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Peroxisome proliferator activated receptors and obesity

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Abstract

The peroxisome proliferator activated receptors (PPARs) are a group of ligand-activated transcription factors that govern numerous biological processes, including energy metabolism, cell proliferation, and inflammation. Three different PPAR isotypes can be distinguished: alpha, beta and gamma. PPARα is mainly present in liver where it has an important role in the regulation of nutrient metabolism, including fatty acid oxidation, gluconeogenesis, and amino acid metabolism. It mediates the effects of fibrates, which are drugs used in the treatment of hyperlipidemia, on DNA transcription. Little is still known about PPARβ. The PPARγ isotype is mainly expressed in adipose tissue where it stimulates adipogenesis and lipogenesis. It is the target of a group of anti-diabetic drugs called thiazolidinediones. As PPARs have a very important role in the regulation of energy metabolism, and as their activity can be modulated by drugs, there is an increasing interest in the potential connection between PPARs and obesity. In this article, the diverse pieces of evidence that have linked PPARs with obesity are reviewed. Furthermore, the association between PPARs and type 2 diabetes is discussed.

Keywords: PPAR (peroxisome proliferator activated receptor); Fibrate; Thiazolidinedione; Steatosis; Adipogenesis; Lipogenesis; Nuclear hormone receptor; Fatty acid oxidation; Fasting

1. Introduction

In most industrialized countries, the prevalence of obesity continues to rise, despite major efforts to reverse this trend. Obesity is also striking at a much earlier age, and since excess weight during childhood invariably leads to adult obesity, it is expected that unless major action will be taken, in less than two decades most of the adult population in the US and several other countries will be obese. Apart from cosmetic reasons, obesity is undesirable because it increases the risk for numerous chronic diseases. Indeed, obesity hardly occurs in isolation but is most often part of an array of metabolic abnormalities that are collected in the term syndrome X. This includes hypertension, hyperinsulinemia/insulin resistance, hypertriglyceridemia, low plasma HDL and hyperuricemia.

Obesity is caused by an imbalance between energy intake and energy expenditure, resulting in a positive energy balance and associated weight gain. Common wisdom tells us that obesity can be easily prevented or treated by lowering food intake combined with increasing energy expenditure. Although theoretically this is supposed to be true, the extremely low success rate of dieting as a means to lose bodyweight and the general aversion towards physical work attest to the difficulty of translating this simple concept into real life. Inasmuch as efforts to change people's dietary habits have failed to yield the desired effect, pharmacological and surgical approaches to treat obesity have become increasingly popular.

Together with the G-protein coupled receptors, the superfamily of nuclear hormone receptors is considered as one of the major targets for drug development. Currently, nuclear hormone receptors are already targeted in the treatment of a variety of conditions such as birth control, cancer, inflammatory diseases, and diabetes. A small group of nuclear hormone receptors that may be of interest for the treatment of obesity is the so-called peroxisome proliferator activated receptors (PPARs). These receptors bind to and are activated by a number of drugs used in the treatment of hyperlipidemia and type 2 diabetes. This review will be devoted exclusively to this highly interesting set of receptors, and will concentrate on the link between PPARs and obesity.

1.1. Peroxisome proliferator activated receptors

The term peroxisome proliferator activated receptors was first coined in 1990 when the first member of the PPAR

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family, PPARα, was discovered (Issemann and Green, 1990). During those heydays of nuclear receptor discovery, PPARα was identified as the receptor that activates DNA transcription in response to a diverse group of compounds called peroxisome proliferators. Certain pesticides, phthalates, and hypolipidemic drugs belong to this category. The term PPAR should have quickly fallen out of grace after two close relatives of PPARα, namely, PPARβ and PPARγ, were identified, neither of which is activated by peroxisome proliferators (Schmidt et al., 1992; Amri et al., 1995; Dreyer et al., 1992; Kliewer et al., 1994; Tontonoz et al., 1994; Zhu et al., 1993). Unfortunately, the names have persisted so the scientific community is now stuck with a rather inaccurate nomenclature.

1.2. Structure and function of PPARs

PPARs are members of the nuclear hormone receptor superfamily, a group of nuclear proteins that mediate the effects of small lipophilic compounds, such as steroids, retinoids, bile acids, and fatty acids, on DNA transcription. They are grouped together based on a common structural motif consisting of a central DNA-binding domain containing two zinc-fingers, and a large C-terminal domain that binds ligand (Aranda and Pascual, 2001). Inasmuch as these receptors govern numerous important biological processes, and as their activity can be influenced by small molecules, nuclear hormone receptors are considered as prime targets for drug development.

As mentioned above, three different PPARs can be distinguished: alpha, beta, and gamma. The PPARbeta isotype is present in numerous tissues, yet its function has largely remained an enigma. It has been proposed to be an intermediate in the tumorigenic pathway of the adenomatous polyposis coli (APC) gene, which when mutated causes the disease familial colorectal polyposis (He et al., 1999). In addition, there is evidence that PPARβ functions as a receptor for prostacyclin in blastocyst implantation (Lim and Dey, 2000). Finally, PPARβ has been implicated in the regulation of lipid metabolism in nerve cells (Basu-Modak et al., 1999) and has been proposed to be the mediator of fatty acid-controlled differentiation of preadipose cells (Amri et al., 1995; Bastie et al., 1999, 2000). The latter function suggests that PPARβ is implicated in adipogenesis and thus may be connected with obesity. However, as only a very limited number of reports have studied the role of PPARβ in adipose tissue, the possible link between PPARβ and obesity will not further be explored in this review. The PPARα isotype is mostly expressed in organs with a high rate of fatty acid catabolism, such as brown adipose tissue, liver, kidney and heart (Braissant et al., 1996). It plays an important role in the regulation of intermediary metabolism, which has been very well studied in liver. There is some evidence connecting PPARα to obesity (see below). The last and also the most studied PPAR isotype, PPARγ, is mainly present in adipose tissue, colon and macrophages (Braissant et al., 1996). Two splice variants of PPARγ are known, PPARγ1 and PPARγ2, both of which are present in white adipose tissue. Because of its abundance in fat, its involvement in adipocyte differentiation, and because it serves as a target for certain anti-diabetic drugs, PPARγ has been strongly associated with obesity. Thus, the majority of this review will be devoted to PPARγ and its possible connection with obesity.

1.3. Synthetic PPAR ligands

PPARs are ligand activated transcription factors, which means that they bind to the promoter of target genes and increase or decrease DNA transcription upon binding of a small lipophilic molecule. Ligands for PPARs can be both endogenous or synthetic and can bind to the receptor with a range of affinities. Potent synthetic ligands for the PPARα receptor are the so-called fibrates, a group of related drugs that improve blood lipid parameters. Treatment with fibrates has become increasingly in vogue, particularly for patients suffering from hypertriglyceridemia, in order to lower plasma triglyceride and increase plasma high density lipoprotein (HDL) levels (Linton and Fazio, 2000). Recently, a newly developed fibrate called micronized fenofibrate was shown to be highly effective in ameliorating lipid profiles in type 2 diabetes patients, and thereby reducing diabetic complications (Anonymous, 2001). Other well-known fibrates are gemfibrozil and bezafibrate. Since most, but not all, of the effects of fibrates go via PPARα, the role of PPARα in human metabolism can partially be extrapolated from the effects of fibrate treatment on human patients, in lieu of experiments in animals.

A group of drugs that also acts via PPARs are the thiazolidinediones. These drugs, of which troglitazone was the first representative, were discovered in the 1980s and were later found to be agonists of PPARγ. As they target the primary defect in type 2 diabetes, which is a lack of insulin responsiveness in peripheral tissues, they were a very welcome addition to the existing arsenal of drugs, consisting of mainly biguanides and sulphonylureas. As with PPARα, the role of PPARγ in energy metabolism can partially be evaluated by observing the effects of PPARγ agonists in humans. However, it should be acknowledged that, even more so than in the case of fibrates and PPARα, many of the effects of thiazolidinediones are independent of PPARγ.

1.4. Natural PPAR ligands

Ever since the cloning of the first PPAR receptor, an intensive search has been going on to identify the natural ligands for PPARs. A unique feature of PPARs is that their ligand-binding pockets are usually large, which allows the receptors to accommodate a range of different ligands (Xu et al., 1999). Although a range of compounds has been shown to activate and bind to PPARs in vitro, including fatty acids, certain prostaglandins, and oxidized phospholipids, which

of these compounds serve as ligands in a living organism is still not fully clear. Elegant experiment with mice lacking peroxisomal acyl-CoA oxidase and/or PPARα suggests that substrates for acyl-CoA oxidase, which are very long chain fatty acids and their acyl-CoA derivatives, serve as natural ligands for PPARα in vivo (Hashimoto et al., 1999). For some putative ligands, such as the PPARγ ligand 15-Deoxy-delta 12, 14-prostaglandin J2 and the PPARα ligand leukotriene B_4, their existence in vivo and their stability in vitro have been challenged. As this information would provide key insight into how the activity of PPARs is governed in vivo, the determination of the physiological ligands for PPARs deserves to remain an important area of study.

2. PPARα

2.1. Function of PPARα

Synthetic ligands for PPARα, collectively known as peroxisome proliferators, are a perfect tool to study the function of PPARα. The most striking effect of the administration of peroxisome proliferators to rodents is a dramatic enlargement of the liver together with, expectedly, a huge increase in the size and number of peroxisomes. Prolonged treatment with peroxisome proliferators causes liver tumor formation, indicating a role of PPARα in hepatocyte proliferation and/or the cell cycle. Experiments with mice lacking PPARα showed that the effect of peroxisome proliferators on tumor formation is dependent on PPARα and may be caused by changes in the expression of important genes involved in cell cycle, such as cyclin-dependent kinase 1 and 4, and c-myc (Peters et al., 1998). Importantly, for reasons that are not completely understood (Lawrence et al., 2001), humans are much less sensitive to the hepatocarcinogenic effect of peroxisome proliferators than rodents.

It is highly unlikely that PPARα evolved solely as a receptor that stimulates liver growth and peroxisome proliferation in response to exposure to certain xenobiotics. Rather, the physiological function of PPARα probably lies in regulating the acute phase response and the adaptive metabolic response to fasting. In this regard, experiments with mice in which the PPARα gene has been deleted have been very illuminating. Whereas these PPARα null mice are without overt symptoms when fed ad libitum, except for moderately elevated plasma triglycerides levels, they show a host of metabolic abnormalities upon starvation (Hashimoto et al., 2000; Kersten et al., 1999; Leone et al., 1999). This includes elevated plasma free fatty acid levels, hypoketonemia, hypoglycemia, elevated plasma urea levels, hypothermia, a decreased metabolic rate, and a fatty liver. Detailed mRNA and protein expression analysis of PPARα null mice has indicated that the metabolic abnormalities are due to altered expression levels of a range of metabolic enzymes (Aoyama et al., 1998; Kersten et al., 1999, 2001). These data have led to the creation of a general picture about the role of PPARα in the regulation of liver intermediary metabolism (Fig. 1). First of all, PPARα stimulates the uptake, binding, activation, and subsequent oxidation of fatty acids (both mitochondrial, peroxisomal, and microsomal). In addition, it stimulates the synthesis of ketone bodies and influences the synthesis of apolipoproteins such

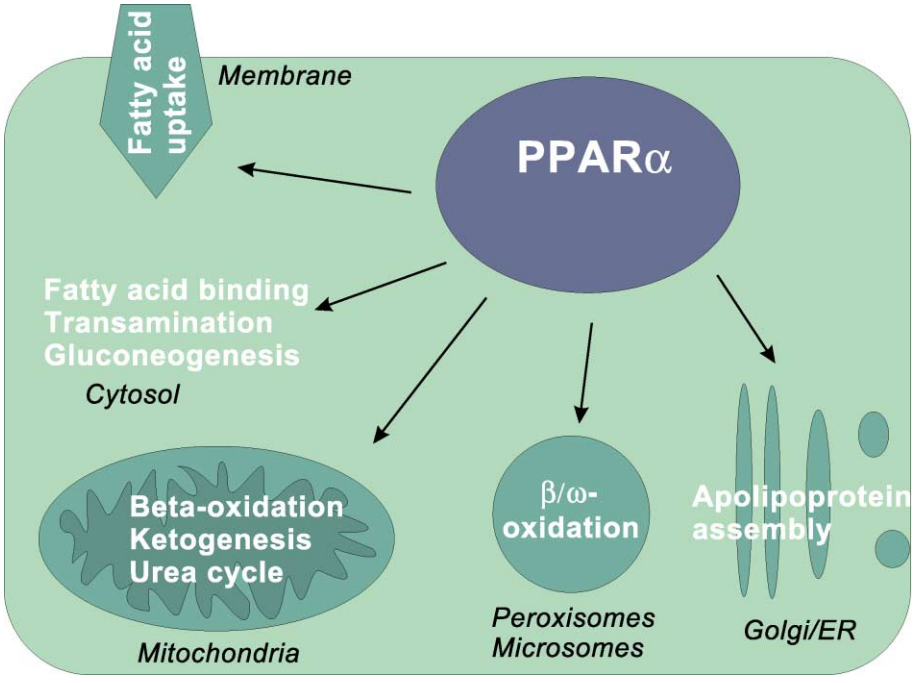

Fig. 1. Regulation of nutrient metabolism in hepatocytes by PPARα. The diverse processes regulated by PPARα in liver are illustrated. Both positive (fatty acid metabolism, gluconeogenesis) and negative regulations (amino acid metabolism) are shown.

as apoAI and apoAII. Furthermore, PPARα promotes the synthesis of glucose by activating the expression of genes involved in gluconeogenesis. Finally, PPARα inhibits the trans- and deamination of amino acids, and the synthesis of urea. In addition to intermediary metabolism, there is now compelling evidence that PPARα has a major role in the regulation of inflammation and the acute phase response (Delerive et al., 1999, 2001; Kockx et al., 1999). However, because this review focuses on the connection between PPARs and obesity, these issues will not further be discussed here.

2.2. PPARα and steatosis

Obesity, especially morbid obesity, is often associated with the development of a fatty liver (steatosis) (Garcia-Monzon, 2001). This is probably due to excess release of fatty acids from the adipose tissue, which accumulate in the liver in the form of triglycerides. The extent of fat accumulation in liver is determined by the balance between fatty acid uptake, -oxidation, -synthesis, and -esterification, and triglyceride secretion. Increased or decreased activity of these pathways has a major impact on fat accumulation in the liver. Several transcription factors are involved in the regulation of the above-mentioned pathways, most notably the sterol regulatory element binding protein 1 (SREBP-1) and PPARα. SREBP-1 stimulates the synthesis of fatty acids and triglycerides, and accordingly, mice that over-express this lipogenic transcription factor display pronounced steatosis (Shimano et al., 1996). Mice lacking PPARα that are put on a high fat diet show a similar phenotype (Kersten et al., 1999). Their ability to influence fat storage in liver suggests that pharmacological activation of these transcription factors may be effective in reducing steatosis. Thus, inasmuch as PPARα is a potent activator of hepatic fatty acid oxidation, activation of PPARα by synthetic PPARα activators may be a possible option to treat obesity-related steatosis and associated complications. So far, a limited number of studies have examined the effect of fibrates on steatosis, but no definitive conclusions have yet been reached (Laurin, 2001).

In line with its purported anti-steatotic role, PPARα has been proposed to play a central role in a pathway that, under conditions of excess dietary energy, serves to minimize fat storage in the central organs at the expense of white adipose tissue (Unger and Orci, 2000). According to this model, rather than preventing obesity, the function of the adipose hormone leptin is to minimize the build-up of triglycerides in tissues such as the pancreatic islets and liver. This is achieved by up-regulating the expression of PPARα, which stimulates the oxidation of incoming fatty acids and thereby prevents their conversion into triglycerides (Wang et al., 1999b). Although many elements of this model need to be confirmed in tissues other than pancreatic islets, it credibly integrates numerous bits and pieces of data for which the physiological significance was not always very clear.

2.3. PPARα and adiposity

Although PPARα is very weakly expressed in white adipose tissue, changes at the level of white adipose tissue have been observed in PPARα null mice. The most striking observation was that mice lacking PPARα suffered from a delayed onset form of obesity (Costet et al., 1998). This was observed particularly in females. However, according to recent results, the differences in adiposity between normal and wild-type mice disappear once the genetic background is cleaned up. Another piece of evidence suggesting a link between PPARα and adipose tissue function is that treatment of rats or mice fed a high fat diet with synthetic PPARα activators reduces adiposity (Guerre-Millo et al., 2000; Mancini et al., 2001; Ye et al., 2001). It is not clear whether the reduced fat gain stems from direct activation of PPARα in white adipose tissue or may be secondary to lowering of the plasma triglyceride level, an effect that originates in the liver. In favor of the first argument, PPARα was found to have an inhibitory effect on the expression of the glucose transporter GLUT4 in white adipose tissue. Further, the PPARα agonist bezafibrate increased fatty acid oxidation in rat primary adipocytes in concert with increased mRNA expression of fatty acid oxidizing enzymes, while the expression of adipocyte marker genes was decreased by bezafibrate (Cabrero et al., 2001). Based on the overall properties of PPARα, the use of synthetic PPARα activators is likely to become more popular in the future, not only to treat hypertriglyceridemia and low plasma HDL levels, but possibly also to ameliorate other symptoms of the obesity syndrome, including steatosis, type 2 diabetes, and adiposity.

There is a large body of evidence showing that conjugated linoleic acid, a fatty acid that is mostly present in meats and dairy products, decreases bodyfat in animals (Whigham et al., 2000), According to very recent studies, this is perhaps also true in humans (Riserus et al., 2001; Blankson et al., 2000). As conjugated linoleic acid is a ligand for PPARα (Moya-Camarena et al., 1999), it has been proposed that the effects of conjugated linoleic acid on diverse processes, including bodyfat, are mediated by PPARα. However, PPARα is just one of many possible targets through which conjugated linoleic acid might act. Thus, the theory linking conjugated linoleic acid to PPARα to adiposity remains premature and requires further verification.

3. PPARγ

3.1. PPARγ and adipogenesis

In recent years, PPARγ has drawn attention from just about any medical specialty including diabetology, gastroenterology, dermatology, oncology, and cardiology. PPARγ has also attracted a lot of interest from researchers studying obesity, for obvious reasons. Not only is PPARγ highly expressed in adipose tissue, it also plays a very important

role in adipogenesis, the process of differentiation of pre-adipocytes into mature fat cells.

The process of adipogenesis can be modeled in cell culture, which has permitted the detailed analysis of the sequence of molecular events that lead to adipocyte differentiation. Readers interested in the molecular regulation of adipogenesis, and the role of PPARγ in this process, are referred to a recent review by Rosen et al. (2000), which nicely summarizes the current state of affairs. In brief, loss and gain of function experiments have shown that the activation of PPARγ is both necessary and sufficient to induce an adipose phenotype, which is defined by lipid accumulation and the expression of fat-specific marker genes such as adipocyte fatty acid binding protein (aP2), lipoprotein lipase, and adipsin. One of the most illuminating set of experiments has been carried out with chimeric mice derived from both wild-type embryonic stem cells and embryonic stem cells with a homozygous deletion of PPARγ (Rosen et al., 1999). It was observed that cells lacking PPARγ were absent from white adipose tissue, but present in other tissues, leading to the important conclusion that PPARγ is required for adipogenesis.

3.2. Lipogenesis

Whereas the function of PPARγ in adipose differentiation is well established, its role in the fully differentiated adult white fat cell is much less clear. It has been argued that, based on experiments with a synthetic PPARγ antagonist, in terminally differentiated adipocytes the PPARγ activity is minimal and has no impact on the expression of PPARγ targets (Camp et al., 2001). However, experiments with heterozygous PPARγ mutant mice suggest that PPARγ, in addition to adipogenesis, may also have an important regulatory role in adult lipogenesis. It was observed that, on a high fat diet, mice with only one copy of the PPARγ gene gained less weight than wild-type mice and had significantly smaller adipose tissue fat stores (Kubota et al., 1999). Unfortunately, homozygous PPARγ null mice are embryonically lethal, which has precluded an investigation of the full impact of PPARγ deletion in adult animals. It is expected that tissue-specific, inducible gene targeting of PPARγ will yield important new insights into the role of PPARγ in fat deposition.

Inasmuch as PPARγ acts by regulating DNA transcription, the identity of genes regulated by PPARγ can provide invaluable information about the function of PPARγ itself. Genes that are under transcriptional control of PPARγ in adipose tissue include lipoprotein lipase, acyl-CoA synthetase, fatty acid translocase (CD36), and fatty acid transport protein, suggesting that PPARγ has an important role in the uptake of fatty acids in adipocytes (Desvergne and Wahli, 1999). Recently, the application of high throughput gene expression profiling, which permits the simultaneous expression monitoring of several thousands of genes, has been extremely useful to identify adipose genes whose expression is regulated by PPARγ. Using a comprehensive mRNA profiling technique called GeneCalling, Way et al. (2001b) were able to show that treating Zucker Diabetic Fatty rats with the non-glitazone PPARγ agonist known as GW1929 resulted in up-regulation of a host of genes in adipose tissue. Among them were numerous genes involved in lipogenesis, such as acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, and several others. Surprisingly, the expression of genes involved in fatty acid oxidation was also stimulated by GW1929. This is unlikely due to cross-activation of PPARα, since the same genes do not seem to be affected by GW1929 in the liver, where PPARα is more highly expressed than in white adipose tissue.

In addition to genes involved in lipogenesis and fatty acid oxidation, GW1929 also caused an up-regulation of the SREBP-1. SREBP-1 is a helix-loop helix transcription factor that stimulates the expression of numerous genes connected with lipogenesis, such as ATP-citrate lyase, acetyl-CoA carboxylase and fatty acid synthase. Because of the involvement of SREBP-1 in lipogenesis, it is unclear whether the effect of the PPARγ agonist GW1929 on lipogenic gene expression is direct or may be secondary to the up-regulation of SREBP-1. Interestingly, not only does PPARγ appear to stimulate SREBP-1 expression, the reverse may also be true. Indeed, SREBP-1 has been shown to activate PPARγ, both by stimulating the production of an endogenous ligand (Kim et al., 1998), as well as by inducing PPARγ promoter activity via a so-called E-box motif (Fajas et al., 1999). These data are suggestive of a feed-forward mechanism, in which PPARγ activates SREBP-1 and vice-versa, and which is aimed at promoting lipogenesis in adipose tissue (Fig. 2).

Aside from up-regulating gene expression, PPARγ is also able to down-regulate the expression of several genes. Two members of this group with direct relevance to obesity are the genes encoding the adipose tissue hormones leptin and resistin. Leptin is the product of the *ob* gene, whose deletion leads to severe obesity in mice and humans. When injected into mice that lack leptin, it potently inhibits food intake and stimulates energy expenditure by inducing lipolysis and fatty acid oxidation (Friedman and Halaas, 1998). Although leptin was initially heralded as the hormone that limits fat storage under conditions of overeating, there is a growing consensus that the decreased leptin concentration during fasting may better reflect the actual physiological function of leptin (Ahima and Flier, 2000). Consistent with its role in promoting lipogenesis, PPARγ down-regulates leptin expression (De Vos et al., 1996; Kallen and Lazar, 1996). Paradoxically, the expression of both PPARγ and leptin is decreased by fasting and increased by feeding. In the latter case, PPARγ may attenuate the increase in leptin expression to limit leptin-induced lipolysis and fatty acid oxidation. The novel hormone resistin was isolated as a negative target of PPARγ agonists and was proposed to serve as a possible mediator between obesity and type 2 diabetes (Steppan et al., 2001). However, follow-up data

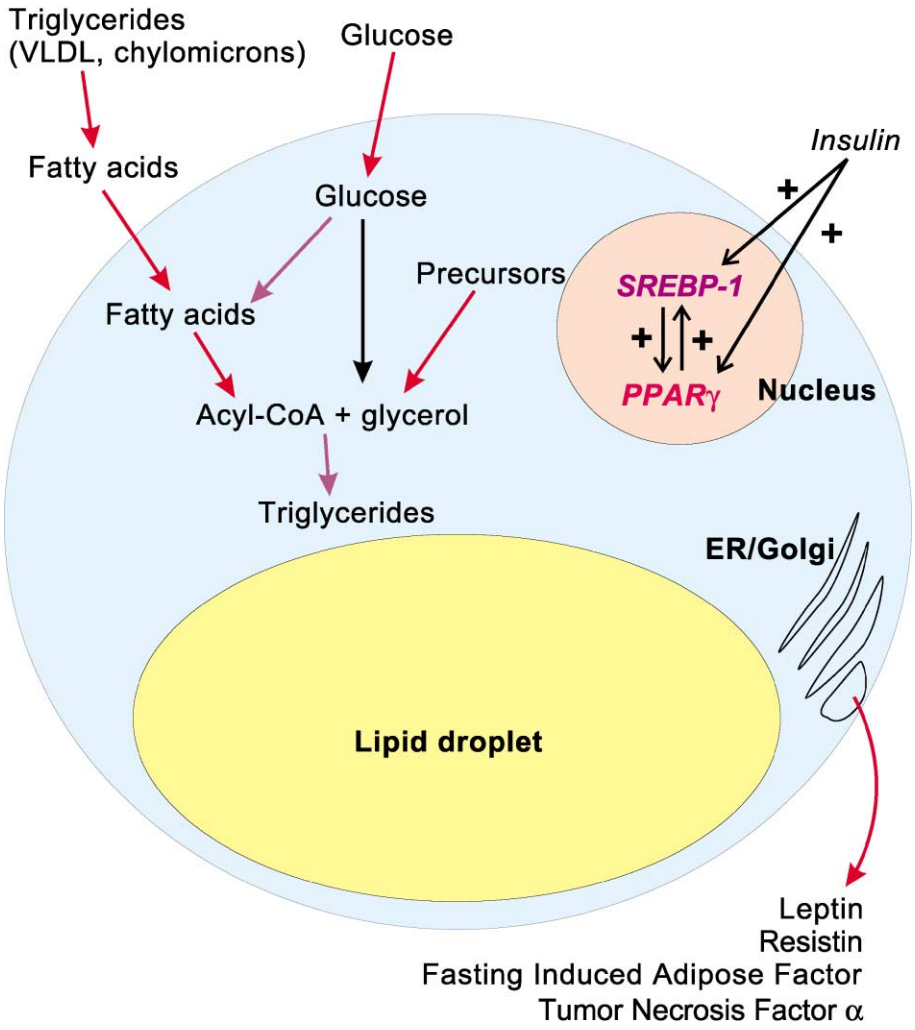

Fig. 2. Regulation of lipid metabolism in white adipose tissue by PPARγ and SREBP-1. The major steps of fat storage from plasma triglycerides and glucose are illustrated. Pathways stimulated by PPARγ are indicated by a red arrow. Pathways stimulated by SREBP-1 are indicated by a purple arrow. Insulin induces both PPARγ and SREBP-1 expression. SREBP-1 stimulates the expression of PPARγ and vice versa.

have indicated that rather than down-regulating resistin expression, synthetic PPARγ agonists increase its expression (Way et al., 2001a). The reason for this discrepancy is presently not clear.

Taken together, based on a diverse set of data, it can be concluded that not only does PPARγ play a pivotal role in adipocyte differentiation, it also is an important stimulator of lipogenesis in differentiated white adipose tissue.

3.3. PPARγ expression and obesity

As PPARγ stimulates adipogenesis and lipogenesis in adipose tissue, it has been hypothesized that obesity may be associated with elevated PPARγ expression levels. In one of the first reports, it was found that PPARγ mRNA levels in white adipose tissue were unaltered in two murine models of obesity (gold thioglucose and ob/ob), but were increased by about 50% after high fat feeding in normal mice (Vidal-Puig et al., 1996). A study with Zucker Diabetic Fatty and lean control rats found no difference in PPARγ1 or 2 expression between these two group either in visceral or subcutaneous adipose tissue (Shimoike et al., 1998). In contrast, in another study PPARγ expression in white adipose tissue was elevated two-fold in obese Zucker rats compared to lean controls (Gorla-Bajszczak et al., 2000). In humans, there are weak indications that PPARγ expression is elevated in obese patients. A significant correlation was found between the ratio of PPARγ2 mRNA to total PPARγ mRNA, and obesity (Hotta et al., 1998). Also, PPARγ mRNA levels were found to be increased in omental fat of obese versus non-obese subjects (Lefebvre et al., 1998). However, these bits of data are counterbalanced by several studies showing no relation between adipose tissue PPARγ mRNA expression and obesity (Auboeuf et al., 1997; Krempler et al., 2000). In conclusion, obesity may increase PPARγ mRNA levels in white adipose tissue, but the effect probably is very small.

Most of the data discussed so far were derived from experiments in animals. These studies have been extremely

important in defining the physiological function of PPARγ and elucidating the molecular mechanisms of its action. However, to evaluate whether PPARγ may be linked to obesity in humans, and to examine whether PPARγ is a suitable pharmacological target for obesity in humans, it is important that studies are carried out in human subjects.

3.4. Human genetic studies

To evaluate whether PPARγ contributes to obesity in humans, genetic studies are an extremely powerful tool. To date, a total of four mutations have been identified in the human PPARγ gene. Except for the common Pro to Ala mutation at position 12 (which therefore deserves to be classified as a polymorphism) these mutations are all extremely rare. Until now, a very small number of individuals (all German) have been identified with a GT base exchange in exon 2 of the PPARγ gene, which is shared by both isoforms, resulting in a substitution of proline to glutamine at position 115. In the original study this mutation was found in 4 out of 121 obese subjects, but not once in 237 normal weight controls (Ristow et al., 1998). Remarkably, this Pro115Gln mutation has failed to emerge in numerous follow-up studies, in which a total of several thousand of obese and normal weight individuals were screened. Two other extremely rare mutations have so far been identified, both of which in patients with severe insulin resistance (Barroso et al., 1999). In two subjects, who were mother and son, proline at position 467 towards the C terminus was mutated into leucine, resulting in a dysfunctional PPARγ protein. Another subject had a valine to methione substitution at codon 290. In contrast to the majority of the patients with insulin resistance, the three afflicted individuals had a relatively normal bodyweight with no evidence of lipoatrophy or altered fat distribution. Unfortunately, no data were presented on possible lipid storage in the skeletal muscle or liver.

The most common mutation in the PPARγ gene is a missense mutation at codon 12 of PPARγ2, with a frequency ranging from 0.12 in Caucasian Americans to 0.01 in Chinese (Yen et al., 1997). In one high profile study, it was found that the Pro12Ala mutation, which reportedly rendered the receptor less active, is associated with increased insulin sensitivity, a lower risk for type 2 diabetes, and a lower body mass index (Deeb et al., 1998). In contrast, in a different study with two different cohorts Beamer et al. (1998) showed that subjects with at least one Ala allele had a significantly higher mean body mass index than subjects homozygous for the Pro allele. Numerous other studies have investigated the relationship between this PPARγ polymorphism and insulin sensitivity and/or obesity. While some have found increased body mass index in subjects with the Pro12Ala mutation (Valve et al., 1999; Meirhaeghe et al., 2000; Cole et al., 2000; Li et al., 2000; Lei et al., 2000), the far majority of the studies have failed to find an association between these two parameters (Hamann et al., 1999; Ringel et al., 1999; Swarbrick et al., 2001; Oh et al., 2000; Clement et al., 2000; Mancini et al., 1999; Mori et al., 1998; Poirier et al., 2000; Schaffler et al., 2001; Vaccaro et al., 2000; Evans et al., 2001). According to one study, the effect of the Pro12Ala mutation may be opposite in lean and obese subjects (Ek et al., 1999). The relationship between the Pro12Ala mutation and obesity may also be influenced by the ratio of dietary polyunsaturated to saturated fat intake, which might partially explain the divergent findings (Luan et al., 2001). The most powerful study thus far was conducted by Altshuler et al. (2000) who, by analyzing over 3000 individuals, found no significant effect of the Pro12Ala mutation on body mass index. In contrast, the risk for type 2 diabetes was modestly yet significantly decreased in carriers of the Ala allele. It was concluded that because the risk allele (Pro) occurs at such high frequency, the modest effect of this polymorphism translates into a large population attributable risk. In conclusion, while it appears that the Pro12Ala mutation in PPARγ may offer some protection against type 2 diabetes, the jury is still undecided on whether it may have any effect on body mass index or obesity.

3.5. PPARγ agonists

In the mid-1990s, the introduction into the market of a new class of insulin sensitizing drugs called thiazolidinediones was heralded as a breakthrough in the pharmacological treatment of type 2 diabetes. The ability of thiazolidinediones to improve insulin sensitivity was first demonstrated in 1988, when a group of researcher from Sankyo showed that the compound troglitazone was very effective in ameliorating plasma glucose, insulin, free fatty acids, triglycerides and ketone body levels in several animal models of type 2 diabetes (Fujiwara et al., 1988). However, it was not until 1994 that the likely mechanism by which thiazolidinediones act became apparent (Ibrahimi et al., 1994). Subsequently, Lehmann et al. (1995) were able to show that thiazolidinediones are able to bind and activate the PPARγ receptor with low to moderate (troglitazone) or moderate to high (rosiglitazone) affinity, seemingly closing the book about what constitutes the primary molecular target of thiazolidinediones. Notwithstanding these results, significant controversy has persisted about whether the insulin sensitizing effects of this group of compounds, which also include pioglitazone, go via PPARγ or are actually PPARγ independent. Indeed, it is known that thiazolidinediones affect certain processes independently of PPARγ. For instance, there is evidence that thiazolidiones influence ion-channels independently of PPARγ (Mishra and Aaronson, 1999; Sunaga et al., 1999; Knock et al., 1999). The same is true for the effect of troglitazone on cholesterol biosynthesis (Wang et al., 1999a). Because of this lack of complete specificity for PPARγ, one should avoid to haphazardly ascribe any effects elicited by thiazolidinediones to PPARγ.

With respect to the insulin-sensitizing activity of thiazolidinediones, evidence in favor of a direct involvement of PPARγ is as follows.

(1) Based on experiments showing that rosiglitazone or troglitazone fails to reduce insulin or glucose levels in A-ZIP/F-1 mice, which lack adipose tissue, it was concluded that adipose tissue, where PPARγ is expressed most abundantly, is required for the anti-diabetic effect of thiazolidinediones (Chao et al., 2000).

(2) Among the thiazolidinediones, there is a positive relationship between the relative insulin sensitizing effect and the binding affinity for PPARγ (King, 2000).

(3) Novel non-thiazolidinedione PPARγ agonists (such as the tyrosine analog GW1929) also have a strong insulin-sensitizing effect (Brown et al., 1999).

(4) Synthetic agonists for the retinoid X receptor (RXR) improve insulin resistance in a mouse model of type 2 diabetes, suggesting that the heterodimer between RXR and PPARγ is the actual functional unit that mediates the effect of thiazolidinediones (Cesario et al., 2001).

Conversely, there is (weaker) evidence suggesting that PPARγ does not mediate the insulin-sensitizing effects of thiazolidinediones.

(1) According to experiments with an alternative mouse model of lipoatrophy, the so-called aP2/DTA mice, white adipose tissue is not required for the anti-diabetic effect of thiazolidinediones (Burant et al., 1997).

(2) Although PPARγ is only weakly expressed in skeletal muscle, this tissue accounts for the majority of the thiazolidinedione-stimulated glucose uptake. To explain this apparent paradox, it is believed that thiazolidinediones influence the secretion of an adipose factor that influences glucose uptake and/or metabolism in skeletal muscle. Candidate factors include free fatty acids, tumor necrosis factor alpha, and the recently discovered hormone resistin. Despite the relatively weak expression of PPARγ in the skeletal muscle, thiazolidinediones are able to increase the glucose transport and translocation of the GLUT4 glucose transporter in L6 myotubes (Yonemitsu et al., 2001).

(3) A PPARγ antagonist that inhibits thiazolidinedione-induced adipocyte differentiation and target gene activation stimulates glucose uptake in 3T3-L1 adipocytes (Camp et al., 2001). Although these data do not completely rule out an involvement of PPARγ, the data do hint that the insulin sensitizing effect of thiazolidinediones may not require PPARγ activation but rather de-activation. In fact, it has been suggested that thiazolidinedione-induced insulin sensitization may stem from their partial antagonist activity (which would antagonize full agonist endogenous ligands) and not from their agonist activity (Olefsky, 2000).

The discussion about whether PPARγ mediates the insulin sensitizing effect of thiazolidinediones is further confounded by a lack of understanding about whether PPARγ itself promotes or inhibits insulin action. Curiously, it was observed that heterozygous PPARγ mutant mice, which would be expected to suffer from insulin resistance based on the assumption that thiazolidinediones improve insulin sensitivity via PPARγ, actually display improved insulin sensitivity on a high fat diet (Kubota et al., 1999; Miles et al., 2000). The latest unpublished news is now that the relation between PPARγ activity and insulin resistance may be U-shaped, which could explain some of the apparent discrepancies (Akiyama et al., 2001). Clearly, the last word about this issue has not yet been spoken.

In the clinic, thiazolidinediones have proven effective in reducing insulin-resistance in type 2 diabetes patients. In addition, they improve plasma lipid parameters such as plasma triglycerides, HDL, and free fatty acid levels, which are often disturbed in these patients. Currently, two types of thiazolidinediones are approved for use in type 2 diabetic patients, either alone or in combination therapy with other anti-diabetic drugs such as metformin. These drugs are rosiglitazone (Avandia™) and pioglitazone (Actos™). The first thiazolidinedione that gained approval, troglitazone (Rezulin™), has since then been withdrawn off the market because its use was associated with hepatocellular damage and hepatic failure in a small number of patients. One of the adverse side-effects of thiazolidinediones in general is that they have the tendency to cause weight gain, part of which is due to fluid retention, but part of which may also be fat. More specifically, thiazolidinediones may induce adipocyte hyperplasia, which is defined as the creation of new fat cells, as opposed to the enlargement of existing fat cells (hypertrophy) (Okuno et al., 1998). This seems to happen only in subcutaneous fat, as opposed to visceral fat (Akazawa et al., 2000). Nevertheless, these side-effects, although undesirable, do under normal circumstances not outweigh the benefits of improved insulin sensitivity, and decreased plasma free fatty acid and triglyceride levels in patients treated with thiazolidinediones.

Taking into account the importance of PPARγ in promoting adipogenesis and lipogenesis, and considering that PPARγ can be easily targeted via small synthetic ligands, full synthetic PPARγ antagonists might theoretically be considered as a possible approach to target adiposity without associated insulin resistance. However, drug-mediated inhibition of fat storage in white adipose tissue faces many hurdles. This is because blocking fat storage in subcutaneous fat tissue, without a concomitant increase in thermogenesis, is likely accompanied by a parallel increase in fat deposition in and around central organs, which will compromise organ function and increase the risk for hyperlipidemia and type 2 diabetes. This point is illustrated by the protease inhibitors for the human immunodeficiency virus, which have been shown to inhibit adipocyte differentiation, possibly via the transcription factor SREBP-1. As a result, patients treated with these drugs develop a form of lipodystrophy, characterized by fat redistribution, insulin resistance and hyperlipidemia. An additional argument against the future use of PPARγ antagonists is that morbid obesity rarely occurs without associated insulin resistance. If we assume that activating PPARγ improves insulin sensitivity (which

according to recent data may not be completely correct), antagonizing PPARγ may evoke or aggravate insulin resistance. Furthermore, plasma lipid profiles may worsen.

In conclusion, PPARs have been linked in numerous ways to obesity. Synthetic agonists for PPARγ and PPARα are used clinically to ameliorate abnormalities in lipid and glucose metabolism, many of which often occur in parallel with obesity, such as dyslipidemia, insulin resistance/type 2 diabetes, and hypercholesterolemia. Activation of PPARα was recently shown to reduce adiposity in rodents, paving the road for possible future studies in humans. One of the most pertinent issues relating to PPARγ that likely will continue to receive major interest in the ensuing years is the question of how PPARγ ligands improve insulin sensitivity and whether this involves their agonistic or partial antagonistic activity. Finally, and which is perhaps a bit disappointing, in spite of strong mechanistic and gene expression data connecting PPARγ with adipo- and lipogenesis, human genetic studies have so far failed to convincingly establish a link between mutations in the PPARγ gene and percent bodyfat, body mass index, or fat distribution.

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