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PPARs: diverse regulators in energy metabolism and metabolic diseases

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The nuclear receptor PPARs are fundamentally important for energy homeostasis. Through their distinct yet overlapping functions and tissue distribution, the PPARs regulate many aspects of energy metabolism at the transcriptional level. Functional impairment or dysregulation of these receptors leads to a variety of metabolic diseases, while their ligands offer many metabolic benefits. Studies of these receptors have advanced our knowledge of the transcriptional basis of energy metabolism and helped us understand the pathogenic mechanisms of metabolic syndrome.
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Introduction

The prevalence of obesity has increased at an alarming rate in the last several decades worldwide. Along with it comes the associated metabolic syndrome, including insulin resistance, glucose intolerance, type 2 diabetes, dyslipidemia (increased serum triglycerides, decreased high-density lipoproteins (HDLs), and increased low-density lipoproteins (LDLs)), hypertension, nonalcoholic fatty liver, and cardiovascular disease [1]. At the center of this medical cluster is insulin resistance, which is often caused by obesity. Insulin resistance not only is an early, fundamental defect in type 2 diabetes [2], but also is the defining feature of metabolic syndrome [1]. Insulin resistance is manifested by hyperinsulinemia, decreased insulin-stimulated glucose uptake into skeletal muscle, and the impaired ability of insulin to inhibit gluconeogenesis in liver and lipolysis in adipose tissue. Type 2 diabetes occurs when the pancreatic β cells are unable to secrete enough insulin to compensate for insulin resistance. Although most insulin-resistant individuals are able to maintain relatively normal glucose levels through hyperinsulinemia during their lifetime, insulin resistance puts these individuals at great risk for the development

of a variety of abnormalities associated with metabolic syndrome [1]. Thus, obesity and insulin resistance play a central role in metabolic syndrome.

In order to better understand the functions of the nuclear receptor PPARs in energy metabolism and metabolic syndrome, it is useful to first summarize the potential mechanisms that could explain the pathogenesis of insulin resistance. Currently two theories are widely accepted. The first one hypothesizes that insulin resistance is tightly linked to disordered fatty acid metabolism and that insulin resistance results from the deleterious effects of lipid overload in the skeletal muscle and liver [3]. Fatty acids are preferably stored as triglycerides in the adipose tissue. However, a dysregulation of fatty acid metabolism can lead to abnormal triglyceride accumulation in skeletal muscle and/or liver that in most cases associates with insulin resistance. This close association between insulin resistance and no-adipose lipid accumulation has been found not only in many animal genetic models, but also in human populations that include obese subjects, lipodystrophic patients, the lean offspring of type 2 diabetes, and the elderly [3]. Interestingly, it is the increased intermediate metabolites of triglycerides, not triglycerides *per se*, that may be responsible for insulin resistance [3]. One such metabolite is diacylglycerol. It is thought that diacylglycerol activates members of protein kinase PKC, which leads to serine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-2 in the skeletal muscle and liver, respectively, thereby interfering

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with insulin action [3]. The second theory links obesity-associated insulin resistance to inflammation. Obesity is considered as a chronic, low-grade inflammatory state. Liver and, in particular, adipose tissue are the sites for this inflammatory response. Obesity leads to an increased production of proinflammatory cytokines by adipose tissue, liver, and their recruited macrophages. These cytokines in turn inhibit insulin signaling both locally and systematically [4, 5]. These two potential mechanisms are not necessarily mutually exclusive. Their relative importance for insulin resistance may depend on the pathophysiological states and affected tissues. Interestingly, PPARs appear to be at the crossroads of lipid metabolism and inflammation, regulating both processes [6].

The PPAR subfamily of nuclear receptors, consisting of three members, PPARα, PPARγ and PPARδ (also known as PPARβ), are ligand-activated transcription factors [6, 7]. They form obligate heterodimers with retinoid X receptors and bind to specific DNA sites composed of a direct repeat of hexameric sequences separated by one base pair, located in the promoter/enhancer region of target genes. Ligand binding triggers a conformation shift that results in release of co-repressors, recruitment of co-activators, and subsequent target gene expression. As nuclear receptors, the PPARs have a typical domain organization, containing an amino-terminal domain, a central zinc-finger DNA-binding domain (DBD), a carboxyl-terminal ligand-binding domain (LBD) that harbors the ligand-dependent activation function, and a small hinge region that links DBD and LBD. The DBD and LBD are highly conserved among the three PPARs. Different from other nuclear receptors, the ligand-binding pocket of the PPARs is unusually large and can accommodate a variety of endogenous lipids, including fatty acids, eicosanoids, oxidized and nitrated fatty acids, and derivatives of linoleic acid [6]. However, these lipids need to be present at high concentrations (micromolar range) to activate PPARs, and are often not highly selective for individual isoforms. Moreover, it has not been shown that any of these lipids is required for PPAR activation. Thus, whether any of them represents physiological ligand remains to be established. Nevertheless, it is clear that the activities and/or expression levels of PPARs are subjected to modulation by diets, nutrient status, and metabolic states. Through their distinct yet overlapping functions and tissue distribution, the three PPARs act as fatty acid sensors to control many metabolic programs that are essential for systematic energy homeostasis. Not surprisingly, they have important implications for human metabolic diseases, as evidenced by the fact that PPARγ and PPARα are respective molecular targets for the type 2 diabetes drug thiazolidinediones (TZDs) and dyslipidemia drug

fibrates. Here I review our current understanding of these receptors in lipid metabolism and associated metabolic syndrome. For the roles of these receptors in inflammatory response that also play a part in metabolic syndrome, readers are referred to recent publications [6, 8, 9].

PPARγ is a master regulator of adipogenesis

Adipose tissue is essential for whole body energy homeostasis [10, 11]. It has two functionally distinct types, white fat (WAT) and brown fat (BAT). WAT serves as a safe place that stores excess energy to avoid lipid build-up in other tissues, and releases the energy when other tissues are in need. It also has an endocrinal function, producing many secreted factors called adipokines that signal whole body metabolism [10, 11]. A large body of work has clearly established that, through its induction of many genes important for fatty acid uptake and storage, PPARγ is both sufficient and necessary for the differentiation of white fat adipocytes [12, 13]. PPARγ is predominantly expressed in the adipose tissue compared to other tissues and is induced during adipocyte differentiation [14]. Ectopic expression of PPARγ in nonadipogenic cells effectively converts them into adipocytes [15], whereas knockout of PPARγ in embryonic fibroblasts abolishes their differentiation into adipocytes [16]. Studies with germline deletion of PPARγ as well as studies with PPARγ hypomorphic mice have demonstrated an essential requirement for this gene in the formation of adipose tissue *in vivo*; that is, animals cannot generate adipocytes in the absence of PPARγ [17-19]. Moreover, mice with PPARγ deleted in differentiated adipocytes develop progressive lipodystrophy, revealing that PPARγ is also important for the survival of differentiated adipocytes [20-22]. Finally, heterozygous, dominant-negative PPARγ mutations cause lipodystrophy in humans [23].

PPARγ-controlled differentiation of white fat adipocytes involves a transcriptional cascade that includes members of C/EBP transcription factors [12, 13] (Figure 1A). *In vivo* studies suggest that C/EBPβ, δ and α are critical for adipogenesis [24, 25]. How do the C/EBPs regulate adipogenesis? Largely based on *in vitro* differentiation data, the current prevalent view is that the three C/EBPs work in concert to induce and maintain PPARγ expression [12, 13]. In addition, C/EBPα appears to be required for the insulin-sensitive properties of adipocytes [26, 27]. Recently, it was observed that C/EBPα co-occupies many of the PPARγ-target gene promoters [28], raising the possibility that C/EBPα may also directly regulate adipogenesis in a cooperative manner with PPARγ. PTIP is a transcriptional co-factor that regulates the expression of C/EBPα and PPARγ through histone methylation of their promoters [29]. The observation that rescue of the
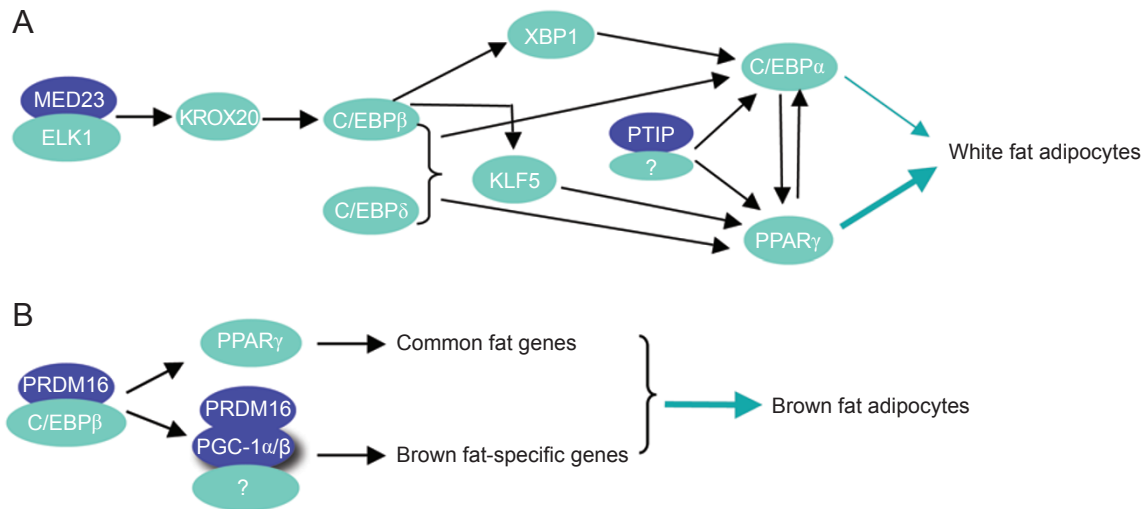
Figure 1 Regulation of white fat **(A)** and brown fat **(B)** adipogenesis by transcriptional cascades. Black arrows indicate increases of gene expression.

adipogenic defect in PTIP-deficient cells requires both PPARγ and C/EBPα is in agreement with this possibility [29]. *In vitro* studies have identified additional transcriptional regulators in adipogenesis, including Med23, ELK1, and Krox20, which act upstream of C/EBPβ [30, 31], and KLF5 and XBP1, which act downstream of C/EBPβ [32, 33] (Figure 1A); however, the *in vivo* requirements for these factors in adipose tissue formation are unclear.

As mentioned above, there are two types of adipose tissue. In contrast to the energy storage function of WAT, BAT is specialized for energy expenditure by dissipating energy as heat [34, 35]. The unique metabolic property of BAT is due to its high mitochondrial density and fuel oxidation capacity, and exclusive expression of the BAT hallmarker, uncoupling protein-1 (UCP1), in the inner mitochondrial membrane. UCP1 is a proton transporter that disrupts the mitochondrial electrochemical gradient by causing protons to leak across the inner mitochondrial membrane. Thus, UCP1 uncouples mitochondrial respiration from ATP production, leading to energy loss in the form of heat. BAT is located in the interscapular region in small mammals. In rodents, it is clear that BAT plays an important part in protection against obesity and obesity-associated metabolic problems. Previously, it was thought that BAT is only present in human neonates and is replaced by WAT in adults. However, recent work has collectively demonstrated that humans do possess functional brown fat depots and that the activity of BAT is inversely associated with obesity [36-38], suggesting that BAT could be important for human physiology and metabolic diseases.

There is a great interest in understanding the development of brown fat. Surprisingly, lineage studies show that BAT and WAT do not share the same precursor cells in early development; instead, BAT originates from Myf5-positive precursor cells that also give rise to skeletal muscle cells [39]. As in WAT, PPARγ is highly expressed in BAT and is required for BAT formation [18]. However, PPARγ alone generates a fat phenotype that is common to both WAT and BAT. The questions are what controls PPARγ expression in the BAT and what determines BAT-specific programs such as UCP1 expression and mitochondrial biogenesis. Spiegelman and co-workers discovered that C/EBPβ and transcriptional co-activators PRDM16, PGC-1α and PGC-1β are critical [39-43]. All these factors are selectively expressed in BAT compared to WAT. During brown fat cell differentiation, PRDM16 forms a complex with C/EBPβ and co-activates C/EBPβ transcriptional activity [42]. This PRDM16/C/EBPβ complex not only induces PPARγ expression, but also induces PGC-1α expression, which is further activated by PRDM16 and is responsible for the BAT-specific program [39, 40, 42] (Figure 1B). Indeed, expression of either PGC-1α or PRDM16 in white fat preadipocytes stimulates the expression of BAT-specific genes during differentiation [40, 41], while brown fat preadipocytes deficient for PRDM16 or PGC-1α and PGC-1β lose differentiation-associated BAT-specific gene expression [40, 43]. Moreover, co-expression of C/EBPβ and PRDM16 is sufficient to convert skin fibroblasts into functional brown fat adipocytes [42]. These pioneering studies

have significantly advanced our understanding of brown fat adipogenesis. While the physiological requirement for C/EBPβ in brown fat development is clear [42], can forced expression of C/EBPα or δ functionally substitute for C/EBPβ? In addition to C/EBPβ, are there other transcription factors mediating the induction of PGC-1α by PRDM16 during adipogenesis? As PPARγ can be co-activated by PRDM16 and PGC-1, does PPARγ directly regulate the expression of some brown fat-specific genes through these co-activators? Interestingly, it was shown that PPARγ is not required for UCP1 expression [40]. Finally, other factors, including Rb and p107 [44], RIP140 [45, 46], and BMP7 [47], have been identified as brown fat differentiation regulators. Their relationships with the molecular pathway described above remain to be determined.

PPARγ and TZD in insulin resistance and the mechanism of action

Members of TZD class drugs are widely used in the treatment of type 2 diabetes. In both animal models and human subjects with type 2 diabetes, TZDs lower glucose levels as well as insulin levels. Glucose clamp experiments demonstrate that TZDs increase skeletal muscle glucose disposal and, to a lesser extent, inhibit hepatic gluconeogenesis. Thus, TZDs work as insulin sensitizers by improving insulin action in the skeletal muscle and liver [48]. However, clinical use of TZDs is associated with side effects, including weight gain, fluid retention, edema, and heart failure [49]. How to separate the side effects from its efficacy is an important task in the future.

TZD drugs and PPARγ were independently discovered. The initial link between them was provided by findings that TZDs are in fact agonists for PPARγ [50, 51]. It is now clear that activation of PPARγ by TZDs is responsible for their beneficial effects on insulin sensitivity. How does activation of PPARγ, a protein that is mainly present in adipose tissue, lead to insulin sensitization in skeletal muscle and liver? Activation of PPARγ in the adipose tissue induces expression of an array of genes for fatty acid transport and storage, as well as promotes *de novo* adipogenesis. One plausible mechanism is that the increased ability to uptake lipids and the expanded capacity to store them allow lipid repartitioning from the skeletal muscle and liver to the adipose tissue, thus eliminating the deleterious effects of lipid on insulin signaling [10, 23]. Interestingly, the expansion of adiposity caused by TZDs in humans occurs in the subcutaneous adipose tissue, not in the visceral depots [23]. Triglycerides stored in the subcutaneous fat are less lipolytic and have less access to portal circulation and the liver [23]. In ad-

dition, PPARγ activation in adipose tissue regulates the production of adiponectin, resistin, and tumor necrosis factor-α (TNF-α). These secreted adipokines can impact insulin sensitivity in skeletal muscle and liver [10, 23]. Taken together, adipose tissue is considered as the primary target of TZD action. In obese, diabetic mice with liver-specific PPARγ deletion, TZDs remain effective in lowering glucose levels, indicating that liver PPARγ is not a target [52]. Since skeletal muscle accounts for 80% of the insulin-stimulated glucose disposal in humans, skeletal muscle-specific PPARγ knockout mice were used to address whether this tissue is also targeted by TZDs. Two studies have come to different conclusions [53, 54]; the reason for this discrepancy is currently unclear.

Pathological roles of PPARγ in non-adipose tissues

In contrast to adipose tissue, liver and heart express very little PPARγ. However, under certain pathological conditions, these tissues can express considerable amounts of PPARγ that have significant impacts on metabolic homeostasis and tissue function.

Hepatic PPARγ expression is markedly increased in several obese or diabetic mouse models that are associated with liver steatosis [23]. Studies suggest the hepatic PPARγ has a similar lipid deposition function as in adipose tissue. Ectopic adenoviral expression of PPARγ in the liver of lean mice causes liver steatosis [55]. In the obese (ob/ob) mice, when PPARγ is specifically deleted in the liver, hepatic triglyceride level is normalized and fatty liver phenotype is ameliorated, accompanied by increased plasma fasting triglyceride and free fatty acid levels [52]. TZD treatment further exacerbates the development of fatty liver in ob/ob mice, but not in ob/ob mice with hepatic PPARγ deletion [52]. These results suggest that the elevated expression of hepatic PPARγ may be responsible for liver steatosis in these mouse models. In contrast, small clinical studies show that TZD treatment reduces hepatic lipid level in humans with nonalcoholic fatty liver [56, 57], indicating that PPARγ function in the adipose tissue continues to be dominant. However, the hepatic levels of PPARγ in these human subjects are not known.

In the heart, fatty acid uptake and oxidation are tightly coordinated. Upregulation of PPARγ causes metabolic perturbations that link to cardiomyopathies, diseases that can ultimately lead to heart failure. In ventricular samples of humans and mice with hypertrophic cardiomyopathy, levels of hypoxia-inducible factor α (HIFα) and PPARγ are increased [58]. Mouse studies suggest that under hypertrophic stress, HIFα induces heart PPARγ expression. The concerted actions of HIFα and PPARγ then lead to cardiac lipid accumulation, apoptosis, and heart dysfunc-

tion [58]. Another study reported that human patients with metabolic syndrome and aortic stenosis express high levels of PPARγ in the heart, which is strongly correlated with cardiac lipid accumulation and poor cardiac function [59]. To directly examine the consequence of increased PPARγ, Goldberg and co-works overexpressed PPARγ in the heart and found that these transgenic mice develop steatosis and dilated cardiomyopathy. Treatment of the transgenic mice with the TZD drug further worsens these phenotypes [60]. These studies together suggest that heart PPARγ, when its level is high under certain pathological conditions, may cause lipid overload that contributes to common forms of cardiomyopathy. One side effect of the TZD drugs in treatment of human diabetic patients is heart failure. While this could be due to fluid retention [49], the involvement of heart PPARγ in subsets of patients should be considered as well.

PPARα regulates hepatic lipid catabolism and fasting response

PPARα is expressed in many metabolically active tissues, but is high in the liver [61]. The effects of PPARα agonist *in vivo* are mainly manifested in the liver [62]. The PPARα null mice display a fatty liver phenotype resulted from a decreased expression of fatty acid oxidation genes [63-67]. Indeed, the major function of PPARα is to promote fatty acid utilization. PPARα upregulates many genes involved in important steps of this process; these include genes for hepatic clearance of very low-density lipoprotein, fatty acid uptake, fatty acid activation and transport into the mitochondria, peroxisomal and mitochondrial β-oxidation, and some enzymes for mitochondrial respiration [68-70]. The fibrate drugs used

for the treatment of dyslipidemia are agonists of PPARα. Through activation of lipid catabolism, fibrates consistently lower plasma triglycerides in patients. Fibrates also raise plasma HDL through induction of apolipoprotein apoA-I and apoA-II in humans [68, 69]. In addition, PPARα upregulates genes for fatty acid and triglyceride synthesis in the liver [70]; the functional importance of this regulation is unknown.

Ketogenesis in the liver is critical to the adaptive response to fasting. During fasting, fatty acids are mobilized in the adipose tissue and uptaken into the liver, where they are oxidized in the mitochondria to produce ketone bodies that provide the large energy source for other tissues. PPARα is induced by fasting and is required for ketogenesis [66]. While the phenotype of the PPARα null mice at fed state is mild (fatty liver), fasting causes a very severe phenotype. This includes massive, exacerbated fatty liver, hypoketonemia, hypoglycemia, hypothermia, increased plasma free fatty acids, and loss of induction of fatty acid oxidation genes by fasting, suggesting that ketogenesis is significantly impaired [66, 67]. Surprisingly, the hormonal peptide fibroblast growth factor 21 (FGF21) appears to be a key component downstream of PPARα. FGF21 is robustly induced during fasting in a large part in a PPARα-dependent manner [71-73]. Introduction of FGF21 into PPARα null mice partially rescues hypoketonemia and fatty liver [73]. On the other hand, knockdown of FGF21 in the liver causes fatty liver, increased plasma free fatty acids and decreased ketone bodies, when mice are fed a ketogenic diet [71]. It was shown that FGF21 regulates the expression of lipases important for fatty acid release in the adipose tissue and the expression of a subset of PPARα target genes important
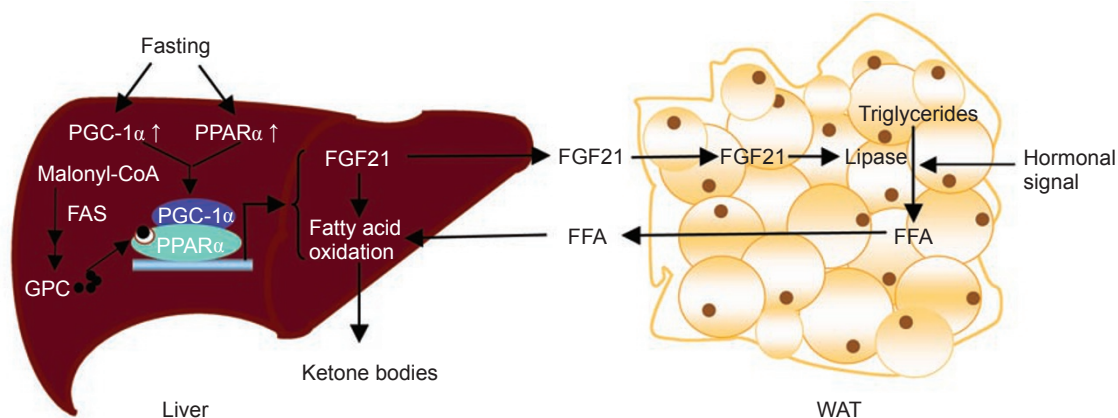

Figure 2 An integrated model of PPARα-regulated ketogenesis. Fasting induces the expression of PPARα and PGC-1α. Activation of PPARα requires *de novo* synthesis of a PPARα ligand (16:0/18:1 GPC). Together, they induce the expression of fatty acid oxidation genes and FGF21. FGF21 in turn promotes lipolysis in the adipose tissue. The released free fatty acids (FFAs) are used as substrates for ketogenesis.

for fatty acid oxidation in the liver [71, 73] (Figure 2).

The PPARα-regulated ketogenesis probably requires PGC-1α co-activation. As a co-activator, PGC-1α is able to interact with and co-activate many transcription factors *in vitro*, including PPARs. Besides being a central regulator in brown fat metabolism as discussed earlier, PGC-1α also plays important roles in energy metabolism in the liver, skeletal muscle and heart [74]. During periods of fasting, hepatic PGC-1α is induced, which in turn regulates fatty acid catabolism and gluconeogenesis [75]. PGC-1α null mice exhibit fasting-induced fatty liver [76]. A direct role of PGC-1α in fasting response was recently demonstrated by a study with liver-specific PGC-1α knockout mice [77]. In this study, to mimic the physiological and pathological fluctuations of hepatic PGC-1α level, one allele of PGC-1α was deleted from the liver by Cre/Lox system; another allele remained intact. These PGC-1α liver heterozygous mice develop fasting-induced fatty liver, impaired ketogenesis, and have reduced expression of hepatic fatty acid oxidation genes, a phenotype that remarkably overlaps with PPARα null mice, indicating that PGC-1α-mediated activation of PPARα is likely to be a major mechanism for hepatic fatty acid catabolism (Figure 2). Transcriptional co-factors Lipin1, Sirt1 and BAF60α were shown to be involved as well [78-80].

A physiologically relevant endogenous PPARα ligand in the liver

Many endogenous lipid species have been considered as ligands for PPARs. However, they usually require presence in micromolar concentration range for PPAR activation, and most importantly, until recently, it has not been shown that lack of any of these lipids would affect the function of PPARs. The recent work by Chakravarthy *et al.* [81] identified the phospholipid 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1 GPC) as a physiologically relevant PPARα ligand in liver. The initial hint of the existence of a physiological ligand for PPARα came from their previous study on the liver-specific fatty acid synthase (FAS) knockout mice (FASKOL) [82]. When these animals were subjected to fasting or placed on a diet that contained no fat (zero-fat diet), they developed a phenotype very similar to PPARα null mice, although the level of PPARα was not affected. Moreover, the phenotype was corrected when these mice were administered with a PPARα agonist. These results are consistent with the idea that FAS is required for the hepatic production of a PPARα ligand that can otherwise be derived from fat in normal diets, but not from fat released from peripheral tissues [82]. To identify this ligand, the authors immunoprecipitated PPARα from liver and analyzed PPARα-associated lipids. The phospholipid 16:0/18:1 GPC was found to be associated with PPARα in the sample from wild-type mice, but not in that from FASKOL mice. This phospholipid potently binds to PPARα, weakly to PPARδ, and has no interaction with PPARγ *in vitro*. Knockdown of CEPT1, a gene required for the synthesis of 16:0/18:1 GPC, downregulates expression of some PPARα target genes in hepatic cells. Infusion of this lipid into mice reduces the liver triglyceride content in a PPARα-dependent manner. These results suggest that 16:0/18:1 GPC is an endogenous PPARα ligand that is indispensable for hepatic PPARα activation and function during fasting (Figure 2). It remains to be determined whether 16:0/18:1 GPC is also a physiological ligand for PPARα in other tissues. Moreover, structural analysis should provide insights into its differential binding affinity with the three PPARs.

PPARα in the skeletal muscle and heart: some unexpected phenotypes

In the PPARα knockout mice, muscle energy metabolism remains normal [83]. A gain-of-function study was performed to address the potential role of PPARα in this tissue [84]. Overexpression of PPARα in the skeletal muscle increases the expression of genes for fatty acid uptake and oxidation, but decreases the expression of the glucose transporter Glut4. Strikingly, under normal chow diet, these transgenic mice have elevated levels of plasma glucose and insulin, are glucose intolerant and insulin resistant; this occurs in the context of normal muscular lipid content and normal insulin signaling. When fed a high-fat diet, the transgenic mice, although resistant to obesity, have significantly higher muscular triglyceride content than controls, and continue to be glucose intolerant and insulin resistant. The effect of the transgene on muscular lipid accumulation is quite unexpected, given the known function of PPARα in fatty acid utilization; a plausible explanation is that the increased fatty acid uptake exceeds the increased muscle capacity for oxidation. This study confirms the role of PPARα in fatty acid catabolism in peripheral tissues. However, it is unclear what causes the severely impaired glucose homeostasis under normal diets. Is it due to the reduced expression of Glut4? Notably, skeletal muscle-specific deletion of Glut4 produces a similar defect in glucose homeostasis [85]. It will be interesting to see whether the PPARα agonists also decrease Glut4 expression or promote lipid accumulation in the skeletal muscle. This question should be readily assessable with the PPARα transgenic mice because of the high expression of the transgene.

PPARα also regulates cardiac metabolism. PPARα null mice have decreased expression of cardiac fatty acid oxi-

dation genes and decreased rates of fatty acid oxidation, but maintain normal cardiac function [83, 86]. Similar to the skeletal muscle PPARα transgene, overexpression of PPARα in the heart increases the expression of genes for fatty acid transport and oxidation, and decreases Glut4 expression; this leads to lipid accumulation, reduced glucose transport and utilization, insulin resistance, and cardiomyopathy [87, 88]. These phenotypes are exacerbated on high-fat diets [89], and are corrected by deficiency of the fatty acid transporter CD36 without affecting fatty acid oxidation [90]. Interestingly, Glut4 expression and glucose transport are increased in the PPARγ cardiac transgenic mice, which also display lipid accumulation and cardiomyopathy [60]. Moreover, in the PPARα muscle transgenic mice, decreased Glut4 expression and defects in glucose metabolism occur in the absence of lipid accumulation [84]. These observations indicate that the decreased Glut4 expression and glucose utilization in the PPARα transgenic mice is a PPAR-isoform-specific phenotype not caused by lipid accumulation or increased fatty acid oxidation *per se*. If this is true, then how this phenotype is corrected by CD36 deficiency is unknown.

PPARδ is an integral component in a transcriptional network regulating brown fat metabolism

PPARδ is ubiquitously expressed in many tissues. Genetic and pharmacological studies reveal its role as a powerful regulator of fatty acid catabolism and energy homeostasis [91]. Initially, due to the unavailability of a specific PPARδ agonist, we used transgenic mice expressing an activated form of PPARδ by fusion with a VP16 activation domain to uncover its tissue-specific function [92]. Adipose tissue expression of this transgene produces lean mice that are resistant to obesity and tissue steatosis induced either genetically or by a high-fat diet. Although WAT expressed a slightly higher level of the transgene than BAT, the induction of the expression of fat-burning genes mainly occurs in BAT. Interestingly, UCP1 induction is the highest among all the genes examined. In WAT, only UCP1 is induced. The reason for this selective induction in BAT vs WAT is unknown. This work helped demonstrate a potential *in vivo* function of brown fat PPARδ in energy expenditure.

To further study the role of PPARδ in brown fat metabolism, we generated an immortalized brown fat preadipocyte cell line from mice containing floxed PPARδ alleles. *In vitro* deletion of the PPARδ alleles by cre recombinase adenovirus-mediated excision, as expected, does not affect brown fat cell differentiation, yet significantly downregulates the expression of fat-burning genes [93]. In particular, UCP1 expression is the mostly decreased, typically by more than 10-fold, while the levels

of PGC-1α, PPARγ and PPARα remain unchanged. This led us to test whether PPARδ acts downstream of PGC-1α. The β3-adrenergic receptor agonist stimulates UCP1 expression through PGC-1α. The PPARδ-deficient brown fat cells are almost completely unresponsive to the β3-adrenergic receptor agonist in the induction of UCP1. Moreover, in the absence of PPARδ, PGC-1α is longer associated with the PPAR-binding site of the UCP1 promoter. Finally, the ability of the PPARδ-fat knockout mice to maintain body temperature during cold exposure is compromised. These results suggest that, in the brown fat, there is a cell-autonomous requirement for PPARδ in the expression of at least some of the PGC-1α target genes and that PPARδ at least partly mediates the action of PGC-1α.

The PGC-1α/PPARδ-regulated brown fat metabolism is fine-tuned by twist-1 [93]. Twist-1 is a critical, physiological negative regulator in brown fat metabolism. It is selectively expressed in the adipose tissue, interacts with PGC-1α, and is recruited to the promoters of PGC-1α's target genes to suppress PGC-1α function. Indeed, twist-1 heterozygous knockout mice are resistant to high fat-induced obesity, whereas the fat twist-1 transgenic mice have the opposite phenotype. Interestingly, upon activation by its ligand, PPARδ is recruited to the twist-1 promoter and induces twsit-1 expression both *in vitro* and *in vivo*, suggesting a negative-feedback mechanism. Thus, PPARδ is an integral component in brown fat metabolism by coordinating the actions of PGC-1α and twist-1.

PPARδ in the skeletal muscle and heart: a comparison with PPARα

Muscle fibers are broadly classified into two groups, oxidative fibers and glycolytic fibers. Oxidative fibers have high mitochondrial content and mainly use mitochondrial oxidative metabolism for energy production, whereas glycolytic fibers have low mitochondrial content and rely on glycolytic metabolism as a major energy source. PPARδ has a higher expression than PPARα in the skeletal muscle [61, 83] and is more abundant in the oxidative fibers [94, 95]. Treatment of skeletal muscle cells with the PPARδ agonists *in vitro* induces the expression of genes for fatty acid catabolism and promotes fatty acid oxidation [83, 92, 96]. To test the *in vivo* function of PPARδ, skeletal muscle PPARδ transgenic mice expressing either a wild type or activated form of PPARδ were generated [94, 97]. There is a remarkable increase of oxidative fibers in these transgenic mice, similar to that observed in the skeletal muscle PGC-1α transgenic mice [98]; however, PGC-1α is not induced in the PPARδ transgenic mice. While the phenotype of the

transgenic mice expressing the wild-type PPARδ remains to be fully characterized, the transgenic mice expressing the activated PPARδ are found to be completely protected against high-fat diet-induced obesity, muscular lipid accumulation, hyperinsulinemia, glucose intolerance, and insulin resistance [94] (our unpublished data). The normalization of insulin sensitivity by the PPARδ transgene on the high-fat diet is most likely due to the lack of muscular lipid accumulation. While both the PPARδ and PPARα muscle transgenic lines are resistant to high-fat diet-induced obesity, the PPARα line, in striking contrast, has increased muscular lipid accumulation and is insulin resistant and glucose intolerant (Figure 3). Both of these two lines display upregulation of genes for fatty acid uptake, β-oxidation, and mitochondrial respiration [84, 94, 97] (our unpublished data). Moreover, although the PPARδ transgene is constitutively active, its expression level is very low; instead, endogenous muscle PPARδ expression is highly elevated so that it may at least partly contribute to the phenotype [94]. Thus, what causes the phenotypic differences between the PPARδ and PPARα transgenic mice is currently unclear and can be either isoform-specific target gene expression or their overall differential effects on metabolic programs. Interestingly, the skeletal muscle PGC-1α transgenic mice, although

showing increased oxidative fibers, not only are not protected against high-fat diet-induced body weight gain, but also are more insulin resistant due to muscular lipid accumulation, compared to control mice [98, 99].

A phenotype opposite to that of PPARδ transgenic mice was revealed in the skeletal muscle-specific PPARδ knockout mice [100]. Deletion of PPARδ decreases the expression of many genes for fatty acid β-oxidation and mitochondrial respiration function, and increases the number of muscle fibers with lower oxidative capacity, leading to an increased body weight gain and insulin resistance. The skeletal muscle of these knockouts also shows a 50% decrease of PGC-1α that could be responsible for the muscle phenotype. However, it is also possible that the decrease in PGC-1α is a secondary chronic effect caused by the increased number of less oxidative fibers. As a similar muscle phenotype was observed when PPARδ was deleted by an inducible cre after the muscle was formed [100], it will be interesting to examine the level of PGC-1α in this setting. Nevertheless, this work demonstrates that PPARδ is required for the formation and maintenance of oxidative muscle fibers.

Cardiac-specific deletion of PPARδ causes lipid accumulation and cardiomyopathy [101]. Unlike PPARα and PPARγ, overexpression of PPARδ in the heart is not as-

	Obesity	Muscular lipid	Plasma insulin	Glucose tolerance	Insulin sensitivity
PPARα	↓	↑	ND	↓	↓
PPARδ	↓	↓	↓	↑	↑
PPARδ ligand	↓	↓	↓	↑	↑

Figure 3 A comparison of the phenotypes among skeletal muscle PPARα transgenic mice, PPARδ transgenic mice, and wild-type mice treated with a PPARδ agonist. Mice were fed a high-fat diet. ND, not determined.

	Cardiac lipid	Glut4 expression	Glucose transport	Cardiomyopathy
PPARγ	↑	↑	↑	Yes
PPARα	↑	↓	↓	Yes
PPARδ	–	↑	↑	NO

Figure 4 A comparison of the cardiac phenotypes of the heart PPAR transgenic mice under a normal diet.

sociated with lipid accumulation and has no detrimental effects on cardiac function [102]. Surprisingly, the transgene increases Glut4 expression and promotes glucose transport in the heart [102] (Figure 4).

Function and mechanism of PPARδ agonist in fat burning

An important question is whether activation of endogenous PPARδ by its agonist would be metabolically beneficial. Treatment of insulin-resistant, obese monkeys for four weeks with the PPARδ agonist lowers plasma insulin and triglyceride levels and increases HDL level, but has no effect on body weight [103]. Increased fatty acid oxidation was observed in the skeletal muscle and BAT, but not in the liver, of PPARδ agonist-treated mice [104]. The PPARδ agonist effectively prevents the high-fat diet-induced obesity, hyperinsulinemia, tissue steatosis, glucose intolerance, and insulin resistance [94, 104]. When given to the diabetic, obese ob/ob mice, the PPARδ agonist produces similar beneficial effects, along with a significant decrease in blood glucose level [104, 105]. However, the body weight reduction in the ob/ob mice is very modest [104], indicating that the agonist is less effective on predisposed obesity. Overall, the PPARδ agonist elicits profound metabolic benefits that essentially recapitulate the phenotypes of the transgenic mice (Figure 3). Future studies with conditional PPARδ knockout mice are needed to address the relative contribution of different tissues to the agonist effects. Presumably, skeletal muscle and BAT are major target tissues.

Is the PPARδ agonist useful for human metabolic syndrome? To date, this has not been extensively studied or reported. In a small pilot study, PPARδ agonist was given to healthy moderately obese subjects for two weeks. Administration of the PPARδ agonist significantly reduces fasting plasma triglycerides, LDL cholesterol, insulin, and liver fat content; in contrast, these changes were not observed in subjects when given the PPARα agonist, except for the lowering of plasma triglycerides [106]. Whether these beneficial effects of the PPARδ agonist can also be observed in a large study remains to be seen.

Conceivably, fat burning in the skeletal muscle requires coordination between fatty acid β-oxidation and mitochondrial respiration. It is clear that PPARδ agonists enhance fatty acid β-oxidation. Are they also capable of promoting mitochondrial respiration function and thereby driving the formation of oxidative muscle fibers? Treatment of fibroblasts or myoblasts *in vitro* with the PPARδ agonist increases the activities of enzymes in the mitochondrial respiration chain [107]. In a mouse model of mitochondrial myopathy, bezafibrate, an agonist for both PPARδ and PPARα, elevates the levels of proteins

for mitochondrial respiration, increases mitochondrial biogenesis, and improves the myopathy phenotype [108]. Treatment of wild-type mice with the PPARδ agonist promotes the formation of oxidative muscle fibers based on succinate dehydrogenase activity staining [109]. However, other *in vivo* studies did not observe any effect of the agonist on expression of a group of genes for mitochondrial respiration function [105] or on the formation of oxidative fibers based on ATPase activity staining [110]; but combined with exercise training, the agonist increases the number of oxidative fibers [110]. Thus, it is likely that PPARδ and its agonist may only regulate a subset of genes for mitochondrial respiration function and that a systematic effect requires additional signaling inputs. Notably, levels of PPARδ and PGC-1α in the skeletal muscle are elevated in several genetic mouse models that display increased mitochondrial biogenesis and oxidative fibers [111, 112].

PPARδ agonists activate gene expression through facilitating the recruitment of transcriptional co-activators. In primary, PGC-1α-deficient myotubes, PPARδ agonist-induced fatty acid oxidation is attenuated but not abolished. These data indicate that co-activation of PPARδ by PGC-1α is partly responsible for the ligand effects in the skeletal muscle and that a PGC-1α-independent mechanism also exits [105]. In agreement with this, we have found that in C2C12 muscle cells where there is little expression of PGC-1α, PPARδ agonist-induced gene expression and fatty acid oxidation are not suppressed by ectopic expression of the PGC-1α inhibitor, twist-1 (our unpublished data).

PPARδ and exercise physiology

Muscle fibers are strongly associated with exercise physiology [113]. On one hand, oxidative muscle fibers confer exercise endurance; on the other hand, regular exercise training promotes the formation of oxidative fibers, which leads to the improvement in metabolic syndrome. Given the effects of PPARδ on muscle fiber and energy metabolism, two related questions become apparent: whether activation of PPARδ promotes exercise endurance and whether PPARδ mediates some of the metabolic benefits of exercise training. The first question has been addressed. When placed on a treadmill, muscle PPARδ transgenic mice are resistant to fatigue and are able to run twice the distance of wild-type mice [94]. Conversely, the muscle PPARδ knockout mice run about 30% less the distance of the wild-type mice [100]. The PPARδ agonist itself is not sufficient to promote exercise performance; however, when combined with exercise training, the agonist significantly increases endurance compared to exercise alone [110]. Part of the
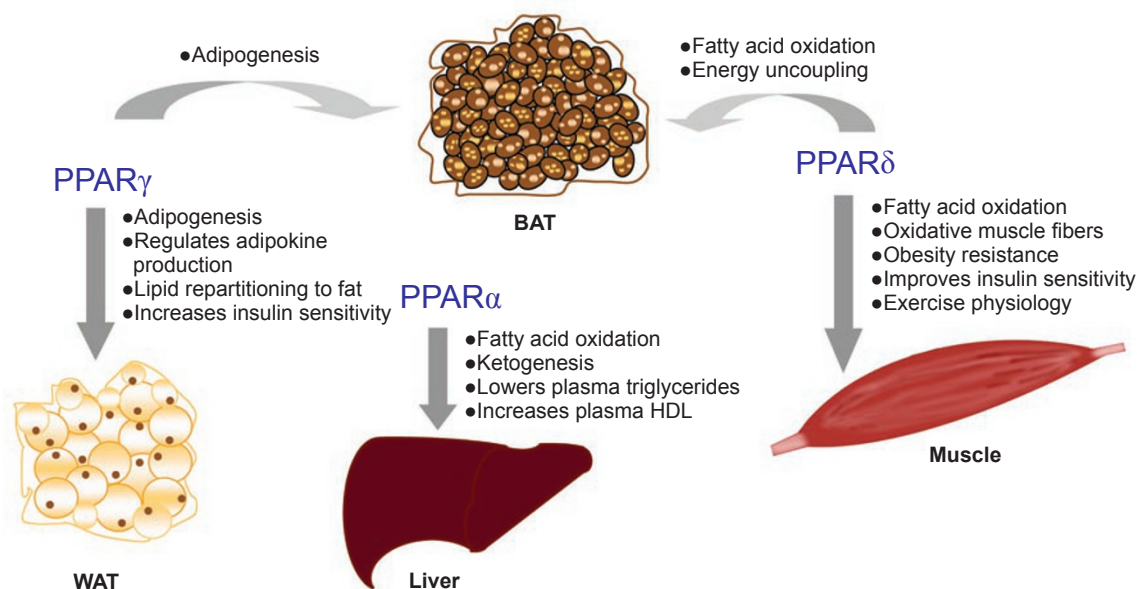
Figure 5 An overview of the physiological and/or pharmacological roles of the PPARs in energy metabolism. Data are summarized from PPAR knockout and ligand studies.

molecular mechanism underlying this synergistic effect on endurance is that exercise activates the AMPK kinase, which further enhances the ligand-activated PPARδ transcriptional activity [110]. This work reveals the AMPK-PPARδ axis as a pharmacological target to reprogram muscle endurance. Does PPARδ in turn contribute to the metabolic benefits pertinent to exercise? This has not been addressed genetically. However, PPARδ expression in the skeletal muscle is increased by exercise training in both rodents and humans [97, 114, 115]. Importantly, there is a strong correlation between exercise-induced PPARδ expression and improvement of clinical parameters in type 2 diabetic patients [115]. These observations, along with the known effects of PPARδ activation on energy metabolism, suggest a formal possibility that PPARδ may mediate some of the metabolic benefits of exercise.

Conclusions

The three PPARs, by acting as lipid sensors, are major metabolic regulators in the body and together they control almost every aspect of fatty acid metabolism (Figure 5). Importantly, through regulation of lipid metabolism, these receptors greatly impact insulin sensitivity and glucose homeostasis. Functional impairment or dysregulation of these receptors is shown to lead to obesity, lipodystrophy, fatty liver, insulin resistance, type 2 diabetes, or cardiomyopathy, whereas proper activation

by ligands offers metabolic benefits. These studies have helped significantly in understanding the transcriptional regulation of energy metabolism and the pathogenesis of metabolic syndrome. However, many interesting questions remain. For example, what are the detailed molecular mechanisms employed by these receptors in gene regulation? What are the causes for the side effects of the PPAR drugs? What are the endogenous, physiological ligands in different tissues? How do the PPARs and their potential endogenous ligands respond to environmental signals? How are their actions coordinated? The three PPARs share a large overlap of target gene profile, but the ensuing phenotypes can be very different. This is probably best exemplified in the skeletal muscle and heart of transgenic mice. How are their distinct functions specified? Addressing these questions will be important for us to fully understand the roles of these receptors in energy metabolism and metabolic syndrome.

Acknowledgments

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