Peroxisome proliferator-activated receptor- γ : too much of a good thing causes harm

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The nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) helps to translate 'what you eat' into 'what you are' because it allows dietary fatty acids (PPAR γ ligands) to modulate gene transcription. Treatments for diabetes include PPAR γ activators, as they sensitize the body to insulin. Our understanding of PPAR γ function has recently been enhanced by a flurry of human and mouse genetic studies, and the characterization of new PPAR γ ligands. This insight has led us to propose that modulating PPAR γ activity, rather than activating it, might be the most effective strategy for treating metabolic disorders, as this will improve glucose homeostasis while preventing adipogenesis.

Keywords: atherosclerosis; integrated metabolism; longevity; mouse models; obesity

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Introduction

The nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) is a transcription factor that is crucial for whole-body energy homeostasis and adipogenesis (reviewed in Auwerx, 1999; Rosen & Spiegelman, 2001). The actions of PPAR γ are mediated by two protein isoforms: the widely expressed PPAR γ 1 and the adipose tissue-restricted PPAR γ 2. The activity of PPAR γ is governed by the binding of small lipophilic ligands—mainly fatty acids—that are derived from nutrition or metabolism, and the activation of PPAR γ leads to adipocyte differentiation and fatty-acid storage. The modern westernized lifestyle—characterized by a high caloric intake and a lack of physical exercise—exposes people to prolonged chronic levels of fatty-acid-like PPAR γ ligands, which, through a feed-forward pathway, often results in obesity (Fig 1) (Burdge *et al*, 2003; Friedman, 2003; Sanders, 2003). Obesity is more prevalent in affluent societies, along with associated metabolic diseases such as hyperlipidaemia, insulin resistance, type 2 diabetes and cardiovascular diseases, which constitute a heavy social and economic burden. It therefore seems ironic that synthetic PPAR γ ligands, such as thiazolidinediones (TZDs), are used to treat dia-

betes because they sensitize the body to insulin; their down side is that they also promote fat accretion, and the long-term consequences of this are unknown. Moreover, the mechanism by which TZDs act and the reasons why they are effective are still not understood. It is therefore essential that these pathways be clearly defined to pave the way for better treatments for these metabolic diseases. Now that recent data have shed more light on the roles of PPAR γ , we propose that insulin sensitization without the accompanying increase in fat deposition might be possible through the controlled regulation of PPAR γ activity.

PPAR γ : a thrifty transcription factor

Four lines of evidence have established PPAR γ as the centrepiece of a feed-forward pathway that favours energy storage by adipocytes and coordinates a 'thrifty response' that once had substantial evolutionary benefits.

The first line of evidence comprises molecular and cellular studies. Early observations showed that the expression of PPAR γ in cells is sufficient to induce adipocyte differentiation (Tontonoz *et al*, 1994). Moreover, targeted mutagenesis of the PPAR γ gene in embryonic stem (ES) cells and knocking down the endogenous PPAR γ 2 in cell lines confirmed the role of PPAR γ in adipocyte differentiation (Rosen *et al*, 1999; Ren *et al*, 2002). Consistent with this, PPAR γ has been shown to increase the expression of genes that promote fatty-acid storage, whereas it represses genes that induce lipolysis and the release of free fatty acids (FFAs) in adipocytes.

Second are human genetic studies. The role of PPAR γ in adipogenesis has been underscored by studies that link the *PPARG* locus on chromosome 3p25–p24 with obesity in Pima Indians (Norman *et al*, 1997). The partial 'loss of function' of the PPAR γ 2-specific Pro12Ala mutation is associated with a decrease in body mass index (BMI), and improvement in insulin sensitivity and lipid profile (Deeb *et al*, 1998; Altshuler *et al*, 2000) (Fig 2). This association between the hypomorphic Ala substitution and insulin sensitivity disappears when the data are corrected for BMI, which indicates a primary effect on body-fat mass accretion (Deeb *et al*, 1998). By contrast, the rare Pro115Gln substitution renders PPAR γ constitutively active and carriers of this mutation are obese but remain insulin sensitive (Hu *et al*, 1996; Ristow *et al*, 1998). The dominant-negative/loss-of-function mutations Phe388Leu, Pro467Leu and Arg425Cys have been associated with partial lipodystrophy, resulting in a loss of fat from some areas of the

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Fig 1 | The balancing act of lipids and tissues in energy homeostasis. Interactions between the main metabolic tissues that store (adipose tissue) and oxidize (skeletal muscle and liver) free fatty acids (FFAs). These interactions are mediated by FFAs and adipokines. Adipokines are subdivided into insulin sensitizing (for example, adiponectin) and insulin resistance (tumour necrosis factor- α) adipokines. The thickness of the arrows reflects the strength of the effects. Three conditions are compared: normal, lipoatrophy and obesity. Both lipoatrophy and obesity are characterized by insulin resistance (IR), which is, however, mediated by different signalling factors.

body (Agarwal & Garg, 2002; Combs *et al*, 2002; Hegele *et al*, 2002; Savage *et al*, 2003), and with severe insulin resistance, diabetes and hypertension (Barroso *et al*, 1999). Overall, PPAR γ activity in humans corresponds directly to adipose mass and not necessarily to insulin sensitivity (Fig 2), which indicates that only part of the effect of PPAR γ on glucose homeostasis is dependent on white adipose tissue (WAT).

Third are mouse genetic studies. Ppar γ ^{-/-} mice that are generated by conventional gene targeting die *in utero* owing to placental and cardiac defects. Although one such Ppar γ ^{-/-} animal was rescued by tetraploid aggregation, it died within days as a result of severe lipodystrophy (Barak *et al*, 1999). This example, together with the characterization of mice that are chimeric for Ppar γ ^{-/-} ES cells (Rosen *et al*, 1999), show the importance of PPAR γ in adipose-tissue development *in vivo*. Interestingly, heterozygous Ppar γ ^{+/-} mice are resistant to the obesity and insulin resistance that is induced by a high-fat diet (Kubota *et al*, 1999; Miles *et al*, 2000). To overcome embryonic lethality, mice with tissue-specific deletions of Ppar γ have been generated to help determine its tissue-specific activities (summarized in Table 1). In contrast to adipose tissue, muscle and liver (discussed below), the deletion of Ppar γ in the pancreas did not result in a metabolic phenotype, but it underscored the antiproliferative role of Ppar γ (Rosen *et al*, 2003).

The specific reduction of PPAR γ in the adipose tissue showed the essential role of PPAR γ in adipogenesis and also highlighted its role in maintaining the integrity and function of the mature adipocyte (He *et al*, 2003; Koutnikova *et al*, 2003). Moreover, the essential role of adipose tissue in whole-body metabolism was exemplified by the significant mortality rate (>40%) of the WAT-specific hypomorphic Ppar γ knockdown mice (PPAR γ ^{hyp/hyp}), which were severely lipodystrophic (Koutnikova *et al*, 2003). When an aP2-driven Cre recombinase transgene was used to excise PPAR γ from the mature adipocytes, a more moderate reduction of adipose mass was observed, which was accompanied by hyperlipidaemia and liver steatosis (He *et al*, 2003). This was in contrast to the surviving adult PPAR γ ^{hyp/hyp} mice, which did not have liver steatosis or dyslipidaemia (Koutnikova *et al*, 2003). Liver steatosis and dyslipidaemia

were predominantly prevented in the Ppar γ ^{hyp/hyp} mice by the efficient oxidation of excess lipids in the muscle by Ppar α and Ppar β/δ . Intriguingly, both adipose Ppar γ -deficient models had relatively normal glucose tolerance (He *et al*, 2003; Koutnikova *et al*, 2003).

An abnormal lipid balance was a constant feature of all tissue-specific knockout models. As expected, when Ppar γ was absent in the adipose tissue, FFAs in the postprandial state were elevated (He *et al*, 2003; Koutnikova *et al*, 2003). Lipid balance was also significantly altered when either muscle or liver Ppar γ was obliterated. Deletion of Ppar γ in the livers of two mouse models with significant steatosis (leptin-deficient *ob/ob* or lipodystrophic A-ZIP/F-1 mice) reduced the triglyceride content of the liver, although it elevated serum FFAs and lipoproteins and induced insulin resistance, which illustrates the role of Ppar γ in liver lipogenesis (Gavrilova *et al*, 2003; Matsusue *et al*, 2003). Postprandial FFAs and serum lipids were also increased in mice lacking Ppar γ specifically in the muscle. These mice had increased adiposity and developed insulin resistance (Hevener *et al*, 2003; Norris *et al*, 2003). It therefore seems that muscle Ppar γ directly or indirectly induces FFA clearance by adipose tissue and FFA combustion in muscle. Ppar γ expression varies significantly between these three metabolic tissues, with Ppar γ expressed predominantly in the adipose tissue, moderately in the liver and only at low levels in the muscle. It is therefore fascinating that, irrespective of the level of Ppar γ expression in each of these tissues, its absence in any of them has an effect on whole-body lipid homeostasis and insulin sensitivity. Each tissue-specific Ppar γ mouse model shows the *in vivo* repartitioning of lipids between metabolic tissues, which illustrates the cross-talk between the liver, adipose and muscle to maintain energy homeostasis. This is, in part, owing to the adaptation of Ppar γ in the non-targeted tissues and by the other Ppar isoforms, Ppar α and Ppar β/δ , which enhance fatty-acid oxidation to minimize hyperlipidaemia and the resulting insulin resistance.

The complete absence of adipose tissue in Ppar γ ^{hyp/hyp} mice established that adipose tissue is crucial for the insulin-sensitizing effects of TZDs, as TZD treatment of these mice ameliorated the glucose intolerance but not the insulin resistance (Koutnikova *et al*, 2003). By contrast, mice with liver or muscle deletions of Ppar γ

Fig 2 | Genetic and pharmacological evidence supporting the relationship of peroxisome-proliferator-activated receptor- γ (PPAR γ) with adipogenesis and insulin sensitivity. Human mutations, mouse models and pharmacological studies show that the level of PPAR γ activity directly corresponds to adipose mass (adipogenesis). By contrast, insulin sensitivity can be achieved by both the inhibition and activation of PPAR γ , as illustrated by human genetic and pharmacological studies.

responded to TZD treatment by reducing serum lipid levels, and by normalizing plasma glucose and insulin levels (Gavrilova *et al*, 2003; Hevener *et al*, 2003; Matsusue *et al*, 2003; Norris *et al*, 2003), which indicates that the muscle and liver Ppar γ are not essential for this response. Despite this, the characterization of mice with a muscle-specific deletion of Ppar γ showed that muscle Ppar γ is important for insulin-stimulated glucose disposal (Hevener *et al*, 2003; Norris *et al*, 2003). Studies with Ppar γ obliterated in the liver showed that, in the presence of WAT, the impact of Ppar γ on glucose homeostasis was minimal. However, in the absence of WAT, the ability of TZDs to lower triglycerides and glucose was dependent on liver Ppar γ (Gavrilova *et al*, 2003). The fact that in this ‘fatless’ model the lowering of FFAs in response to TZDs was not dependent on liver Ppar γ further points to a role for muscle Ppar γ in FFA combustion. Ppar γ in each of these metabolic tissues therefore contributes, in part, to maintaining whole-body insulin sensitivity. Taken together, these results indicate that WAT, rather than liver and muscle, is predominantly required for the insulin-sensitizing effects of Ppar γ (Gavrilova *et al*, 2003; He *et al*, 2003; Koutnikova *et al*, 2003; Matsusue *et al*,

2003; Norris *et al*, 2003). Therefore, Ppar γ is not only the master regulator of adipogenesis *in vivo*, but also a driving force of glucose and lipid homeostasis. The knowledge obtained from the tissue-specific deletions of Ppar γ will be further refined by the generation of mouse models that carry specific Ppar γ mutations, such as the recently described knock-in of Ala at position 112 (S112A). This mouse, in which Ppar γ is constitutively active (similar to the human Pro115Gln mutation), retains insulin sensitivity during diet-induced obesity (Rangwala *et al*, 2003).

The fourth and final line of evidence comprises pharmacological studies. PPAR γ binds multiple ligands that can modulate its activity and induce a range of activities from full inhibition (antagonist) to activation (agonist) (Fig 2). The widely differing effects of various PPAR γ ligands are attributed to differential cofactor recruitment (Rocchi *et al*, 2001). Moreover, selective PPAR modulators (SPPARMs) are compounds that activate or inactivate the PPAR isoforms in a tissue-specific manner. Full PPAR γ agonists, such as the TZDs, improve insulin sensitivity, glucose tolerance and the lipidemic profile in type 2 diabetic patients. TZDs increase WAT mass, redistribute WAT from visceral to subcutaneous deposits and induce the appearance of small, newly differentiated adipocytes (reviewed in Picard & Auwerx, 2002). The observation that PPAR γ agonists increase fat mass and improve glucose control supports the theory that WAT mediates some of their effects on glucose homeostasis. Besides FFAs, WAT secretes several adipokines, such as TNF α , leptin, resistin and adiponectin, which affect insulin signalling in other tissues (reviewed in Aldhahi & Hamdy, 2003). Whereas enhanced PPAR γ activity is invariably associated with increased fat mass, sub-optimal PPAR γ activation or PPAR γ antagonism is neutral or even reverses weight gain. This principle is illustrated by the binding of the partial agonist FMOC-L-Leu to PPAR γ , which induces the differential recruitment of co-regulators to PPAR γ such that glucose levels are still lowered but there is no weight gain (Rocchi *et al*, 2001). Other weak or partial PPAR γ agonists, such as NC-2100 (Fukui *et al*, 2000) and MCC-555 (Reginato *et al*, 1998), also have little effect on adipocyte differentiation, but have potent antidiabetic activities. Partial inhibition of either PPAR γ or its heterodimerization partner the retinoid X receptor (RXR) by antagonists (Mukherjee *et al*, 2000; Yamauchi *et al*, 2001; Rieusset *et al*, 2002) also improves insulin

Table 1 | Comparison of PPAR γ tissue-specific knockout mouse models

	Adipose	Muscle	Liver	Pancreas
Adiposity	Congenital lipodystrophy ¹ Progressive lipodystrophy ²	Normal ³ ↑adipose mass ⁴	Progressive obesity ⁵ Normal ⁶	Normal
Plasma profile	↑glucose, ↑insulin, ↑TG, ↑FFA ¹ Normal glucose, insulin, ↑TG, ↑FFA ²	Normal glucose, insulin ³ Normal glucose, ↑insulin, ↑TG, ↑FFA ⁴	↑glucose, ↑TG, ↑insulin ⁵ Normal glucose ⁶	Normal
Adipokine production	↓leptin, ↓adiponectin ^{1,2}	Normal leptin ³ ↑leptin, ↓adiponectin ⁴	↑leptin, ↓adiponectin ⁵	N/D
Liver steatosis	Normal liver TG ¹ Steatosis ²	Normal liver TG ³ Steatosis ⁴	Normal liver TG ^{5,6}	Normal
Insulin resistance	IR ^{1,2}	IR ^{3,4}	IR ⁵ No IR ⁶	Normal
Background (age in months)	Mixed, SV129 & C57BL/6J(5) ¹ or C57BL/6J (14) ²	Mixed (12) ³ or C57BL/6J (14) ⁴	Mixed (40) ⁵ or mixed (10) ⁶	Mixed (13)
References	¹ Koutnikova <i>et al</i> , 2003 ² He <i>et al</i> , 2003	³ Norris <i>et al</i> , 2003 ⁴ Hevener <i>et al</i> , 2003	⁵ Gavrilova <i>et al</i> , 2003 ⁶ Matsusue <i>et al</i> , 2003	Rosen <i>et al</i> , 2003

The different mouse models have various age- and diet-dependent responses; therefore, for simplicity, the descriptions are for the adult phenotype in the postprandial state on a chow diet. FFA, free fatty acid; IR, insulin resistance; N/D, not determined; TG, triglycerides.

sensitivity, which is consistent with human and mouse genetic studies. Taken together with the mouse models of ablated PPAR γ expression in metabolic tissues and human mutational analysis, these pharmacological studies show that PPAR γ activity corresponds directly to adiposity in a linear fashion (Fig 2). However, unlike the relationship between PPAR γ and fat mass, PPAR γ activity is not linearly related to insulin sensitivity, as both inactivation and activation of PPAR γ can enhance insulin sensitivity (Fig 2). This nonlinear relationship indicates that insulin sensitivity is an integrated effect, which is achieved predominantly by modulating PPAR γ actions in the adipose tissue with effects on adipokine secretion and lipid storage, in addition to other tissue-specific PPAR γ responses, as shown by tissue-specific PPAR γ -obliterated models.

PPAR γ : from inflammation to atherosclerosis

In addition to these thrifty activities in WAT, other PPAR γ activities might also be beneficial, such as its role in modulating lipid homeostasis in arterial macrophages. Macrophage uptake of atherogenic lipoproteins in the arterial wall results in cholesteryl ester deposition and foam-cell formation, which are hallmarks of early and late atherosclerosis. PPAR γ targets both lipoprotein uptake and cholesterol efflux, which are two competing processes that are involved in macrophage lipid homeostasis. PPAR γ activation induces expression of the scavenger receptor CD36, thereby promoting oxidized low-density lipoprotein (oxLDL) uptake and foam-cell formation. In addition to the acquisition of cholesterol, however, macrophage uptake of oxLDL provides the cell with naturally occurring PPAR γ ligands, thereby promoting further PPAR γ activation and CD36 upregulation. Such a feed-forward cycle predicts that PPAR γ is predominantly pro-atherogenic (Nagy *et al*, 1998; Tontonoz *et al*, 1998). PPAR γ activation, however, might also be anti-atherogenic by reducing the expression of another scavenger receptor, scavenger receptor type AI/II (Moore *et al*, 2001), and by decreasing inflammatory cytokine production by macrophages (Jiang *et al*, 1998; Marx *et al*, 1998; Ricote *et al*, 1998). In addition to its effect on lipoprotein uptake, PPAR γ activation promotes the removal of cholesterol from macrophages through enhancing the cholesterol efflux mediated by ATP-binding cassette transporter A1 (ABCA1). This stimulates high-density lipoprotein (HDL) formation and reverse cholesterol transport. The expression of ABCA1 is tightly regulated by cellular cholesterol content through the oxysterol-dependent activation of another nuclear receptor, the liver X receptor (LXR). PPAR γ has been shown to activate ABCA1 expression indirectly through enhanced transcription of LXR (Chawla *et al*, 2001; Claudel *et al*, 2001).

These data emphasize the potential involvement of PPAR γ in the pathogenesis of atherosclerosis, which was underscored in humans by the association of the PPAR γ Pro12Ala polymorphism with protection from coronary heart disease (Ridker *et al*, 2003). The efficacy of PPAR γ agonists in reducing atherosclerosis in mice (Chen *et al*, 2001; Claudel *et al*, 2001; Collins *et al*, 2001; Li *et al*, 2000) and exerting vasculoprotective effects in humans (Takagi *et al*, 2000; Koshiyama *et al*, 2001; Sekiya *et al*, 2001; de Dios *et al*, 2003) can be attributed to its effects on both inflammation and cholesterol efflux. Further studies are required to define how much these effects are due to the beneficial systemic metabolic effects of PPAR γ versus its vascular and immune effects, which themselves might be indirect and mediated through LXR. It is also interesting to speculate that the increased frequency of atherosclerosis could be a PPAR γ -driven maladapted macrophage response that occurs when the inherent

beneficial effects (stimulation of the innate immune response and cholesterol efflux) are overwhelmed by the pro-atherogenic effects (increased oxLDL uptake). Such conditions are probably created by the chronic overload of lipids that is associated with an affluent westernized lifestyle.

PPAR γ , caloric restriction, fat and ageing

From an evolutionary perspective, a 'thrifty response' clearly favours survival. However, caloric restriction, which refers to a dietary regimen that is low in calories without undernutrition, is also known to extend lifespan in species ranging from yeast to non-human primates (Weindruch, 1988; Sohal & Weindruch, 1996; Lane *et al*, 2001) (Fig 3). The beneficial effects of caloric restriction have been associated with alterations in metabolism, particularly the insulin/insulin-like growth factor 1 (IGF-1) pathways, and a decreased fat mass (Weindruch, 1988). These pathways converge on the FOXO forkhead transcription factors, which are activated when insulin/IGF-1 signalling decreases, and this translates ultimately into increased stress resistance, which is the hallmark of caloric restriction (Guarente, 2000; Nemoto & Finkel, 2002; Tran *et al*, 2002). The most plausible hypothesis to explain the anti-ageing effects of caloric restriction states that the reduced flow of carbon through the glycolytic pathways slows down the conversion of NAD $^{+}$ to NADH, which is recognized by neuroendocrine sensors that ultimately

Fig 3 | The effect of caloric restriction on energy metabolism translates into longevity. Caloric restriction decreases energy levels, which leads to the activation of a signalling cascade to enhance longevity. Decreased glucose intake reduces the flow of carbon through the glycolytic pathway and slows down the conversion of ADP to ATP, which eventually alters the NAD $^{+}$:NADH ratio. SIRT1, which is a NAD $^{+}$ -regulated chromatin deacetylase, prolongs lifespan in response to caloric restriction in lower organisms. Also, signalling by insulin/insulin-like growth factor 1 (IGF-1) is attenuated under these conditions, which allows the FOXO forkhead transcription factors to increase stress resistance (pro-ageing). As extremes in fat mass are inversely related to lifespan, this indicates that PPAR γ could also affect longevity, although the mechanisms are not yet established.

reduce the production of growth hormone by the pituitary (and, in turn, IGF-1) and of insulin by the pancreas (Guarente, 2000). It has been proposed that the sirtuins, which are a family of NAD⁺-regulated protein deacetylases, have a role in these signalling events (Koubova & Guarente, 2003). The importance of insulin/IGF-1 signalling in longevity is supported by the extended lifespan of humans, mice, *Caenorhabditis elegans* and *Drosophila* that carry mutations in the insulin/growth hormone/IGF-1/FOXO signalling pathways (Bluhner *et al*, 2003; and reviewed in Koubova & Guarente, 2003; Tatar *et al*, 2003). In contrast to caloric restriction, mice with increased fat mass have a shorter lifespan (Harrison *et al*, 1984; Smith *et al*, 1991). It is, at present, unclear how PPAR γ interfaces with this signalling pathway, but the fact that PPAR γ coordinates adipogenesis and glucose homeostasis leads us to speculate that it might also affect longevity (Fig 3).

Too much of a good thing

Collectively, these data indicate that moderate levels of PPAR γ activation coordinate an evolutionarily beneficial and adaptive response (Auwerx, 1999). Throughout human evolution, longevity has been improved because efficient energy conservation and storage has allowed survival through periods of food shortages, whereas an enhanced innate immune response combated infections, uncontrolled cell proliferation and cancers. Our present affluent lifestyle, which exposes us to excessive levels of natural PPAR γ activators, throws this tightly regulated system out of balance. Being born with a 'silver spoon in your mouth' (that is, enjoying an affluent and often sedentary lifestyle) now turns this once favourable energy conservation response into a detrimental one, which contributes to the pathogenesis of lifestyle-associated diseases, such as obesity, type 2 diabetes and atherosclerosis. This also indicates that modulating (or inhibiting) PPAR γ activity, rather than activating it, might be the preferred therapeutic strategy to treat metabolic disorders, in order to improve glucose homeostasis yet prevent adipogenesis.

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