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PPARs and adipocyte function.

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Abstract:

For long viewed as passive lipid storage depots, adipocytes are now recognised as key players in the pathogenesis of insulin resistance and metabolic disease. In parallel, the last two decades of research have seen the emergence of transcription factors of the peroxisome proliferator-activated receptor (PPAR) family as central regulators of lipid and glucose homeostasis and molecular targets for drugs to treat hyper-lipidaemia and type 2 diabetes mellitus. In this review we discuss the characteristics of PPARs and the role of the different isotypes in adipocyte biology.

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Introduction

We are currently in the midst of a global obesity epidemic. Aside from being a social stigma, obesity causes or exacerbates many health problems. In particular, it is associated with the development of insulin resistance, linked (at least in part) to the development of glucose intolerance, hypertension, hyper-lipidaemia, and atherosclerosis - the so-called Metabolic Syndrome (MetS) [1]. For long viewed as passive vessels for lipid storage, adipocytes are now recognised as central players in the pathogenesis of insulin resistance and the MetS. First, they act as a 'safe' free fatty acid (FFA) storage depot, being able to accumulate large amounts of lipid in a manner that is non-toxic to the cell or whole organism. Exceeding the capacity of white adipose tissue (WAT) to store FFA as occurs in lipodystrophy or some types of obesity leads to extra-adipose lipid accumulation (in muscle, liver, and pancreas), lipotoxicity and insulin resistance [2]. Second, adipocytes secrete multiple hormones and cytokines termed 'adipokines' (e.g. leptin and adiponectin) which directly regulate whole-body insulin sensitivity. Deregulated adipokine secretion from the WAT of obese individuals also contributes to the development of systemic insulin resistance and metabolic disease [3]. Thus, dissecting the molecular pathways under-pinning adipocyte differentiation and function is fundamental in designing rational and effective therapies to prevent and treat the MetS. In this respect, over the past twenty years transcription factors of the peroxisome proliferator-activated receptor (PPAR) family have emerged as central regulators of lipid homeostasis and molecular targets for drugs to treat hyper-triglyceridaemia and type 2 diabetes mellitus. The parallel identification of PPAR γ as an important regulator of adipogenesis has added further fuel to interest in the role of PPARs in adipocyte biology which is the topic of this review.

The PPAR family

PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor super-family [4]. The name PPAR derives from the ability of the first identified family member, PPAR α , to induce peroxisome proliferation in rodent hepatocytes. This function is not shared by the two other PPAR homologues, namely PPAR γ and PPAR δ

(also known as PPAR β); instead, PPARs have emerged as major regulators of lipid and carbohydrate metabolism.

PPARs possess the classic domain structure of nuclear receptors [4]. Specifically, their N-terminal region displays a ligand-independent, weak trans-activation domain called activation function 1 (AF1). This is followed by a two zinc finger motif comprising DNA-binding domain. The C-terminal region contains the ligand-binding pocket and a ligand-dependent, major trans-activation domain, termed AF2. The C-terminus is also responsible for dimerisation with the retinoid X receptor (RXR) and for the docking of regulatory co-factor proteins (see below).

PPARs activate transcription by binding to specific DNA response elements termed PPAR response elements (PPREs) as obligate hetero-dimers with RXR. Ligand binding promotes a conformational change favouring recruitment of chromatin-decondensing co-activator and dismissal of co-repressor complexes. In contrast to other nuclear receptors, the binding properties of PPARs are rather promiscuous consequent to a ligand-binding pocket with markedly open conformation. Indeed, PPARs are activated by a wide range of naturally occurring or metabolised FFA and eicosanoid FFA derivatives. For instance oxidized FFAs [5] and prostaglandins [6, 7] are reported to bind and activate PPAR γ with micro-molar range affinity. Nitrated FFAs also potently activate PPAR γ at nano-molar concentrations [8]. Remarkably, a recent structural study has raised the intriguing idea that PPAR γ may simultaneously bind two different natural FFAs [9]. Whilst the studies described above utilised radio-ligand binding assays to demonstrate on the whole weak (i.e. micro-molar range) PPAR binding to endogenous ligands, such assays are known to underestimate the affinities of binding proteins for lipids. In this respect, more recent work utilising direct ligand binding assays indicates that unsaturated FFAs and fatty acyl CoAs are high affinity (nano-molar range) ligands of PPARs such as PPAR α [10, 11]. These observations have led to the suggestion that PPARs act as generic “lipid sensors” translating nutritional signals into metabolic responses. PPARs are also important pharmaco-therapeutic targets and specific synthetic ligands exist for the different isotypes. Thiazolidinediones (TZDs) are PPAR γ ligands currently in use for the

treatment of type 2 diabetes whilst fibrates are PPAR α agonists used in the management of hyper-lipidaemia. Apart from ligands the trans-activation potential of PPAR γ and PPAR α is regulated by phosphorylation [12, 13]. In addition, PPARs can negatively influence (e.g. pro-inflammatory) gene expression in a ligand-dependent manner through competition with other transcription factors. PPAR-mediated gene ‘trans-repression’ does not entail binding to PPREs and involves several proposed mechanisms. These include direct protein-protein interactions between PPARs and members of the nuclear factor kappa-B (NF- κ B) and activator protein-1 (AP-1) families, inhibition of mitogen-activated protein kinase activity, induction of inhibitor of NF- κ B (I κ B α) expression, competition for a limiting pool of co-activators, and (sumoylation-dependent) PPAR targeting to gene promoters where they act to block signal-dependent clearance of co-repressor complexes [14]. In the following paragraphs we discuss the characteristics of PPARs and their roles in adipocyte differentiation and function.

PPAR γ

Since its discovery in the nineties PPAR γ has emerged as the prime regulator of adipocyte differentiation. In addition, as already alluded to, it serves as the receptor for the TZD class of insulin sensitising drugs. PPAR γ is transcribed into three splice variants giving rise to two distinct protein isoforms. PPAR γ 1 and PPAR γ 3 transcripts give rise to the PPAR γ 1 protein. PPAR γ 2 mRNA encodes for a protein containing an additional 28 or 30 amino acids at its extreme N terminus (in mouse and human respectively). Under physiological conditions PPAR γ 2 protein is produced almost exclusively in WAT and brown adipose tissue (BAT). On the other hand, though also predominantly produced in adipose, PPAR γ 1 is additionally detected in other tissues including immune cells, intestine, kidney, and liver [15, 16].

PPAR γ and adipocyte biology

As already mentioned, adipocytes play a key role in the control of systemic glucose and lipid homeostasis and it is now established that PPAR γ is an important regulator of adipogenesis. In this respect no transcriptional regulator has been discovered that

promotes adipocyte differentiation in the absence of PPAR γ and thus other adipogenic factors must act (at least in part) by activating PPAR γ expression or activity. The importance of PPAR γ in adipogenesis has been demonstrated by extensive cell culture and *in vivo* studies. PPAR γ is induced during *in vitro* pre-adipocyte differentiation and its ectopic expression in non-adipogenic fibroblasts stimulates adipogenesis in the presence of PPAR γ ligands [17]. More compellingly, PPAR γ -null fibroblasts and embryonic stem cells are differentiation-incompetent *in vitro* [18, 19]. Likewise, dominant negative PPAR γ mutants inhibit adipogenesis in cultured pre-adipocytes [20].

In vivo loss-of-function studies with PPAR γ were made difficult by embryonic lethality among null embryos due to placental insufficiency [18, 21]. To circumvent this difficulty Rosen et al. [19] created chimeric mice and showed that PPAR γ -null cells were unable to contribute to the formation of adipocytes in these animals. These results were corroborated by Barak et al [21] which utilised a tetraploid-rescue approach that bypasses placental defects to generate a single PPAR γ -null pup that completely lacked WAT (and BAT). More recently, using Cre-Lox technology to inactivate PPAR γ in the embryo but not the trophoblasts, Duan et al. [22] also generated generalised PPAR γ deficient mice which similarly displayed severe lipodystrophy in addition to insulin resistance and surprisingly hypotension. Conditional knockout strategies have also allowed several groups to examine the consequences of adipose-selective ablation of PPAR γ thereby establishing its critical role in post-differentiation survival of mature white (and brown) adipocytes [23-25].

Naturally occurring PPAR γ mutations in human subjects also support an important role for this transcription factor in WAT development [26, 27]. Specifically, individuals carrying dominant-negative mutations in PPAR γ display partial lipodystrophy concomitant with insulin resistance, dys-lipidaemia, and hypertension. Interestingly, lipodystrophy in these subjects occurs in a stereotypic pattern, with preferential loss of subcutaneous WAT in the limbs and gluteal region and preserved subcutaneous and visceral abdominal fat. Likewise, the progressive lipodystrophy manifesting as a result of

targeted deletion of PPAR γ in adipose tissue occurs at an accelerated rate in subcutaneous compared to epididymal adipocytes [23]. Reciprocally, PPAR γ agonist treatment in humans leads to peripheral redistribution of WAT [27]. Finally, mice harbouring dominant negative PPAR γ mutations similarly exhibit altered fat pad distribution (albeit one characterised by decreased intra-abdominal relative to extra-abdominal WAT) [28, 29] indicating that PPAR γ also plays a role in determining WAT distribution.

In order to address the contribution of different PPAR γ isoforms in adipocyte differentiation, Ren and co-workers silenced expression of PPAR γ 1 and PPAR γ 2 in 3T3-L1 cells using zinc finger repressor proteins [30]. By employing this approach it was demonstrated that ectopic expression of PPAR γ 2 but not PPAR γ 1 is able to restore adipogenesis. However, experiments using PPAR γ null fibroblast cell lines have shown that both PPAR isoforms are competent to promote differentiation although PPAR γ 2 appears more potent [31]. That PPAR γ 1 harbours adipogenic activity is also supported by mouse models in which the PPAR γ 2 isoform was specifically (and globally) disrupted. Whilst these animals exhibit no [32] or significant impairment [33] in WAT development (depending on host genetic background and gender) the fact that adipose tissue is still able to form is testament to this conclusion.

PPAR γ is subject to various post-translational modifications which modulate its transcriptional activity and thereby ability to promote adipogenesis. Phosphorylation of PPAR γ 2 at serine residue 112 within the AF1 domain by growth factor (extra-cellular signal-regulated kinases 1/2) or stress-induced (p38/Jun NH₂-terminal kinase 1/2) kinases represses PPAR γ transcriptional activity [34]. Indeed, over-expression of a phosphorylation defective PPAR γ mutant (in which serine 112 is mutated to alanine [PPAR γ S112A]) in mouse NIH-3T3 fibroblasts or 3T3-L1 pre-adipocytes results in enhanced adipogenesis compared with wild-type protein [12, 35]. *In vivo*, homozygous PPAR γ S112A knock-in mice are protected from high-fat diet-induced insulin resistance and display decreased adipocyte size, elevated serum adiponectin, and reduced FFA levels [36]. PPAR γ also undergoes sumoylation at lysine 107 in the AF1 and lysine 395

in the AF2 region [37]. Sumoylation of the AF1 domain represses PPAR γ transcriptional activity and accordingly a sumoylation-defective PPAR γ 2 mutant (bearing a lysine 107 to arginine substitution) promoted adipogenesis more efficiently than wild-type PPAR γ 2 when ectopically expressed in NIH-3T3 cells [38-40]. In contrast, lysine 395 sumoylation is not involved in the regulation of direct PPAR γ target genes but rather in the trans-repression of inflammatory genes by PPAR γ in macrophages [41]. Finally, PPAR γ also undergoes poly-ubiquitination which is linked in turn to its proteasomal degradation. Interestingly, PPAR γ ubiquitination is positively linked to PPAR γ transcriptional activity [37].

In addition to being indispensable for adipose tissue development, PPAR γ also plays a key role in promoting FFA uptake and triglyceride storage in WAT. Accordingly, its expression is highest post-prandially and is down-regulated by fasting consistent with the stimulatory effect of insulin on PPAR γ expression [42, 43]. In the context of a high-fat diet, docking protein 1 (DOK1)-mediated insulin signalling also enhances PPAR γ activity by counteracting the inhibitory effect of extra-cellular signal-regulated kinase (ERK)-mediated PPAR γ phosphorylation (thereby promoting adipocyte hypertrophy). Activation of PPAR γ in adipocytes leads to up-regulation of genes that stimulate FFA release from lipoprotein-contained triglycerides (lipoprotein lipase), FFA uptake (fatty acid transport protein 1), intra-cellular FFA transport (fatty acid-binding protein 4), FFA activation (acyl-CoA synthase), and FFA esterification (phosphoenolpyruvate carboxykinase 1). In addition, by promoting the expression of the lipid droplet associated proteins fat-specific protein 27 (FSP27), cell death-inducing DFFA-like effector A (CIDEA), and perilipin PPAR γ promotes efficient storage of triglycerides in unilocular lipid droplets [44, 45]. Indeed, young (≤ 6 months) adipose-specific PPAR γ knock-out mice display grossly elevated plasma levels of FFA and triglycerides despite modest lipodystrophy [23, 25]. In addition, the WAT anti-lipolytic effect of insulin is blunted in these animals [23]. Similarly, in addition to WAT loss an associated decrease in the ability of WAT to store dietary lipids is thought to contribute significantly (and may even be pathogenetically more important) to the development of metabolic complications in patients with

dominant-negative PPAR γ mutations. In this respect, despite a fairly moderate degree of lipodystrophy these subjects often display extreme hyper-triglyceridaemia [27]. Additionally, direct measurement of WAT triglyceride trapping in an individual with the PPAR γ P467L mutation revealed this to be markedly reduced [46].

Whilst most extensively studied in models of WAT differentiation, PPAR γ is also indispensable for BAT development and function. In contrast to white fat cells which specialise in storing energy in the form of triglycerides, brown adipocytes function to catabolise FFA and dissipate energy in the form of heat. The thermogenic properties of BAT are conferred by its high mitochondrial density and fuel oxidation capacity, coupled with its exclusive expression of uncoupling protein 1 (UCP1), a mitochondrial proton transporter that uncouples respiration from oxidative phosphorylation. In physiological terms, BAT is thought to function in adaptive thermogenesis to provide defence against cold and protection against obesity. Until recently, brown fat was thought to be of metabolic importance only in smaller mammals and infant humans. Studies using PET scanning, however, have conclusively shown the existence of discrete, metabolically active BAT depots in adult humans [47-49]. Furthermore, these studies suggest that brown fat might make an important contribution to whole-body energy balance thus re-invigorating interest in therapeutic strategies aimed at promoting the amount and/or activity of brown adipocytes as a means of combating obesity and related metabolic disorders.

PPAR γ is abundantly expressed in adult BAT and during embryonic development appears exclusively in this tissue at a time when identifiable brown fat depots can first be observed in the embryo [50]. *In vitro*, PPAR γ expression is high in proliferating brown pre-adipocytes and increases further following induction of differentiation [50]. Furthermore, activation of PPAR γ by TZDs in the HIB-1B brown clonal pre-adipocyte cell line potently stimulates adipogenesis and UCP1 expression. PPAR γ is likewise able to drive BAT formation *in vivo* as evidenced by the ability of TZDs to increase BAT accumulation in rodents [50, 51]. Interestingly, PPAR γ agonists also induce UCP1 expression and “browning” of white cells following exposure either in culture or *in vivo*

[52]. Reciprocally, genetic animal models with generalised or adipose-selective PPAR γ ablation display deficient brown adipocyte development and survival [22, 24].

While undoubtedly important for BAT development, PPAR γ does not control determination of brown adipocyte fate. Instead, as shown recently (and conclusively) this function resides with the transcriptional regulator PRD1-BF1-RIZ1 homologous domain containing protein 16 (PRDM16) [53, 54]. Surprisingly, PRDM16 specifies the brown fat lineage from a progenitor that expresses myoblast markers and is not involved in white adipogenesis. PRDM16 functions to stimulate adipogenesis in these mesenchymal precursors by interacting with, and co-regulating the activity of other transcription factors and co-activators, including PPAR γ coactivator-1 α (PGC1 α) (see below). Crucially, (ligand-independent) binding to and co-activation of PPAR γ is essential for the adipogenic function of PRDM16 [53, 54].

In addition to interacting with PRDM16, PPAR γ also binds to and cooperates with PGC1 α to modulate brown adipocyte function [55, 56]. PGC1 α is selectively expressed in BAT (*vs.* WAT) and is critical in activating the thermogenic programme in brown adipocytes. PGC1 α binds to and co-activates PPAR γ in a ligand independent manner. This in turn initiates a broad program of mitochondrial gene expression, thermogenesis, and increased cellular respiration through PGC1 α co-activation of other transcription factors (e.g. PPAR δ and PPAR α) is also necessary to mediate these effects. Notably, members of the steroid receptor co-activator (SRC) family of transcriptional regulators influence brown fat development and function by modulating the interaction of PPAR γ with PGC1 α [56]. Additionally, the lysine 9 of histone H3 (H3K9)-specific demethylase JMJC domain-containing histone demethylase 2A (JHDM2A) was recently shown to contribute to β -adrenergic-mediated UCP1 activation in BAT by augmenting recruitment of PPAR γ , RXR and PGC1 α (along with SRC1 and p300) to the PPRE of the UCP1 enhancer [57]. Correspondingly, JHDM2A knockout mice displayed defective adaptive thermogenesis and cold-induced UCP1 induction was completely blocked in the BAT of

these animals. Furthermore, JHDM2A null mice became obese in adulthood and displayed dys-lipidaemia despite normal caloric intake.

PPAR γ and insulin resistance

Multiple experimental evidence supports the conclusion that TZDs exert their biological effects on insulin sensitivity through activation of PPAR γ . First, the clinical potencies of the various TZDs correlate closely with their *in vitro* potency in PPAR γ binding or trans-activation assays [58, 59]. Second, non-TZD PPAR γ agonists also improve insulin sensitivity *in vivo* with potencies that correlate with *in vitro* PPAR γ binding affinity [60]. Finally, as mentioned above, point mutations in the ligand or DNA-binding domain of PPAR γ are associated with severe insulin resistance in human subjects [26, 27]. The majority of data also supports the conclusion that WAT is the principal tissue responsible for the therapeutic effects of TZDs although extra-adipose actions also contribute to improved insulin sensitivity. Consistent with this, mice lacking adipose tissue are refractory to the glucose and insulin lowering TZD effects [61]. Furthermore, mice with adipose selective PPAR γ ablation are insulin resistant and deficient in their response to TZD administration [23]. Finally, TZDs retain their glucose-lowering potential in liver [62] and at least one muscle-specific PPAR γ knockout model [63], the two main organs of insulin-mediated glucose-disposal.

Multiple potential mechanisms have been proposed whereby PPAR γ activation in adipose tissue may improve insulin sensitivity (Fig. 1). One such mechanism is altered FFA partitioning [27]. Type 2 diabetes is associated with increased plasma FFA levels and inappropriate lipid deposition in extra-adipose sites including liver and skeletal muscle. Accumulation of FFA in these tissues promotes lipotoxicity and insulin resistance [2]. Activation of PPAR γ target gene expression in WAT is believed to enhance the capacity of adipose tissue to store dietary FFA by stimulating lipid trapping and storage as discussed earlier. PPAR γ agonists also increase adipose tissue mass *in vivo* by promoting *de novo* adipocyte differentiation [64]. As a consequence, FFA are safely partitioned into WAT and sequestered away from tissues where their accumulation could give have

detrimental effects on insulin action. Consistent with this hypothesis, TZDs effectively lower FFA levels in humans and rodents [27]. Furthermore, although protected from obesity leptin deficient (*ob/ob*) mice which lack PPAR γ 2 or carry a heterozygous dominant negative PPAR γ mutation (P465L) develop severe insulin resistance [28, 65].

Another potential mechanism whereby activation of PPAR γ in adipose tissue may impact whole-body insulin sensitivity is by altering the adipokine milieu. For instance, PPAR γ agonists inhibit the expression and/or secretion of tumour necrosis factor α (TNF α) and plasminogen activator inhibitor 1 (PAI1), both of which promote insulin resistance [66, 67]. Administration of TZDs in rodents also leads to a reduction in the number of adipose macrophages. WAT macrophage infiltration has been shown to be pathogenetically linked to the development of obesity-related insulin resistance through the induction of local and (by altering the WAT adipokine profile) systemic inflammation [68]. In parallel, PPAR γ agonists stimulate the production of adiponectin, a direct PPAR γ target gene in rodent and human adipocytes [69, 70]. Adiponectin promotes FFA oxidation and insulin sensitivity in muscle and liver via activation of AMP-activated protein kinase. Accordingly, mice lacking adiponectin show impaired responses to TZDs [71].

Finally, in humans PPAR γ agonist treatment also leads to WAT redistribution. Specifically, TZDs cause a preferential accretion of subcutaneous WAT with concomitant lack of change or reduction in the size of visceral depots [72]. Visceral obesity is more strongly linked to insulin resistance compared to gluteo-femoral obesity [73]. According to the “portal theory” this is consequent to visceral fat drainage (of FFA, adipokines, and cortisol) directly into the liver via the portal vein [74] although several recent studies also indicate that various WAT depots may be derived from distinct precursors with different metabolic properties [75].

PPAR δ

Despite its near-ubiquitous distribution in mammalian tissues PPAR δ (also called PPAR β) exerts powerful regulatory functions in adipose tissue metabolism and energy homeostasis as demonstrated by pharmacologic and genetic studies. *In vitro*, expression

of PPAR δ is induced during the early phase of white pre-adipocyte differentiation in murine clonal cell lines [76]. PPAR δ may serve to stimulate adipogenesis (perhaps in response to activation by long chain FFA) by inducing PPAR γ expression and promoting mitotic clonal expansion [77-79]. PPAR δ gene expression likewise increases during early brown adipogenesis [80]. While PPAR δ -deficient brown fat cells differentiate normally *in vitro* they display dramatically decreased expression of UCP1 and mitochondrial metabolic genes (in the context of normal PPAR γ , PPAR α , and PGC-1 α expression). Furthermore, they are almost completely unresponsive to β 3-adrenergic receptor agonist induction of UCP1 expression. This phenotype is remarkably similar to that of PGC-1 α acute knock-down [81].

In vivo, PPAR δ deficiency results in frequent (>90%) embryonic lethality due to placental defects. Surviving PPAR δ null mice are healthy and fertile and exhibit a striking (>60%) reduction in adiposity (uniformly affecting WAT and BAT depots) relative to wild-type animals [82]. This effect however, is not recapitulated in mice harbouring an adipose tissue-specific deletion of PPAR δ , suggesting that the phenotype is fat-non-autonomous and may evolve from perturbations in systemic lipid metabolism [82]. Alternatively, PPAR δ may exert its adipogenic action only during the early steps of differentiation which would be unaffected by the late deletion using the fatty acid-binding protein 4 (FABP4) promoter.

PPAR δ null mice also expend less energy and consequently display increased susceptibility to weight gain coupled with blunted BAT UCP1 expression on a high-fat diet [83]. Consistent with deficient adaptive thermogenesis, these animals also have compromised ability to maintain their body temperature during cold exposure [81]. In contrast, targeted activation of PPAR δ in adipose tissue specifically induces expression of genes required for FFA oxidation and energy dissipation in BAT and UCP1 expression in WAT thereby leading to reduced adiposity and improved lipid profiles. In addition to being lean, these animals are also resistant to both high-fat diet-induced and genetic (*db/db*) obesity, hyper-lipidaemia, and hepatic steatosis [83].

These genetic models collectively suggest that activation of PPAR δ protects against obesity by stimulating thermogenesis in adipose tissues. Importantly, PPAR δ ligands mimic these effects. Specifically, pharmacological activation of PPAR δ in 3T3-L1 (and skeletal muscle) cells enhanced FFA oxidation and utilisation [83]. *In vivo*, short term administration of the synthetic PPAR δ agonist GW501516 to genetically obese (*db/db*) mice reduced intracellular lipid accumulation in BAT and liver [83]. Most tantalisingly, PPAR δ ligands ameliorated diet-induced obesity (and insulin resistance) in high-fat diet-fed mice [84]. While short-term (4-month) treatment of obese rhesus monkeys with GW501516 did not affect body weight [85], acute (2-week) administration of the same compound to six healthy, male human subjects was associated with a weight-loss trend [86].

PPAR δ functions to enhance BAT metabolism by mediating, at least in part, the function of PGC1 α which constitutes the central regulator of BAT thermogenesis. Interestingly, TWIST1, a helix-loop-helix containing transcriptional regulator selectively expressed in adipose tissue was recently demonstrated to act as a negative-feedback regulator of PGC1 α /PPAR δ -mediated brown fat metabolism [81]. TWIST1 was shown to interact with PGC1 α , and to be recruited to PGC1 α 's target gene promoters to suppress mitochondrial oxidative metabolism and uncoupling (by promoting histone deacetylation). *In vivo*, transgenic mice expressing TWIST1 in adipose tissue were prone to high-fat-diet-induced obesity, whereas TWIST1 heterozygous knockout mice were obesity resistant. These phenotypes were consequent to altered mitochondrial metabolism and uncoupling in brown fat. Interestingly, PPAR δ was shown to induce TWIST1 expression (both *in vitro* and *in vivo*) in response to pharmacological activation, suggesting a negative-feedback regulatory mechanism [81].

PPAR α

PPAR α is predominantly expressed in the liver and to a lesser extent in the heart, skeletal muscle, small intestine, and kidney where it has a crucial role in controlling FFA

oxidation [87]. Although this transcription factor is expressed at low levels in WAT, its expression level in BAT is four times higher than in liver.

In vitro, the level of PPAR α in white adipocyte cell lines is similarly very low suggesting a limited role for this isotype during adipogenesis. Indeed, pharmacologic activation of endogenous PPAR α (by the specific agonist WY-14643) failed to promote adipocyte differentiation of 3T3-L1 cells [60]. Of note however, when co-expressed with PGC1 α PPAR α co-operatively induced expression of FFA oxidation enzymes and increased cellular palmitate oxidation rates in 3T3-L1 pre-adipocytes [88]. Exposure of rat primary adipocytes to bezafibrate likewise led to increased expression of genes involved in FFA uptake and mitochondrial β -oxidation coupled with enhanced palmitate oxidation [89]. On the other hand, whilst not detected in HIB-1B clonal adipocytes (irrespective of whether they are exposed to norepinephrine) PPAR α mRNA is expressed in differentiated primary murine brown adipocytes [80]. In contrast to PPAR γ and PPAR δ expression however, which is attained early (while cells do not show signs of adipocyte conversion) PPAR α mRNA becomes detectable and increases during terminal differentiation co-incident with UCP1 expression [80].

In vivo, WAT is normally developed in animals lacking the PPAR α gene [90]. However, these animals display spontaneous, sexually dimorphic, late onset obesity (despite stable caloric intake) which is associated with adipocyte hypertrophy [91]. Furthermore, they are prone to diet-induced obesity which paradoxically is associated with enhanced insulin sensitivity [92]. In contrast, clofibrate treatment was shown to lead to a 'browning' of retroperitoneal WAT as evidenced by enhanced UCP1 expression [93]. In parallel, pharmacologic PPAR α activation reduced adiposity in a variety of mouse models of diet-induced and genetic obesity. PPAR α agonist treatment also lowered hyper-insulinaemia and when present hyperglycaemia [94]. Conflicting these findings, however, transgenic over-expression of PPAR α in muscle whilst also protecting from obesity, was associated with peripheral insulin resistance and glucose intolerance [92].

PPAR α null mice also display normal BAT development. Furthermore, these animals are able to defend their core-temperature when exposed to cold and exhibit similar BAT UCP1 expression (both baseline and following cold exposure) to control mice [95]. Nonetheless, pharmacologic PPAR α activation induces UCP1 gene expression in primary brown adipocytes and BAT *in vivo* [93, 96]. Indeed, in transient transfection assays PPAR α expression vectors stimulated activity of reporter genes driven by UCP1 promoter constructs. In these studies PGC1 α [and CREB-binding protein (CBP)] synergistically co-activated PPAR α transcriptional activity [96]. In parallel PPAR α expression (and its nuclear translocation) was induced by cold exposure in rodent BAT [57, 97]. This effect is likely to be mediated (at least in part) by the H3K9-specific demethylase JHDM2A. Specifically, JHDM2A was shown to directly bind to the PPRE of PPAR α and reduce the level of dimethylation of lysine 9 of histone H3 in muscle cells. As expected PPAR α expression was reduced in JHDM2A knockout BAT and cold-induced PPAR α (and UCP1) induction was defective in these animals which displayed obesity (with normal caloric intake) and defective adaptive thermogenesis [57].

Conclusions

Abundant experimental and clinical data supports a crucial role for the PPAR transcription factor family in adipocyte biology. Whilst PPAR γ plays a key role in adipocyte differentiation and lipid/energy accrual, adipose PPAR δ and PPAR α are primarily involved in adaptive thermogenesis and lipid/energy utilisation (Fig. 2). Consequently, selective and potent PPAR α and/or PPAR δ agonists may, by (in part) augmenting BAT metabolism and inducing ‘browning’ of WAT, prove useful agents to treat obesity and related metabolic disorders. In this respect use of the PPAR δ agonist GW501516 has shown clinical promise in a small scale trial involving healthy male volunteers [86]. In parallel the current (and future) development of selective modulators of PPAR γ activity promises to provide novel therapies to treat insulin resistance without the concomitant side effects of weight gain, fluid retention, and potentially increased cardiovascular risk associated with the use of TZDs. Ultimately the success of these therapies depends on developing a better understanding of the physiology of PPARs and

the molecular networks whereby they modulate adipose (and whole body) lipid metabolism. Dissecting these pathways will be a challenging task. Such an undertaking however, is certain to lead to many exciting discoveries to parallel those of the last two decades.

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Figure legends

Figure 1. Mechanisms whereby TZD-mediated PPAR γ activation in adipose tissue improves insulin sensitivity.

Figure 2. Effects of physiologic and pharmacologic PPAR activation on (A) white and (B) brown adipocyte biology. Actions of the different PPAR isoforms are indicated in different colours. MSC, mesenchymal stem cell; FFA, free fatty acids; UCP1, uncoupling protein 1.

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Figure 1

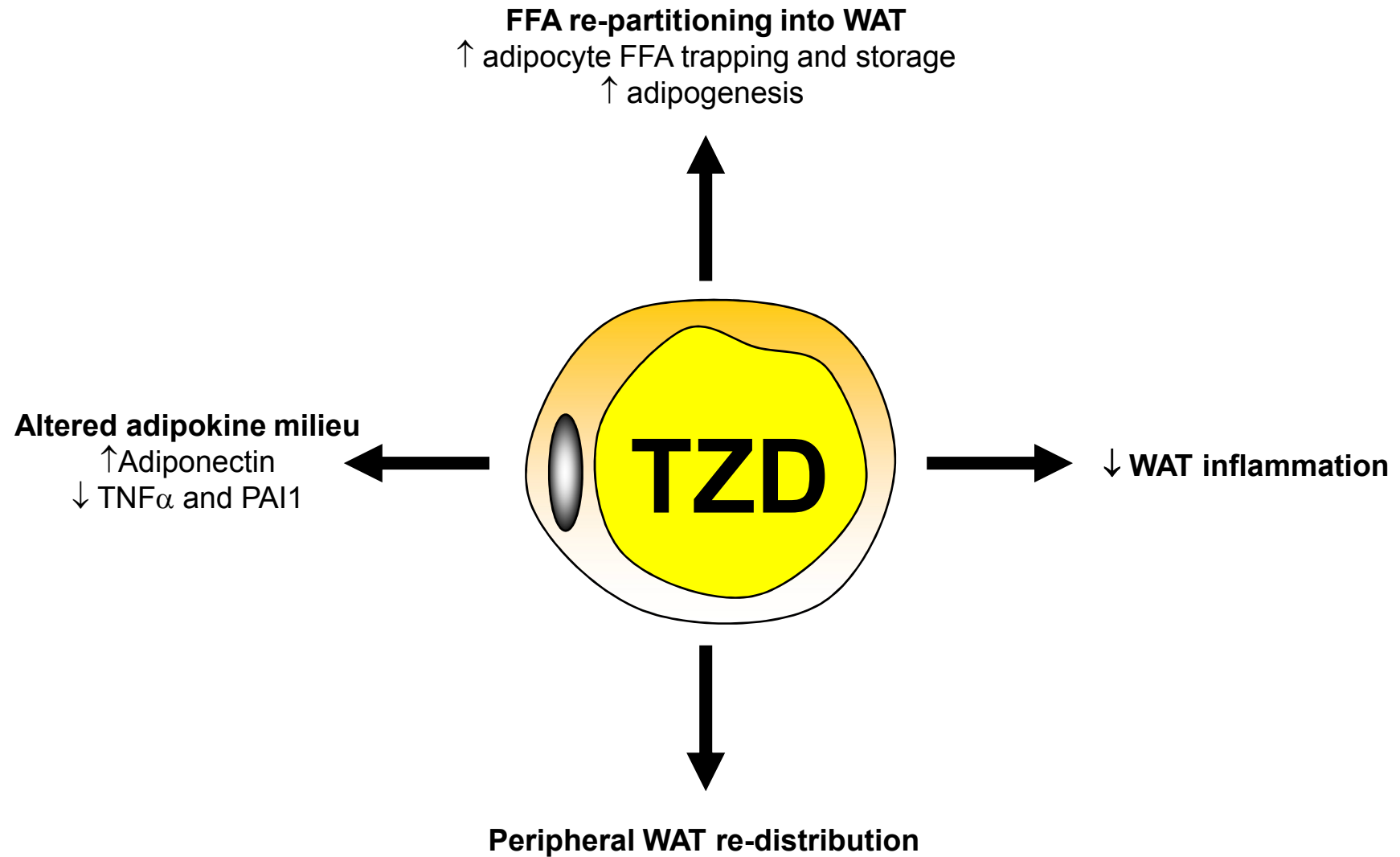
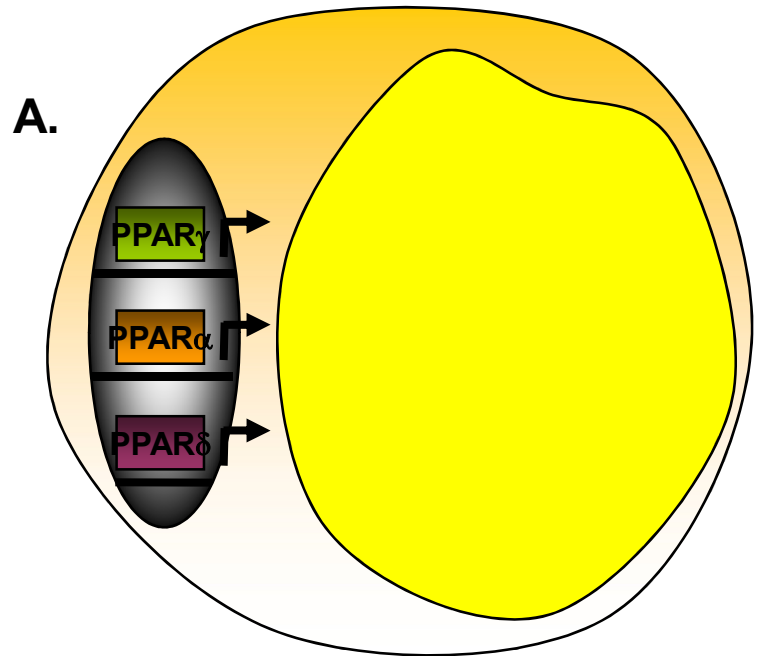


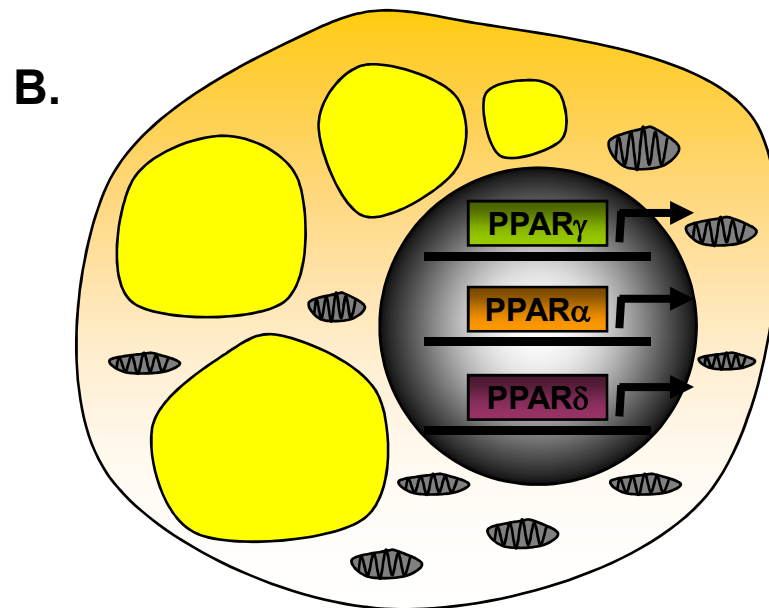
Figure 2



MSC fate determination
 Adipocyte differentiation and survival
 FFA trapping and efficient storage
 Mitochondrial biogenesis, FFA oxidation and uncoupling
 Peripheral adipose distribution

FFA oxidation and uncoupling

? Adipogenesis
 UCP1 expression



Adipogenesis
 Adaptive thermogenesis

UCP1 expression

Adaptive thermogenesis