage [29–32]. Other metabolic pathways under control of PPARα include gluconeogenesis [33], biotransformation [34], and cholesterol metabolism [35]. While the function of PPARα in mouse liver is relatively well defined, much less is known about its role in human liver. Experiments with "humanized" PPARα mice have revealed that there are intrinsic differences in the properties of the human and mouse PPARα protein [36]. In general, research on the role of PPARα in human liver is hampered by the low expression levels of PPARα in human hepatoma cell lines [37].

Besides governing metabolic processes, PPARα also regulates inflammatory processes, mainly by inhibiting inflammatory gene expression. Hepatic PPARα activation has been repeatedly shown to reduce hepatic inflammation elicited by acute exposure to cytokines and other compounds. In recent years, several molecular mechanisms responsible for the immunosuppressive effects of PPARα have been uncovered [38]. These include interference with several proinflammatory transcription factors including signal transducer and activator of transcription (STAT), activator protein-1 (AP-1), and NF-κB by PPARα [39]. The latter mechanism involves stimulation of expression of the inhibitory protein IκBα, which retains NF-κB in a nonactive state, leading to suppression of NF-κB DNA-binding activity [40]. Detailed molecular studies have further revealed that PPARα diminishes the activity of the proinflammatory transcription factor CAATT/enhancer binding proteins (C/EBP) via sequestration of the coactivator glucocorticoid receptor-interacting protein-1/transcriptional intermediary factor-2 (GRIP1/TIF2) [41]. Finally, PPARα can also inhibit cytokine signaling pathways via downregulation of the IL-6 receptor [42] and upregulation of sIL-1 receptor antagonist [Stienstra et al., in press], leading to diminished inflammatory responses. Interestingly, in humans, specific PPARα activation using fenofibrate has been shown to decrease plasma levels of several acute phase proteins that are normally increased during inflammatory conditions [42].

2.1. PPARα and steatosis

In mice fed a high-fat diet, proper functioning of PPARα is essential to prevent the liver from storing large amounts of fat [43]. By inducing mitochondrial, peroxisomal, and microsomal fatty acid oxidation, PPARα reduces hepatic fat accumulation in the liver during the development of fatty liver disease, and thus prevents steatosis [31, 44, 45]. It can be hypothesized that since PPARα has a potent anti-inflammatory activity in liver, the progression of steatosis towards steatohepatitis might be counteracted by PPARα. Indeed, several studies in mice have shown that PPARα activation is able to reduce or even reverse steatohepatitis induced by feeding a methionine- and choline-deficient (MCD) diets [31, 45, 46].

In a mouse model of steatohepatitis, the presence and activation of PPARα prevented the induction of COX-2 expression [47]. Since upregulation of COX-2 is seen in alcoholic steatohepatitis and nonalcoholic steatohepatitis and has been directly linked to the progression of steatosis to steatohepatitis, the inhibitory effect of PPARα on COX-2 may reduce steatohepatitis. An anti-inflammatory role of PPARα in the development of steatohepatitis is further supported by a study in which wild-type and PPARα $-/-$ mice were fed a high-fat diet to induce obesity. Although both genotypes developed a fatty liver after chronic high-fat feeding, animals lacking PPARα developed steatohepatitis accompanied by an increased number of infiltrated lymphocytes and macrophages. By suppressing the expression of specific chemokines involved in attracting macrophages and other immune-related cell types, PPARα might moderate steatohepatitis [Stienstra et al., submitted]. These results are in line with a study performed in APOE2 knock-in mice fed a western-type high-fat diet [48]. When the animals were cotreated with fenofibrate, macrophage infiltration of the liver was prevented.

2.2. PPARα and atherosclerosis

Inflammation in the arterial wall is known to promote the process of atherosclerosis [49]. In addition to suppressing the inflammatory response in liver, PPARα may also influence inflammatory reactions in the arterial wall. As PPARα is expressed in various cell types present in atherosclerotic lesions, the effect of PPARα on lesion development is rather complex. Immune-modulating effects of specific PPARα activation have been reported in various cell types. However, some controversy still exists about the exact role of PPARα in the vascular wall as both pro- and antiatherogenic effects of PPARα have been demonstrated.

An antiatherogenic effect of PPARα via suppression of several proinflammatory genes like MCP-1, TNFα, vascular cell adhesion molecule-I (VCAM I), intercellular adhesion molecule-I (ICAM I), and interferon-γ (IFNγ) has been reported in the vascular wall of animals with extensive atherosclerosis [50]. Other studies have shown that the anti-inflammatory role of PPARα in the vascular wall seems to be dependent on the severity of inflammation or vascular lesion. In the absence of inflammation or in early lesions, the effects

of PPARα are mainly proatherogenic [51, 52], whereas the development of severe lesions accompanied by inflammation is strongly reduced by PPARα activation.

Several acute phase proteins have been linked to the development of atherosclerosis [53]. This includes CRP, which is currently used as a marker for systemic inflammation and linked to CVD, and serum amyloid A (SAA), which has been shown to be involved in the development of atherosclerosis [54]. As PPARα activation downregulates plasma concentrations of acute phase proteins including CRP and SAA in humans [42], it might indirectly prevent or slow down the progression of atherosclerosis.

2.3. PPARα and adiposity

Although expression of PPARα in WAT is much lower compared to PPARγ, evidence abounds that PPARα may also influence adipose tissue function. It has been shown that PPARα $-/-$ mice gain more adipose mass compared to wild-type animals [55], which may be via local or systemic effects of PPARα. An antiobesity role for PPARα is supported by several studies in which obese rodents were administered synthetic PPARα agonists [56–58]. While it is true that PPARα agonists have a clear anorexic effect resulting in decreased food intake, evidence is accumulating that PPARα may also directly influence adipose tissue function, including its inflammatory status.

A recent study revealed that treatment of obese diabetic KKAy mice with Wy-14643 decreased adipocyte hypertrophy as well as macrophage infiltration [59]. In PPARα $-/-$ mice chronically fed a high-fat diet (HFD), expression of inflammatory genes in adipose tissue was more pronounced compared to wild-type mice. In addition, fractionation of adipose tissue in adipocytes and stromal vascular cells revealed higher gene expression levels of the specific macrophage marker F4/80+ in the stromal vascular fraction of PPARα $-/-$ mice [Stienstra et al., submitted].

PPARα may govern adipose tissue inflammation in three different ways: (1) by decreasing adipocyte hypertrophy, which is known to be connected with a higher inflammatory status of the tissue [3, 11, 59], (2) by direct regulation of inflammatory gene expression via locally expressed PPARα, or (3) by systemic events likely originating from liver. Full clarification of the role of locally expressed PPARα in adipose tissue will have to await the availability of adipose tissue-specific PPARα $-/-$ mice.

Thus, while evidence is mounting that PPARα activation reduces adipose inflammation as observed during obesity, it is unclear whether the anti-inflammatory effects of PPARα in WAT are caused by direct or indirect mechanisms.

3. PPARβ/δ

Compared to PPARα and PPARγ, much less is known about PPARβ/δ and its natural ligands. Due to its ubiquitous expression profile, lack of specific ligands and, until recently, lack of availability of knock-out models, the role of PPARβ/δ in many tissues has been poorly explored. Fortunately, the recent generation of PPARβ/δ $-/-$ mice has provided a strong impetus for the characterization of the function of PPARβ/δ [60]. Several abnormalities have been observed in mice lacking PPARβ/δ which include impaired wound healing, a decrease in adipose mass, and disturbed inflammatory reactions in skin [61].

PPARβ/δ has been directly linked to the development of obesity. Indeed, several groups have reported a decrease in adiposity after PPARβ/δ activation. By stimulating fatty acid oxidation, PPARβ/δ activation leads to loss of adipose mass in different mouse models of obesity [62]. Similar effects on fatty acid oxidation have been observed in heart, resulting in improved muscle contraction [63]. In addition to increasing fatty acid oxidation, activation of PPARβ/δ in muscle also increases the number of type I muscle fibers, which leads to enhanced endurance performance [64].

The number of studies that have addressed the role of PPARβ/δ during inflammation is limited. So far, an anti-inflammatory effect has been observed in macrophages suggesting a possible role for PPARβ/δ in the process of atherogenic inflammation. It appears that PPARβ/δ acts as an inflammatory switch in which inactivated PPARβ/δ is proinflammatory and activated PPARβ/δ promotes an anti-inflammatory gene expression profile. The proposed switch of PPARβ/δ is linked to the B cell lymphoma-6 (BCL-6) protein which functions as inflammatory suppressor protein [65]. In the unliganded state, BCL-6 is part of the PPARβ/δ-RXRα transcriptional complex. Upon ligand activation, corepressors including BCL-6 are dissociated and PPARβ/δ-dependent gene transcription ensues. The released BCL-6 subsequently acts as a repressor of proinflammatory gene expression in macrophages.

3.1. PPARβ/δ and steatosis

It can be hypothesized that the stimulatory effect of PPARβ/δ on fatty acid oxidation in muscle and adipose tissue might also extend to liver, which would render PPARβ/δ an anti-steatotic role in liver. Within the liver, PPARβ/δ expression is found in different cell types although the highest levels are found in hepatic endothelial cells [66].

According to a recent report by Nagasawa et al., activation of PPARβ/δ may diminish fatty liver disease. In this study, mice were fed an MCD diet to induce steatohepatitis. Administration of the PPARβ/δ agonist GW501516 not only decreased hepatic lipid content, yet it also reduced inflammatory gene expression. PPARβ/δ decreased fat storage in liver mainly by activation of genes involved in fatty acid oxidation. Furthermore, the elevated mRNA levels of transforming growth factor-β1 (TGF-β1), TNFα, MCP-1 and interleukin-1β (IL-1β) that accompany the development of steatohepatitis were counteracted by PPARβ/δ activation [67]. Which liver cell types and molecular mechanisms contribute to the observed regulation is unknown.

3.2. PPARβ/δ and atherosclerosis

Due to the anti-inflammatory properties of PPARβ/δ in macrophages, it is plausible that atherosclerosis is affected by PPARβ/δ-activation. By feeding low-density lipoprotein

receptor (LDLR) −/− mice a hypercholesterolemic diet supplemented with a specific PPARβ/δ ligand, it was shown that PPARβ/δ is able to interfere with the inflammatory process underlying the development of atherosclerosis. Whereas lesion development itself was not prevented by PPARβ/δ activation, inflammatory gene expression was blunted compared to untreated mice [50]. The anti-inflammatory action of PPARβ/δ was mainly achieved by a strong inhibition of VCAM-1, MCP-1, and IFN-γ expressions, genes that are associated with the development of atherosclerosis. A recent study in which LDLR −/− mice were treated with the PPARβ/δ agonist GW0742X revealed an antiatherosclerotic effect of PPARβ/δ, in addition to an anti-inflammatory effect. Lesion development was strongly inhibited and inflammatory gene expression in macrophages was decreased [68].

While in mice there is compelling evidence for an anti-inflammatory role of PPARβ/δ in the atherosclerosis, the role of PPARβ/δ in humans is relatively unknown. Remarkably, PPARβ/δ was shown to strongly promote lipid accumulation in human macrophages, thereby supporting the development of atherosclerosis [69]. Whether PPARβ/δ influences inflammatory gene expression in human cells needs further study.

3.3. PPARβ/δ and adiposity

Recently, it was shown that activation of PPARβ/δ in adipose tissue causes a marked decrease in fat mass which is mainly achieved by activation of fatty acid oxidative pathways [62]. Moreover, high-fat-diet-induced adiposity was strongly inhibited by activation of PPARβ/δ in adipose tissue. Whether PPARβ/δ is able to control inflammatory gene expression in WAT during diet-induced obesity is still unclear. Inasmuch as inflammatory gene expression is linked to adiposity, it could be hypothesized that inflammatory gene expression will be suppressed by PPARβ/δ activation. Also, since expressions of IL-1β, MCP-1, and TNFα are controlled by PPARβ/δ in liver [67], it is tempting to speculate that inflammatory gene expression is under control of PPARβ/δ in adipose tissue as well.

4. PPARγ

PPARγ is considered the master regulator of adipogenesis, and accordingly has been extensively studied in the context of obesity. In humans, PPARγ is most highly expressed in adipose tissue, yet reasonable levels of PPARγ mRNA can also be found in other organs including skeletal muscle, colon, and especially lung [70]. The latter is probably due to the abundance of macrophages in lung. At least two different isoforms of PPARγ are known: PPARγ1, which is the form expressed in nonadipose tissues, and PPARγ2, which is adipose-tissue specific. Unsaturated fatty acids and several eicosanoids serve as endogenous agonists of PPARγ, while antidiabetic drugs belonging to the thiazolidinediones act as synthetic agonists of PPARγ. Target genes of PPARγ are involved in adipocyte differentiation, lipid storage, and glucose metabolism, and include lipoprotein lipase, CD36, phosphoenolpyruvate carboxykinase, aquaporin 7, and adiponectin [71].

Gain and loss of function studies have shed more light on the specific functions of PPARγ in different tissues. While homozygous PPARγ-deficient animals are embryonically lethal, specific ablation in adipose tissue revealed the indispensable role of PPARγ in adipocyte differentiation and function [72]. In liver, PPARγ is involved in triglyceride homeostasis and contributes to steatosis. At the same time, hepatic PPARγ protects other tissues from triglyceride accumulation and insulin resistance [73].

Similar to PPARα, PPARγ is involved in governing the inflammatory response, especially in macrophages. Currently, two different molecular mechanisms have been proposed by which anti-inflammatory actions of PPARγ are effectuated: (1) via interference with proinflammatory transcription factors including STAT, NF-κB, and AP-1 [74], and (2) by preventing removal of corepressor complexes from gene promoter regions resulting in suppression of inflammatory gene transcription [75]. This mechanism involves ligand-dependent SUMOylation of PPARγ followed by binding of PPARγ to nuclear receptor corepressor (NCoR)-histone deacetylase-3 (HDAC3) complexes localized on inflammatory gene promoters. The binding of PPARγ prevents the removal of corepressor complexes, thus retaining inflammatory genes in a suppressed state.

4.1. PPARγ and adiposity

PPARγ is indispensable for adipocyte differentiation both in vivo and in vitro [76–78]. In spite of its vital role in adipogenesis and lipogenesis, PPARγ expression itself is not strongly influenced during obesity. As discussed above, diet-induced obesity is associated with increased inflammatory gene expression in adipose tissue via adipocyte hypertrophy and macrophage infiltration. It has been shown that PPARγ is able to reverse macrophage infiltration, and subsequently reduces inflammatory gene expression [18]. Adipose expression of inflammatory markers A disintegrin and metallopeptidase domain-8 (ADAM8), macrophage inflammatory protein-1α (MIP-1α), macrophage antigen-1 (MAC-1), F4/80+, and CD68 was downregulated by specific PPARγ activation. Inflammatory adipokines mainly originate from macrophages which are part of the stromal vascular fraction of adipose tissue [18, 19], and accordingly, the downregulation of inflammatory adipokines in WAT by PPARγ probably occurs via effects on macrophages. By interfering with NF-κB signaling pathways, PPARγ is known to decrease inflammation in activated macrophages [74]. PPARγ may also influence inflammatory gene expression via effects on adipocyte morphology. Indeed, smaller adipocytes are known to secrete less inflammatory markers compared to larger adipocytes [3]. Treatment of obese rats with the synthetic PPARγ agonist troglitazone dramatically reduced the size of adipocytes without changing the total weight of WAT. In parallel, the expression levels of the inflammatory marker TNFα were normalized compared to those of untreated rats [79]. Furthermore, by inducing the expression of adiponectin in adipocytes [80], PPARγ may directly contribute to suppression of chronic inflammation accompanying obesity.

Summarizing, the anti-inflammatory effects of PPARγ activation in adipose tissue are presumably achieved by effects on both adipocytes and adipose tissue-resident macrophages. Interestingly, PPARγ is induced both during macrophage and adipocyte differentiation [71]. Since preadipocytes that are present in adipose tissue have the ability to differentiate towards macrophage-type cells and towards adipocytes depending on the local environment [81], the role of PPARγ in determining the fate of preadipocytes is of interest. It can be hypothesized that activation of PPARγ might favor adipocyte differentiation resulting in a decreased inflammatory status of adipose tissue during obesity.

4.2. PPARγ and atherosclerosis

PPARγ is expressed in white blood cells and differentiated macrophages and has been implicated in the process of atherosclerosis. Initially, PPARγ activation was proposed to be proatherogenic by stimulating uptake and storage of oxidized lipids in macrophages via upregulation of the scavenger receptor/fatty acid transporter CD36. This process leads to foam cell development and is a key event in the development of atherosclerosis [82]. In contrast, treatment with thiazolidinediones has been shown to reduce the development of atherosclerosis in mouse models [50, 71], suggesting that PPARγ is antiatherogenic. The inhibitory effect on atherosclerosis may be mediated by upregulating expression of the ABCA1 transporter in macrophages, thereby promoting cholesterol efflux. Furthermore, PPARγ activation strongly reduces inflammatory gene expression in macrophages, including MCP-1, VCAM-1, ICAM-1, IFNγ, and TNFα [50]. Several human studies also point to antiatherogenic effects of PPARγ in type II diabetic patients. Daily administration of 400 mg troglitazone or 30 mg pioglitazone for 6 months resulted in a reduction of common carotid arterial intimal and medial complex thickness which is used as a noninvasive method to monitor early atherosclerotic lesions [83, 84]. In a randomized controlled trial using 5238 patients with type II diabetes, treatment with 15 mg to 45 mg pioglitazone improved cardiovascular outcome [85]. Whether these protective effects in humans are achieved by inhibiting inflammation remains to be determined.

4.3. PPARγ and steatosis

It has been well established that in mouse models of steatosis, the development of fatty liver is associated with increased hepatic expression of PPARγ. In a nonfatty liver, the role of PPARγ appears to be limited and is probably restricted to stellate cell function during liver injury-induced fibrogenesis [86]. During the development of steatosis, hepatocytes become lipid-loaden and gain phenotypical characteristics of adipocytes which include the formation of large lipid droplets. In parallel, expression of adipogenic and lipogenic genes such as sterol regulatory element binding protein (SREBP), Adipose differentiation-related protein (ADRP) and PPARγ are strongly upregulated in steatotic livers [87, 88]. Likely, the upregulation of PPARγ contributes to the phenotype, since adenoviral-mediated hepatic overexpression of PPARγ1 on a PPARα $-/-$ background dramatically increases hepatic lipid accumulation and adipogenic gene expression in mice [89]. Also, marked upregulation of PPARγ in livers of PPARα $-/-$ mice fed a high-fat diet leads to increased expression of adipocyte markers and might contribute to the fatty liver phenotype [43]. In contrast, mice that specifically lack PPARγ in liver are protected from hepatic steatosis and show decreased expression levels of lipogenic genes compared to wild-type mice [73, 90]. Thus, PPARγ induction appears to be necessary and sufficient for hepatic steatosis.

The development of steatosis and progression into steatohepatitis is closely linked to an increased inflammatory state of the liver [4]. Recent data suggest that activation of PPARγ in fatty liver may protect against inflammation. Microarray analysis revealed that several inflammatory genes that are upregulated in fatty livers of mice fed a high-fat diet were strongly downregulated by PPARγ overexpression in liver [89]. These genes include SAA, Chemokine (C-X-C motif) ligand 10 (CXL10)/IP10 and interferon-γ-inducible protein, 47 kd. Data from our own group showed that hepatic PPARγ activation by rosiglitazone under steatotic conditions results in downregulation of multiple proinflammatory genes. Thus, although activation of PPARγ in liver contributes to the development of steatosis, inflammatory gene expression is suppressed.

Several small clinical human studies have been performed to evaluate the effects of thiazolidinediones in patients diagnosed with NASH. After treatment, the degree of steatosis and inflammation improved in a number of patients indicating that PPARγ may be an interesting pharmacological target [91]. Apart from weight gain, no side effects were reported in these studies. However, more studies are needed to assess the potentially beneficial effects of PPARγ activation on liver function.

5. CONCLUSION

An elevated inflammatory status is increasingly believed to be an important mediator that links excess (visceral) fat mass with numerous metabolic abnormalities, including insulin resistance. PPARs may influence the inflammatory response either by direct transcriptional downregulation of proinflammatory genes via mechanisms involving transrepression, or indirectly via their transcriptional effects on lipid metabolism. Numerous animal studies have demonstrated a role for PPARs in counteracting obesity-induced inflammation in liver, adipose tissue, and the vascular wall. The ability to reduce inflammatory cell infiltration further underlines the central role of PPARs in obesity-induced inflammation (Figure 1).

A growing number of studies strongly support anti-inflammatory properties of PPARs in human obesity as well. Several clinical trials in type II diabetic or hyperlipidemic patients have clearly shown that PPARα agonists including fenofibrate, ciprofibrate, and gemfibrozil can effectively reduce circulating levels of TNFα, IL-6, fibrinogen, and CRP [92]. Rosiglitazone, a selective PPARγ agonist, exerts anti-inflammatory effects in both obese and type II

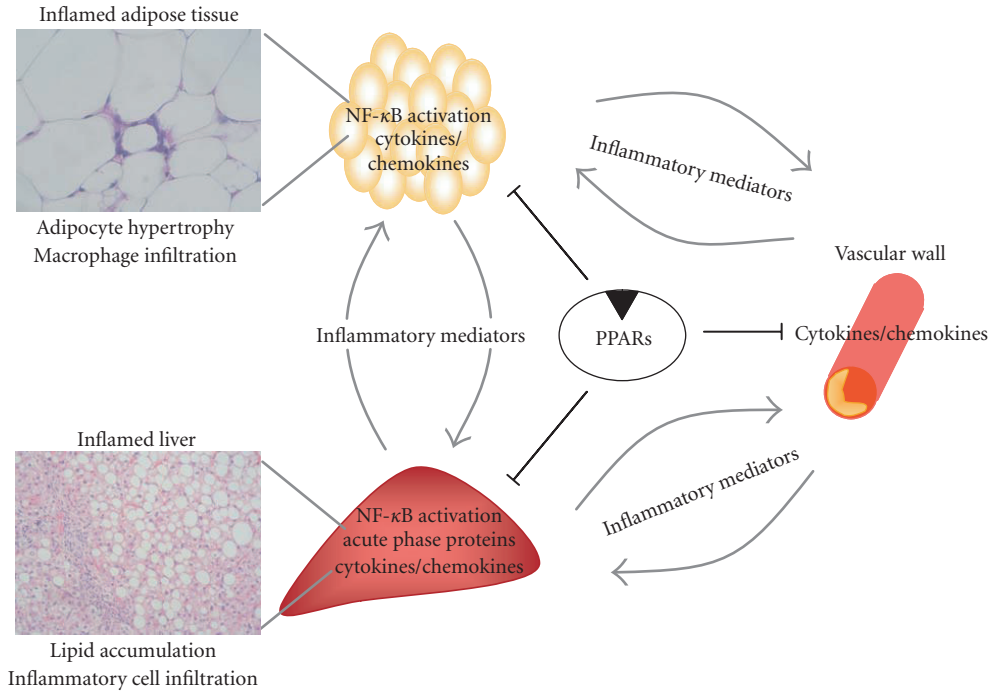


Figure 1: Central role of PPARs in obesity-induced inflammation. (Visceral) obesity and associated fatty liver stimulate inflammation in adipose tissue and liver via increased recruitment and infiltration of macrophages, resulting in increased production of proinflammatory cytokines. By downregulating proinflammatory genes in liver, adipose tissue and the vascular wall, PPARs have a major influence on the progression of obesity-related inflammation and its complications.

diabetic individuals by decreasing plasma concentrations of C-reactive protein, serum amyloid-A, and matrix metalloproteinase [93, 94].

Since synthetic PPARα and PPARγ agonists independently ameliorate obesity-induced inflammation, agonists that activate both PPARα and PPARγ (the so-called dual PPARα/PPARγ agonists) might be even more effective. Unfortunately, the development and clinical trials of these compounds have been hampered by serious concerns regarding their safety. Many dual PPARα/PPARγ agonists once in clinical development have since been abandoned, often for reasons of toxicity, including most recently the dual agonist tesaglitazar.

In conclusion, although more work is needed to evaluate their full potential in humans, especially in terms of safety, PPAR agonists nevertheless represent a promising strategy to mitigate obesity-associated inflammation.

REFERENCES

[1] A. H. Mokdad, B. A. Bowman, E. S. Ford, F. Vinicor, J. S. Marks, and J. P. Koplan, "The continuing epidemics of obesity and diabetes in the United States," *Journal of the American Medical Association*, vol. 286, no. 10, pp. 1195–1200, 2001.

[2] E. Malecka-Tendera and A. Mazur, "Childhood obesity: a pandemic of the twenty-first century," *International Journal of Obesity*, vol. 30, supplement 2, pp. S1–S3, 2006.

[3] M. Jernås, J. Palming, K. Sjöholm, et al., "Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression," *The FASEB Journal*, vol. 20, no. 9, pp. 1540–1542, 2006.

[4] G. C. Farrell and C. Z. Larter, "Nonalcoholic fatty liver disease: from steatosis to cirrhosis," *Hepatology*, vol. 43, no. 2, supplement 1, pp. S99–S112, 2006.

[5] G. M. Reaven, "Banting Lecture 1988. Role of insulin resistance in human disease," *Diabetes*, vol. 37, no. 12, pp. 1595–1607, 1988.

[6] Y. Matsuzawa, "Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease," *Nature Clinical Practice Cardiovascular Medicine*, vol. 3, no. 1, pp. 35–42, 2006.

[7] G. S. Hotamisligil, N. S. Shargill, and B. M. Spiegelman, "Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance," *Science*, vol. 259, no. 5091, pp. 87–91, 1993.

[8] P. Trayhurn and I. S. Wood, "Signalling role of adipose tissue: adipokines and inflammation in obesity," *Biochemical Society Transactions*, vol. 33, no. 5, pp. 1078–1081, 2005.

[9] H. Florez, S. Castillo-Florez, A. Mendez, et al., "C-reactive protein is elevated in obese patients with the metabolic syndrome," *Diabetes Research and Clinical Practice*, vol. 71, no. 1, pp. 92–100, 2006.

[10] S. M. Haffner, "The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease," *American Journal of Cardiology*, vol. 97, no. 2, supplement 1, pp. 3A–11A, 2006.

[11] A. S. Greenberg and M. S. Obin, "Obesity and the role of adipose tissue in inflammation and metabolism," *The American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 461S–465S, 2006.

[12] G. S. Hotamisligil, "Inflammatory pathways and insulin action," *International Journal of Obesity and Related Metabolic Disorders*, vol. 27, supplement 3, pp. S53–S55, 2003.

[13] V. Aguirre, E. D. Werner, J. Giraud, Y. H. Lee, S. E. Shoelson, and M. F. White, "Phosphorylation of Ser^{307} in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action," *Journal of Biological Chemistry*, vol. 277, no. 2, pp. 1531–1537, 2002.

[14] M. T. A. Nguyen, H. Satoh, S. Favelyukis, et al., "JNK and tumor necrosis factor-α mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 280, no. 42, pp. 35361–35371, 2005.

[15] Z. Gao, D. Hwang, F. Bataille, et al., "Serine phosphorylation of insulin receptor substrate 1 by inhibitor κB kinase complex," *Journal of Biological Chemistry*, vol. 277, no. 50, pp. 48115–48121, 2002.

[16] Y.-H. Yu and H. N. Ginsberg, "Adipocyte signaling and lipid homeostasis: sequelae of insulin-resistant adipose tissue," *Circulation Research*, vol. 96, no. 10, pp. 1042–1052, 2005.

[17] T. Kadowaki and T. Yamauchi, "Adiponectin and adiponectin receptors," *Endocrine Reviews*, vol. 26, no. 3, pp. 439–451, 2005.

[18] H. Xu, G. T. Barnes, Q. Yang, et al., "Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1821–1830, 2003.

[19] S. P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante Jr., "Obesity is associated with macrophage accumulation in adipose tissue," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1796–1808, 2003.

[20] M. C. Arkan, A. L. Hevener, F. R. Greten, et al., "IKK-β links inflammation to obesity-induced insulin resistance," *Nature Medicine*, vol. 11, no. 2, pp. 191–198, 2005.

[21] D. Cai, M. Yuan, D. F. Frantz, et al., "Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB," *Nature Medicine*, vol. 11, no. 2, pp. 183–190, 2005.

[22] H. Kanda, S. Tateya, Y. Tamori, et al., "MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity," *Journal of Clinical Investigation*, vol. 116, no. 6, pp. 1494–1505, 2006.

[23] P. Angulo, "Nonalcoholic fatty liver disease," *New England Journal of Medicine*, vol. 346, no. 16, pp. 1221–1231, 2002.

[24] G. Stoll and M. Bendszus, "Inflammation and atherosclerosis: novel insights into plaque formation and destabilization," *Stroke*, vol. 37, no. 7, pp. 1923–1932, 2006.

[25] E. Bonora, "The metabolic syndrome and cardiovascular disease," *Annals of Medicine*, vol. 38, no. 1, pp. 64–80, 2006.

[26] V. Bocher, I. Pineda-Torra, J.-C. Fruchart, and B. Staels, "PPARS: transcription factors controlling lipid and lipoprotein metabolism," *Annals of the New York Academy of Sciences*, vol. 967, pp. 7–18, 2002.

[27] S. A. Smith, "Peroxisomal proliferate-activated receptors and the regulation of lipid oxidation and adipogenesis," *Biochemical Society Transactions*, vol. 25, no. 4, pp. 1242–1248, 1997.

[28] S. Mandard, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor α target genes," *Cellular and Molecular Life Sciences*, vol. 61, no. 4, pp. 393–416, 2004.

[29] S. Kersten, J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1489–1498, 1999.

[30] Y. Harano, K. Yasui, T. Toyama, et al., "Fenofibrate, a peroxisome proliferator-activated receptor α agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver," *Liver International*, vol. 26, no. 5, pp. 613–620, 2006.

[31] E. Ip, G. C. Farrell, G. Robertson, P. Hall, R. Kirsch, and I. Leclercq, "Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice," *Hepatology*, vol. 38, no. 1, pp. 123–132, 2003.

[32] C. J. Chou, M. Haluzik, C. Gregory, et al., "WY14,643, a peroxisome proliferator-activated receptor α (PPARα) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice," *Journal of Biological Chemistry*, vol. 277, no. 27, pp. 24484–24489, 2002.

[33] D. Patsouris, S. Mandard, P. J. Voshol, et al., "PPARα governs glycerol metabolism," *Journal of Clinical Investigation*, vol. 114, no. 1, pp. 94–103, 2004.

[34] O. Barbier, L. Villeneuve, V. Bocher, et al., "The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor α and γ target gene," *Journal of Biological Chemistry*, vol. 278, no. 16, pp. 13975–13983, 2003.

[35] P. Lefebvre, G. Chinetti, J.-C. Fruchart, and B. Staels, "Sorting out the roles of PPARα in energy metabolism and vascular homeostasis," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 571–580, 2006.

[36] K. Morimura, C. Cheung, J. M. Ward, J. K. Reddy, and F. J. Gonzalez, "Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor α to Wy-14,643-induced liver tumorigenesis," *Carcinogenesis*, vol. 27, no. 5, pp. 1074–1080, 2006.

[37] J. W. Lawrence, Y. Li, S. Chen, et al., "Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) α. PPARα fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expression," *Journal of Biological Chemistry*, vol. 276, no. 34, pp. 31521–31527, 2001.

[38] W. Vanden Berghe, L. Vermeulen, P. Delerive, K. De Bosscher, B. Staels, and G. Haegeman, "A paradigm for gene regulation: inflammation, NF-κB and PPAR," *Advances in Experimental Medicine and Biology*, vol. 544, pp. 181–196, 2003.

[39] P. Delerive, K. De Bosscher, S. Besnard, et al., "Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1," *Journal of Biological Chemistry*, vol. 274, no. 45, pp. 32048–32054, 1999.

[40] P. Delerive, P. Gervois, J.-C. Fruchart, and B. Staels, "Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators," *Journal of Biological Chemistry*, vol. 275, no. 47, pp. 36703–36707, 2000.

[41] P. Gervois, N. Vu-Dac, R. Kleemann, et al., "Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor α agonists via inhibition of CCAAT box/enhancer-binding protein β," *Journal of Biological Chemistry*, vol. 276, no. 36, pp. 33471–33477, 2001.

[42] P. Gervois, R. Kleemann, A. Pilon, et al., "Global suppression of IL-6-induced acute phase response gene expression after chronic in vivo treatment with the peroxisome proliferator-activated receptor-α activator fenofibrate," *Journal of Biological Chemistry*, vol. 279, no. 16, pp. 16154–16160, 2004.

[43] D. Patsouris, J. K. Reddy, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression," *Endocrinology*, vol. 147, no. 3, pp. 1508–1516, 2006.

[44] J. K. Reddy and M. S. Rao, "Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 290, no. 5, pp. G852–G858, 2006.

[45] E. Ip, G. Farrell, P. Hall, G. Robertson, and I. Leclercq, "Administration of the potent PPARα agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice," *Hepatology*, vol. 39, no. 5, pp. 1286–1296, 2004.

[46] P. V. Kashireddy and M. S. Rao, "Lack of peroxisome proliferator-activated receptor α in mice enhances methionine and choline deficient diet-induced steatohepatitis," *Hepatology Research*, vol. 30, no. 2, pp. 104–110, 2004.

[47] J. Yu, E. Ip, A. Dela Peña, et al., "COX-2 induction in mice with experimental nutritional steatohepatitis: role as pro-inflammatory mediator," *Hepatology*, vol. 43, no. 4, pp. 826–836, 2006.

[48] R. Shiri-Sverdlov, K. Wouters, P. J. van Gorp, et al., "Early diet-induced non-alcoholic steatohepatitis in APOE2 knock-in mice and its prevention by fibrates," *Journal of Hepatology*, vol. 44, no. 4, pp. 732–741, 2006.

[49] A. J. Lusis, "Atherosclerosis," *Nature*, vol. 407, no. 6801, pp. 233–241, 2000.

[50] A. C. Li, C. J. Binder, A. Gutierrez, et al., "Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARα, β/δ, and γ," *Journal of Clinical Investigation*, vol. 114, no. 11, pp. 1564–1576, 2004.

[51] H. Lee, W. Shi, P. Tontonoz, et al., "Role for peroxisome proliferator-activated receptor α in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 interleukin-8 by endothelial cells," *Circulation Research*, vol. 87, no. 6, pp. 516–521, 2000.

[52] E. Teissier, A. Nohara, G. Chinetti, et al., "Peroxisome proliferator-activated receptor α induces NADPH oxidase activity in macrophages, leading to the generation of LDL with PPAR-α activation properties," *Circulation Research*, vol. 95, no. 12, pp. 1174–1182, 2004.

[53] Å. Sjöholm and T. Nyström, "Endothelial inflammation in insulin resistance," *Lancet*, vol. 365, no. 9459, pp. 610–612, 2005.

[54] K. E. Lewis, E. A. Kirk, T. O. McDonald, et al., "Increase in serum amyloid a evoked by dietary cholesterol is associated with increased atherosclerosis in mice," *Circulation*, vol. 110, no. 5, pp. 540–545, 2004.

[55] P. Costet, C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau, "Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis," *Journal of Biological Chemistry*, vol. 273, no. 45, pp. 29577–29585, 1998.

[56] M. Guerre-Millo, P. Gervois, E. Raspé, et al., "Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity," *Journal of Biological Chemistry*, vol. 275, no. 22, pp. 16638–16642, 2000.

[57] M. Vázquez, N. Roglans, À. Cabrero, et al., "Bezafibrate induces acyl-CoA oxidase mRNA levels and fatty acid peroxisomal β-oxidation in rat white adipose tissue," *Molecular and Cellular Biochemistry*, vol. 216, no. 1-2, pp. 71–78, 2001.

[58] F. P. Mancini, A. Lanni, L. Sabatino, et al., "Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats," *FEBS Letters*, vol. 491, no. 1-2, pp. 154–158, 2001.

[59] A. Tsuchida, T. Yamauchi, S. Takekawa, et al., "Peroxisome proliferator-activated receptor (PPAR)α activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARα, PPARγ, and their combination," *Diabetes*, vol. 54, no. 12, pp. 3358–3370, 2005.

[60] J. M. Peters, S. S. Lee, W. Li, et al., "Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$.," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[61] G. D. Barish, V. A. Narkar, and R. M. Evans, "PPARδ: a dagger in the heart of the metabolic syndrome," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 590–597, 2006.

[62] Y.-X. Wang, C.-H. Lee, S. Tiep, et al., "Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity," *Cell*, vol. 113, no. 2, pp. 159–170, 2003.

[63] L. Cheng, G. Ding, Q. Qin, et al., "Cardiomyocyte-restricted peroxisome proliferator-activated receptor-δ deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy," *Nature Medicine*, vol. 10, no. 11, pp. 1245–1250, 2004.

[64] Y.-X. Wang, C.-L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[65] C.-H. Lee, A. Chawla, N. Urbiztondo, D. Liao, W. A. Boisvert, and R. M. Evans, "Transcriptional repression of atherogenic inflammation: modulation by PPARδ," *Science*, vol. 302, no. 5644, pp. 453–457, 2003.

[66] M. Hoekstra, J. K. Kruijt, M. Van Eck, and T. J. C. Van Berkel, "Specific gene expression of ATP-binding cassette transporters and nuclear hormone receptors in rat liver parenchymal, endothelial, and Kupffer cells," *Journal of Biological Chemistry*, vol. 278, no. 28, pp. 25448–25453, 2003.

[67] T. Nagasawa, Y. Inada, S. Nakano, et al., "Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARδ agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet," *European Journal of Pharmacology*, vol. 536, no. 1-2, pp. 182–191, 2006.

[68] T. L. Graham, C. Mookherjee, K. E. Suckling, C. N. A. Palmer, and L. Patel, "The PPARδ agonist GW0742X reduces atherosclerosis in LDLR$(-/-)$ mice," *Atherosclerosis*, vol. 181, no. 1, pp. 29–37, 2005.

[69] H. Vosper, L. Patel, T. L. Graham, et al., "The peroxisome proliferator-activated receptor δ promotes lipid accumulation in human macrophages," *Journal of Biological Chemistry*, vol. 276, no. 47, pp. 44258–44265, 2001.

[70] M. A. Lazar, "PPARγ, 10 years later," *Biochimie*, vol. 87, no. 1, pp. 9–13, 2005.

[71] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[72] H. Koutnikova, T.-A. Cock, M. Watanabe, et al., "Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPARγ hypomorphic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 24, supplement 2, pp. 14457–14462, 2003.

[73] O. Gavrilova, M. Haluzik, K. Matsusue, et al., "Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass," *Journal of Biological Chemistry*, vol. 278, no. 36, pp. 34268–34276, 2003.

[74] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[75] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[76] J. Auwerx, T. A. Cock, and C. Knouff, "PPAR-γ: a thrifty transcription factor," *Nuclear Receptor Signaling*, vol. 1, p. e006, 2003.

[77] I. Tzameli, H. Fang, M. Ollero, et al., "Regulated production of a peroxisome proliferator-activated receptor-γ ligand

during an early phase of adipocyte differentiation in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 279, no. 34, pp. 36093–36102, 2004.

[78] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[79] A. Okuno, H. Tamemoto, K. Tobe, et al., "Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats," *Journal of Clinical Investigation*, vol. 101, no. 6, pp. 1354–1361, 1998.

[80] T. Yamauchi, J. Kamon, H. Waki, et al., "The mechanisms by which both heterozygous peroxisome proliferator-activated receptor γ (PPARγ) deficiency and PPARγ agonist improve insulin resistance," *Journal of Biological Chemistry*, vol. 276, no. 44, pp. 41245–41254, 2001.

[81] G. Charrière, B. Cousin, E. Arnaud, et al., "Preadipocyte conversion to macrophage: evidence of plasticity," *Journal of Biological Chemistry*, vol. 278, no. 11, pp. 9850–9855, 2003.

[82] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[83] J. Minamikawa, S. Tanaka, M. Yamauchi, D. Inoue, and H. Koshiyama, "Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 5, pp. 1818–1820, 1998.

[84] H. Koshiyama, D. Shimono, N. Kuwamura, J. Minamikawa, and Y. Nakamura, "Rapid communication: inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 7, pp. 3452–3456, 2001.

[85] J. A. Dormandy, B. Charbonnel, D. J. Eckland, et al., "Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial in macroVascular Events): a randomised controlled trial," *Lancet*, vol. 366, no. 9493, pp. 1279–1289, 2005.

[86] L. Yang, C. C. Chan, O. S. Kwon, et al., "Regulation of peroxisome proliferator-activated receptor-γ in liver fibrosis," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 291, no. 5, pp. G902–G911, 2006.

[87] S. E. Schadinger, N. L. R. Bucher, B. M. Schreiber, and S. R. Farmer, "PPARγ2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes," *American Journal of Physiology - Endocrinology and Metabolism*, vol. 288, no. 6, pp. E1195–E1205, 2005.

[88] W. Motomura, M. Inoue, T. Ohtake, et al., "Up-regulation of ADRP in fatty liver in human and liver steatosis in mice fed with high fat diet," *Biochemical and Biophysical Research Communications*, vol. 340, no. 4, pp. 1111–1118, 2006.

[89] S. Yu, K. Matsusue, P. Kashireddy, et al., "Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor γ1 (PPARγ1) overexpression," *Journal of Biological Chemistry*, vol. 278, no. 1, pp. 498–505, 2003.

[90] K. Matsusue, M. Haluzik, G. Lambert, et al., "Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes," *Journal of Clinical Investigation*, vol. 111, no. 5, pp. 737–747, 2003.

[91] H. Reynaert, A. Geerts, and J. Henrion, "Review article: the treatment of non-alcoholic steatohepatitis with thiazolidinediones," *Alimentary Pharmacology and Therapeutics*, vol. 22, no. 10, pp. 897–905, 2005.

[92] A. Zambon, P. Gervois, P. Pauletto, J.-C. Fruchart, and B. Staels, "Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-α activators: clinical and experimental evidence," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 5, pp. 977–986, 2006.

[93] H. Ghanim, S. Dhindsa, A. Aljada, A. Chaudhuri, P. Viswanathan, and P. Dandona, "Low-dose rosiglitazone exerts an antiinflammatory effect with an increase in adiponectin independently of free fatty acid fall and insulin sensitization in obese type 2 diabetics," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3553–3558, 2006.

[94] P. Mohanty, A. Aljada, H. Ghanim, et al., "Evidence for a potent antiinflammatory effect of rosiglitazone," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2728–2735, 2004.