Perspectives in Diabetes

PPAR-γ: Adipogenic Regulator and Thiazolidinedione Receptor

B.M. Spiegelman

The past several years have seen an explosive increase in our understanding of the transcriptional basis of adipose cell differentiation. In particular, a key role has been illustrated for PPAR-γ, a member of the nuclear hormone receptor superfamily. PPAR-γ has also been recently identified as the major functional receptor for the thiazolidinedione class of insulin-sensitizing drugs. This review examines the evidence that has implicated this transcription factor in the processes of adipogenesis and systemic insulin action. In addition, several models are discussed that may explain how a single protein can be involved in these related but distinct physiological actions. I also point out several important areas where our knowledge is incomplete and more research is needed. Finally, I discuss how advances in our understanding of nuclear receptor function, particularly the docking of cofactors in a ligand-dependent fashion, should lead to improved drugs that utilize the PPAR-γ system for the treatment of insulin resistance. *Diabetes* 47:507–514, 1998

Adipose tissue has been viewed historically as a passive player in the regulation of energy homeostasis, storing energy in times of nutritional excess and releasing that energy when needed in times of nutritional deprivation. However, the last decade or so has seen a rather startling revision of this view. It is now appreciated that adipose cells secrete a large number of bioactive molecules, including adipsin, angiotensinogen, tumor necrosis factor-α (TNF-α), leptin, ACRP30/AdipoQ, and plasminogen activator inhibitor 1 (1). Although the roles of these molecules (and probably more to come) remain to be fully elucidated, it is clear that TNF-α and leptin are bona fide signaling molecules, involved in insulin resistance and the control of food intake, respectively. This new appreciation of the interactive role of the adipose cell in energy metabolism and insulin sensitivity has added a further dimension to our understanding of this cell type. It has also added urgency to our need to understand adipose cell differentiation and gene expression.

This review will describe the identification and role of PPAR-γ as a central regulator of fat cell differentiation. It will also review the discovery and function of this protein as the major receptor for the thiazolidinedione (TZD) class of insulin-sensitizing drugs. With the introduction of troglitazone (under brand-names such as Rezulin, Romozin), the first PPAR-γ ligand of the TZD class, into clinical practice in 1997 for the treatment of NIDDM, it is especially important to point out where the major gaps in our understanding of this receptor-ligand system lie. Finally, I will describe some potential new therapeutic opportunities that have been opened up by the recent progress.

PPAR-γ AND ADIPOGENESIS

The cloning of mammalian PPAR-γ and its link with adipogenesis came from our analysis of the adipose-specific enhancer from the aP2 gene, an abundant adipocyte-specific fatty-acid binding protein. Mutational analysis and study of protein binding to this 500–base-pair piece of DNA identified a key nuclear factor, termed ARF6, that bound to two sites (ARE6 and ARE7) in this enhancer (2). This DNA binding activity was observed only in extracts of fat cells. Cloning of this factor (3) revealed it to be a member of the peroxisome proliferator activated receptor (PPAR) subfamily of nuclear hormone receptors and, in particular, the mammalian homolog of Xenopus PPAR-γ, which had been cloned earlier (4). Several other labs also cloned mammalian PPAR-γ independently, through homology screens that sought new members of the PPAR family (5,6). It is now accepted that there are three related but quite distinct PPAR proteins, PPAR-α, PPAR-δ (also called PPAR-β, Nuc-1, or FAAR), and PPAR-γ. PPAR-γ is expressed in an adipose-selective fashion in both rodents and humans, being 10- to 30-fold higher in fat than in most other tissues (3). Interestingly, two forms of the protein, γ1 and γ2, exist as products of alternative promoter usage. The two forms differ in that PPAR-γ2 has an NH_2-terminal extension of 30 amino acids. In addition, PPAR-γ2 is found selectively in fat tissue, whereas γ1 is expressed at low levels in many tissues (7,8). The functional meaning of these splice variants is not yet clear.

Our early work indicated that PPAR-γ, like other PPAR-s, heterodimerizes with the retinoid X receptor (RXR) and activates the aP2 enhancer in fibroblasts, a cell type in which this enhancer ordinarily has little or no activity (3). The

From the Department of Cancer Biology, Dana-Farber Cancer Institute, and the Department of Cell Biology, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Bruce M. Spiegelman, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115. E-mail: bruce_spiegelman@dfci.harvard.edu.

Received for publication 30 December 1997 and accepted in revised form 5 January 1998.

B.M.S. has received honoraria from Parke Davis-Warner Lambert.

ADD1/SREBP1, adipocyte determination and differentiation factor 1/sterol response element binding protein 1; C/EBP, CAAT/enhancer binding protein; ETYA, 5,8,11,14-eicosatetraynoic acid; K_d, dissociation constant; PPAR-γ, peroxisome proliferator activated receptor-γ; RXR, retinoid X receptor; TNF-α, tumor necrosis factor-α; TZD, thiazolidinedione.

PPAR-γ/RXR heterodimer binds to direct repeats of hormone response elements separated by one base, so-called DR-1 sites. While no bona fide ligands for PPAR-γ were known at this time, a diverse group of activators, mainly fatty-acid derivatives such as 5,8,11,14-eicosatetraynoic acid (ETYA), were known to activate PPAR-α (4); high levels of this compound will also activate PPAR-γ. Strong activation of the aP2 enhancer by PPAR-γ and RXR requires the application of a PPAR activator, an RXR ligand, or both. PPAR-γ and RXR also transactivated another bona fide fat enhancer, that from the PEPCK gene (9). This led to the most ambitious question: were PPAR-γ expression and activation sufficient to give a full adipocyte differentiation response in fibroblasts? In fact, retroviral expression of PPAR-γ in many fibroblast cell lines, followed by application of an activator such as ETYA, gave abundant differentiation that included lipid accumulation, changes in cell morphology, and expression of most if not all of the genes that characterize the adipocyte phenotype (10). With the identification of relatively high-affinity ligands (see below) it is now clear that PPAR-γ, expressed at or below the levels seen in fat tissue, can convert nearly every fibroblastic cell in a given culture into a fully differentiated adipocyte.

PPAR-γ interacts with other transcription factors in potentially important ways (11), as illustrated in Fig. 1. CAAT/enhancer binding protein (C/EBP)-α, though expressed in many tissues, is induced in adipogenesis and itself has adipogenic action when expressed at high levels (12). When expressed at levels equivalent to those seen in fat, it can cooperate powerfully with PPAR-γ, even allowing a significant adipogenic response in the absence of added PPAR-γ ligands. Precisely how these two transcription factors interact remains an important mechanistic problem. This ability of PPAR-γ and C/EBP-α to promote differentiation is not limited to fibroblasts, as simultaneous expression of both of these factors and activation of PPAR-γ can cause "transdifferentiation" of cultured myoblasts to adipocytes (13).

Another factor that can cooperate with PPAR-γ is adipocyte determination and differentiation factor 1/sterol response element binding protein 1 (ADD1/SREBP1). This transcription factor, a member of the basic helix-loop-helix family, was independently identified as a potential regulator of adipogenesis and fatty-acid metabolism (14), and as a key factor in cholesterol homeostasis (15). Coexpression of ADD1/SREBP1 with PPAR-γ increases the transcriptional activity of PPAR-γ, even without adding a PPAR-γ ligand (16). Because ADD1/SREBP1 can increase the expression of several key genes of fatty-acid metabolism, such as fatty acid synthetase and lipoprotein lipase, it seems plausible that this cooperation is through the generation of endogenous ligands for PPAR-γ. Other key players in the transcriptional control of adipogenesis are C/EBP-β and -δ. These factors appear to be very important in the induction of PPAR-γ in adipogenic differentiation. Indeed, conditional expression of C/EBP-β and -δ has been shown to yield expression levels of PPAR-γ equivalent to normal fat cells; a very strong differentiation response can be seen in these cells upon application of PPAR-γ ligands (17). This data has led to a model of a transcriptional cascade that is dependent on the expression of C/EBP-β and -δ to turn on PPAR-γ. The binding of ligands, perhaps generated by the action of ADD1/SREBP1, triggers the full differentiation response.

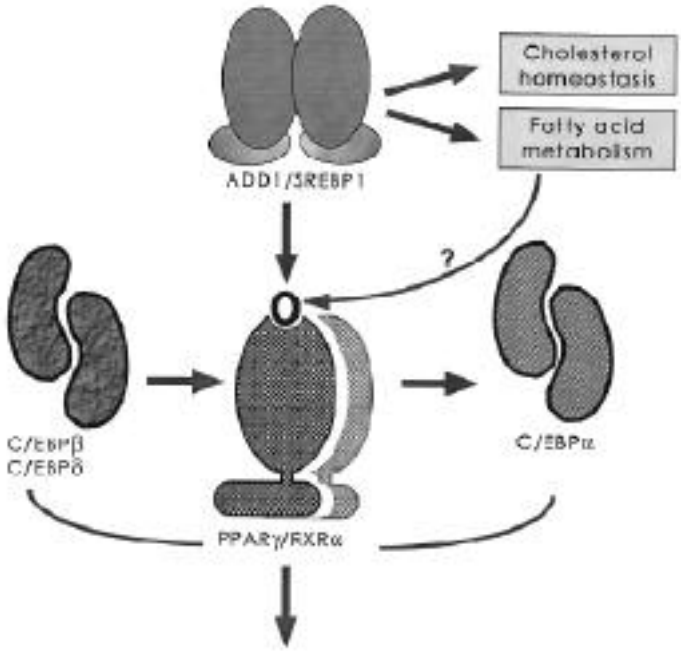

FIG. 1. Transcriptional cascade in adipogenesis. PPAR-γ and the C/EBP family interact to control adipose-cell differentiation. C/EBP-β and -δ are involved in the transcriptional control of PPAR-γ. Adipogenesis is stimulated upon PPAR-γ activation by ligands. C/EBP-α is activated later in the differentiation process but can functionally synergize with PPAR-γ and may also be involved in maintaining high levels of PPAR-γ expression. ADD1/SREBP1 has been implicated in the control of several key genes of fatty-acid metabolism and can promote the transcriptional activity of PPAR-γ, probably through the formation of endogenous ligands.

C/EBP-α both cooperates with PPAR-γ to yield a more powerful differentiation and may be needed to maintain PPAR-γ expression at high levels. It may be noteworthy that a binding site for C/EBP family members has been noted in both the PPAR-γ1 and -γ2 promoters (18).

TZDs AS LIGANDS FOR PPAR-γ

A key report that led to identification of TZDs as ligands for PPAR-γ came from Harris and Kletzien (19), who showed that pioglitazone increased transcriptional activity from the aP2 enhancer and apparently did so through the differentiation-linked DNA site (ARE6) described above. When we cloned and identified the ARE6 binding factor as the PPAR-γ/RXR heterodimer, two groups independently asked whether the TZD drugs were acting as direct agonists for PPAR-γ. These studies identified BRL49653 and pioglitazone as direct ligands of PPAR-γ with positive activity on gene transcription (20,21). Importantly, the TZDs were also shown to be highly selective for PPAR-γ, as they had very minimal activity toward PPAR-α or PPAR-δ. As expected from the data cited above, the TZDs were potent and effective at stimulating adipogenesis in cells containing endogenous or ectopically expressed PPAR-γ (20,21). This data also made sense of earlier reports in which the TZDs had been shown to stimulate adipogenesis in preadipocyte cell lines, though the mechanism of action of the drugs in these early studies was not known.

The evidence that PPAR-γ is the major receptor mediating the antidiabetic activity of the TZDs is now very strong, based on the following multiple lines of pharmacological evidence.

1. Each of the TZD drugs binds to and activates PPAR-γ in the same concentration range that has antidiabetic activity (22).
2. Among many TZDs surveyed, the rank order of potency of their antidiabetic activities closely matches the rank order of their affinities for PPAR-γ (22).
3. Potent and selective ligands for PPAR-γ outside of the TZD class have now been developed on the basis of their activation of PPAR-γ. These have antidiabetic actions in preclinical models of insulin resistance and diabetes (T. Willson, personal communication).
4. Ligand stimulation of RXR, the heterodimeric partner of PPAR-γ, also improves insulin sensitivity in vivo (23).
5. No other receptor for the TZD drugs has been identified.

Taken together, these data make a compelling case that PPAR-γ is the major functioning receptor for the common TZD actions in diabetes. However, it is possible that individual drugs of this class may have additional targets that contribute to their therapeutic actions or to their side effects.

One concern surrounding the clinical use of TZDs is that their adipogenesis-promoting effects could be detrimental to patients with NIDDM, who all too often are overweight to begin with. In therapeutic doses, it is clear that the TZDs do promote weight gain and increase fat deposition in rodent models (e.g., 24). Whether this is primarily due to increased insulin sensitivity, more fat cell differentiation, or a combination of the two is not clear. However, clinical use in humans has not shown these drugs to induce significant weight gain. This may reflect the fact that increased adipogenesis per se would not necessarily cause obesity; recent evidence indicates that TZDs may increase fat cell number while simultaneously decreasing fat cell size (24). Alternatively, it may indicate that preadipocytes in adult humans are relatively resistant to the differentiative effects of the TZDs. Three other side effects observed with the TZDs deserve mention. A small increase in plasma volume is consistently observed in patients undergoing treatment with troglitazone (25). If and how this relates to PPAR-γ activation is not clear, but this phenomenon does not seem to limit clinical utility. Of more concern is an increased adipose cell formation observed in the bone marrow of rodents treated with certain TZDs. Fatty transformation of bone marrow is thought to be a very serious condition, and the considerable published data linking PPAR-γ and adipogenesis, including stromal cells of the bone marrow (26), suggest that this may well be a consequence of PPAR-γ activation. On the other hand, this is only observed at high doses of TZDs, so it is likely that a reasonable therapeutic window is available between beneficial effects in NIDDM and the potentially deleterious effects on stromal elements in the bone marrow. Most recently, liver toxicity has been observed in a small percentage of patients taking troglitazone (27), leading to a withdrawal of this drug from the market in England. It is unclear whether the hepatatoxic effect is mediated by activation of PPAR-γ or whether it represents a nonspecific effect of troglitazone. In the U.S., the drug is now given with recommendations that liver enzyme levels be periodically monitored.

Natural ligands. Radiolabeled TZD ligands have enabled development of a displacement assay that allowed a search for natural ligands of PPAR-γ. This question is interesting in two ways. First, the identity of natural ligands may provide insights into new therapeutic approaches. Second, knowledge of the endogenous ligand will allow investigation of whether some insulin-resistant states are due to a deficiency in the endogenous ligand for PPAR-γ. This seems plausible when one considers that much endocrine pathology—such as hypothyroidism and adrenal insufficiency (Addison's disease) or excess (Cushing's Syndrome)—results from dysregulation of the endogenous ligands for other nuclear receptors.

The initial screening for natural ligands examined many fatty acids and fatty-acid derivatives for binding activity. The first natural ligand described was an unusual prostanoid 15-deoxy$\Delta^{12,14}$PG J2 (21,28). More recently, several polyunsaturated fatty acids, such as linoleic acid, have also been found to bind directly to PPAR-γ (29). Although all of these molecules must be considered as potentially important ligands in vivo, it should be emphasized that their affinities are relatively low, in the range of 2–50 μmol/l. This contrasts with the higher affinities that most nuclear receptors have for their endogenous ligands (dissociation constants [K_ds] in the low nmol/l range). Although 2–50 μmol/l is not necessarily out of the concentration range at which fatty acids such as polyunsaturated fatty acids circulate in vivo, it is not clear that these molecules can reach the nucleus of relevant tissues (fat, muscle, liver) at these concentrations. Fatty-acid levels inside cells are usually tightly regulated through the actions of binding proteins in the cell membrane and cytoplasms, as well as by the fatty-acid acylation machinery. Still, the notion of whether PPAR-γ is a "promiscuous" receptor for many fatty acids with low affinity or has a more limited number of specific, high-affinity ligands has been the subject of much speculation and interesting debate. More research is required.

INSULIN SENSITIZATION BY TZDs: TISSUE TARGETS AND MECHANISMS

The work described above identifying PPAR-γ as the primary target for the antidiabetic action of the TZDs has also presented two paradoxes. How can a receptor expressed predominantly in fat tissue improve insulin sensitivity in all of the major insulin-sensitive tissues? Additionally, given the well-established connection between obesity and insulin resistance, how can we reconcile the enhancement of insulin sensitivity by a receptor known to promote adipogenesis? There are no clear answers to these questions yet, but it is important to recognize that adipose cell differentiation is not identical to obesity. Obesity is primarily a disorder of energy balance, where energy intake exceeds energy expenditure. Although an increase in fat cell number may accompany great obesity, an increased fat cell number per se without an increase in total energy stored would not necessarily lead to insulin resistance. There are several possible cellular and/or molecular explanations for how the TZDs might work in vivo (Fig. 2).

Direct effects on fat, muscle, and liver. Although PPAR-γ levels are 10–30 times higher in fat than in muscle or liver, this receptor is expressed in these latter tissues. Pharmacological doses of TZDs may be sufficient to stimulate PPAR-γ at all of these sites and hence achieve alterations in gene expression that can reduce insulin resistance. In addition,

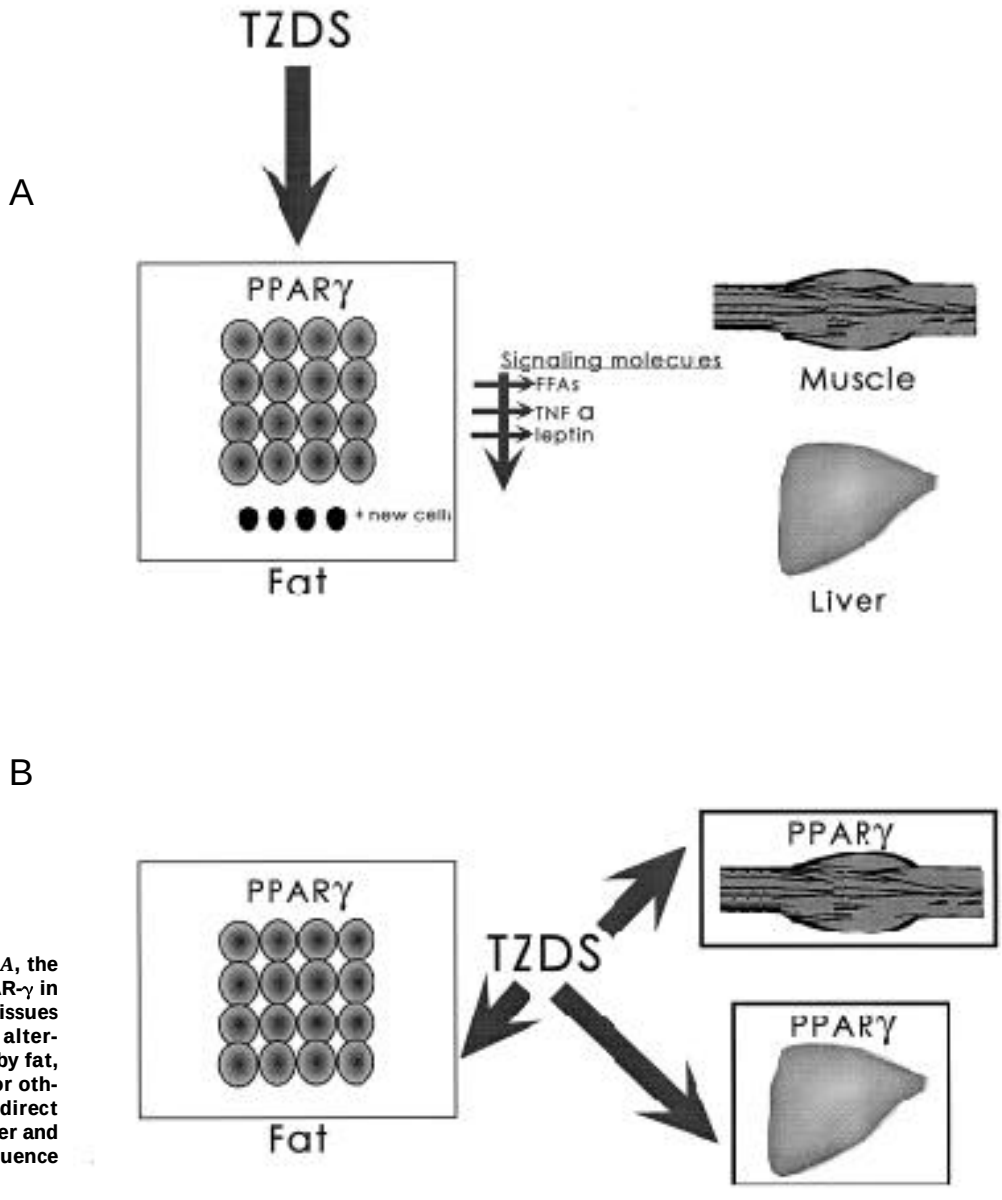

FIG. 2. Tissue targets for TZD drugs. In *A*, the primary tissue target for the TZDs is PPAR-γ in fat. Effects on insulin action in other tissues would then occur as a consequence of alterations in signaling molecules produced by fat, such as free fatty acids, TNF-α, leptin, or others. Alternatively (*B*), TZDs may have direct actions on PPAR-γ in fat, muscle, and liver and control the expression of genes that influence insulin action in these tissues.

there is evidence from experiments in cell culture that activation of PPAR-γ by ligands can increase the expression of this receptor in a positive-feedback loop. It is entirely possible that PPAR-γ levels are initially low in insulin-resistant patients but become significantly elevated in muscle and liver during TZD treatment. Most recently, troglitazone has been shown to improve insulin sensitivity in an experimental model of lipodystrophy in mice, suggesting that important regulation of PPAR-γ can occur in the absence or near absence of fat (30).

Effects on fat cell differentiation. As described above, activation of PPAR-γ by TZDs promotes adipose-cell differentiation. In the absence of increased energy storage, this would be expected to produce more fat cells of a smaller average size. Because smaller adipose cells are usually more sensitive to insulin, such a differentiative response would be expected to produce greater insulin-dependent glucose uptake (24). In addition, because insulin is a powerful antilipolytic agent, smaller fat cells with increased insulin sensitivity would be expected to have lower relative rates of lipolysis. Because high levels of free fatty acids may be causally involved in the induction of insulin resistance, this could affect insulin sensitivity at distant sites such as muscle and liver (e.g., 31,32). This mechanism would also be compatible with the so-called Randle Effect, where the tendency of muscle to utilize glucose as an energy source is inversely correlated with the use of fatty acids as an energy source. There are also reports that TZD administration to rodents greatly increases the amounts of brown adipose tissue (33,34). Considering that this tissue functions to dissipate energy through the oxidation of fatty acids, it could also reduce circulating lipid levels and have a beneficial effect on insulin sensitivity, as discussed above.

Control of adipose-cell signaling. Since it is now appreciated that adipose cells send molecular signals to other tissues participating in energy metabolism, it is possible that PPAR-γ activation controls one or more genes that regulate systemic insulin sensitivity. Two interesting candidate genes

in this regard are TNF-α and leptin. A large and increasing body of data suggests that TNF-α is produced by adipose cells and is overexpressed in obesity and insulin resistance (1). In experimental obese animals, neutralization of TNF-α with a soluble receptor-IgG fusion protein showed a significant improvement in systemic insulin sensitivity (35). Most recently, experiments using genetic "knockouts" of the TNF-α ligand or the p55 TNF receptor definitively demonstrate a crucial role for this cytokine in insulin resistance in vivo (36). The role of TNF-α in human insulin resistance remains to be determined, though a small study using an anti–TNF-α antibody in established NIDDM did not ameliorate hyperglycemia or hyperinsulinemia (37). TZDs apparently have significant effects on two branches of the TNF-α system. Treatment of obese mice with pioglitazone for 2 weeks decreased adipose TNF-α mRNA by 50% (38). Most recently, TZDs have been shown to block the ability of TNF-α to interfere with the most proximal events of insulin signaling (39). Although TNF-α treatment of cultured adipocytes blocks insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate 1, these tyrosine phosphorylations occur normally in the presence of TNF-α if cells are pretreated for several hours with TZDs. This effect of the TZDs has an apparent specificity for the insulin-signaling cascades because TNF-α is able to induce the transcription factor NFκB whether or not TZDs are added. In an independent series of experiments, similar effects of TZDs have also been observed in vivo, as application of troglitazone to rats prevented the induction of insulin resistance caused by an infusion of TNF-α (40). TZDs have also been implicated in the regulation of leptin expression. Application of TZDs in vivo or to cultured adipose cells can cause a reduction in the expression of leptin mRNA and protein (41,42). Although the role of leptin in insulin resistance is a controversial one, some reports indicate that leptin might interfere with insulin signaling in certain cell types (e.g., 43). Hence, a role of the TZDs through leptin must be considered possible.

Finally, as mentioned above, the release of fatty acids during lipolysis has taken on potentially great significance, with the recognition that these substrates may be important signaling molecules themselves. Improvement of insulin signaling in fat by the TZDs would be expected to reduce lipolysis and diminish whatever impact elevated circulating fatty acids have in systemic insulin resistance. Taken together, these and other secreted fat-derived signaling molecules are good candidates to play some considerable role in systemic insulin resistance. Regulation of these processes by TZDs through PPAR-γ seems likely to contribute to their actions. The quantitative nature of this contribution in different animal models and patient subsets remains to be determined. Ultimately, the contribution of PPAR-γ in individual tissues to the overall antidiabetic effects of the TZDs will be determined through tissue-specific genetic ablation in mice. This is well under way in several laboratories.

Target genes for PPAR-γ. Of course, it is not possible to determine which potential target genes for PPAR-γ are most relevant for the antidiabetic action of the TZDs until the tissue(s) directly affected by these drugs are clarified. Most of the work done to date has been on adipose cells; and even here, the target genes relevant for amelioration of insulin resistance have not been clearly determined. Similarly, the downstream targets that trigger adipogenesis per se are not known. In differentiated cells and tissues, TNF-α and leptin expression are reduced by PPAR-γ activation. In addition, two genes of fatty-acid metabolism, lipoprotein lipase and the fatty-acid binding protein aP2, appear to be direct targets of PPAR-γ activation. Increased levels of lipoprotein lipase in fat have been shown to occur as a consequence of TZD treatment in vivo (44). This would be expected to increase uptake of triglycerides by fat and thereby improve insulin signaling in muscle and liver. Increased aP2 could affect many aspects of fatty-acid metabolism, though a "knock-out" of aP2 function in mice has shown a decreased tendency to develop insulin resistance (45). The resolution of this potential paradox awaits further studies on the exact function of aP2. Another potentially important target gene for PPAR-γ is GLUT4. The expression of this gene is increased in cultured adipocytes and fat tissue through PPAR-γ activation by TZDs (46,47); presumably, this could contribute to reduced hyperglycemia. Of course, because muscle is the major sink for insulin-dependent glucose disposal, it will be important to determine if GLUT4 is induced under these conditions in muscle.

FUTURE DEVELOPMENTS: BETTER PPAR-γ LIGANDS?

The TZDs hold enormous promise for the treatment of patients with NIDDM. Because these drugs were originally developed without any knowledge of their molecular targets, there is hope that the research described above will lead to the development of new and better PPAR-γ ligands. This development is important because for all the promise of these drugs, they do not normalize the glucose levels of most diabetic patients when used alone, and many patients do not respond well clinically even when TZDs are used in combination with insulin.

The word "better" can be thought of in at least two ways: potency and efficacy. Given that the potency of a particular ligand will ultimately depend on its affinity for PPAR-γ, there is probably much room for improvement. Most of the antidiabetic TZDs have affinities for PPAR-γ between 30 nmol/l (BRL49653) and 700 nmol/l (troglitazone). With the use of modern drug-screening procedures, large chemical libraries, and combinatorial chemistry methods, it is very likely that agents with higher affinity for PPAR-γ can be found. Presumably, this will include many agents from outside the TZD class.

Efficacy may be a trickier issue. Although the affinity of troglitazone for PPAR-γ is relatively low, normal therapeutic doses of this drug result in blood levels that exceed its K_d for the receptor. Hence, further increases in receptor occupancy may not be possible. Indeed, clinical studies suggest that efficacy of troglitazone is maximal in the 400–600 mg/day range, with little or no benefit resulting from further dose escalation (25).

Improvements in efficacy may reside in two areas: ligands that have more full agonist activity on PPAR-γ or agents that stimulate the PPAR-γ/RXR heterodimer in ways that troglitazone or the other TZDs do not. In this regard, a recent report that RXR agonists could have antidiabetic action in obese/diabetic rodents carries exciting promise (23). These drugs working together with a PPAR-γ ligand could theoretically lower glucose more than either one alone. However, it is not clear from the studies done to date whether maximal costimulation of this heterodimeric receptor pair gives a larger glucose-lowering effect than does maximal stimulation of PPAR-γ alone. Another key issue will be whether stimula-

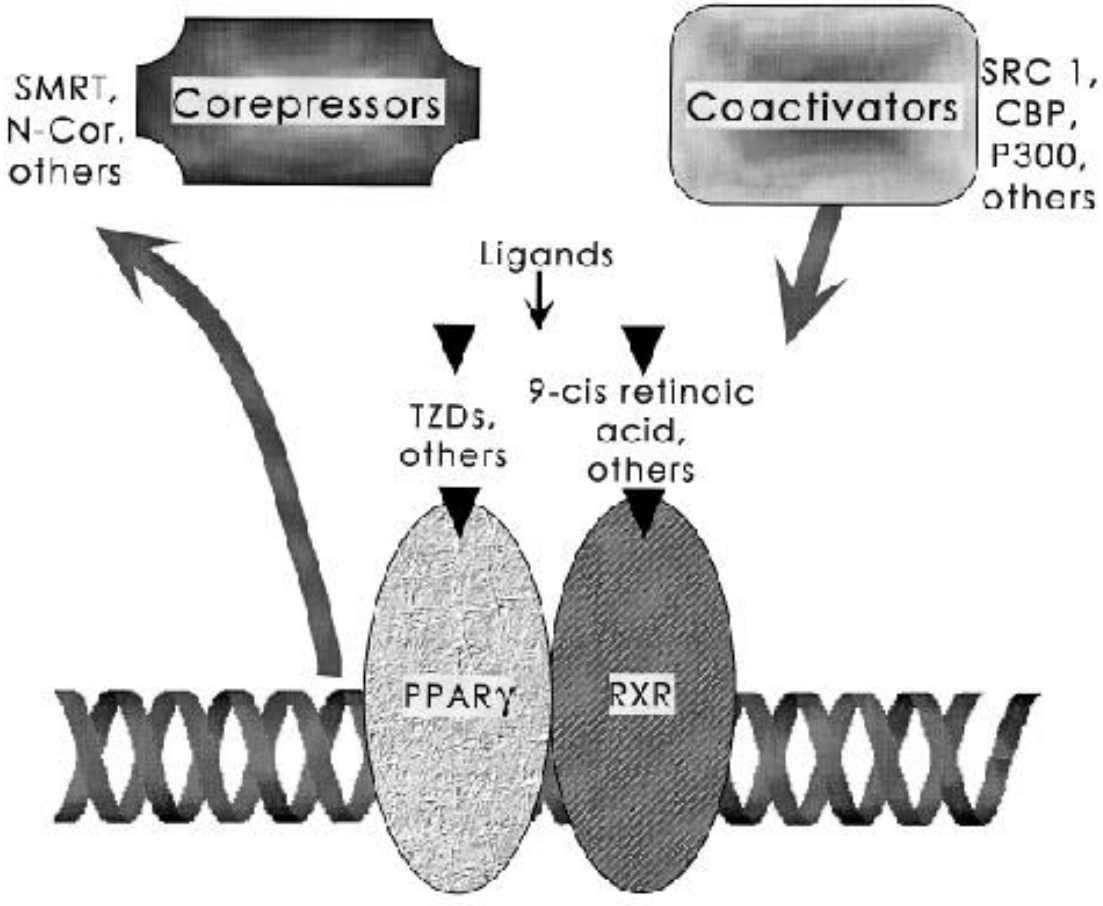

FIG. 3. Docking of cofactors for PPAR-γ through ligand binding. The PPAR-γ/RXR heterodimer binds to distinct DNA sequence elements called DR-1 sites. The binding of ligands to nuclear receptors is now understood to stimulate release of negative factors (corepressors) and trigger binding of positive cofactors (coactivators). Many of these proteins modulate the acetylation state of histones and serve to open chromatin for more efficient transcription.

tion of RXR, a heterodimeric partner of many other nuclear receptors—such as the other PPAR-s, the thyroid hormone receptor, vitamin D receptor, and retinoic acid receptors—will result in unacceptable side effects. Although none have been observed in the early studies in rodents, the chronic nature of diabetes suggests that careful long-term studies will be required to assess the issue of side effects.

Several recent studies show that the transcriptional activity of PPAR-γ stimulated by TZDs can be sharply reduced as a result of phosphorylation by the enzyme mitogen-activated protein kinase (48,49). Studies to date also suggest that under most common culture conditions, some significant portion of PPAR-γ is in the less active, phosphorylated state. The biochemical basis for this decreased activity is not yet known, but it could theoretically involve a decreased affinity for ligand by the phosphorylated receptor. Alternatively, phosphorylation could alter interactions with important (and as yet undefined) protein factors of PPAR-γ, such as corepressors or coactivators. In fact, it is now known that nuclear receptors function as ligand-gated platforms for the assembly of these cofactors into large protein complexes on specific DNA sequences (50–52) (Fig. 3). Some of these coactivator proteins (CBP/p300, SRC1, pCAF) have histone acetyltransferase activity that functions to "open" the configuration of chromatin, allowing more efficient transcription. One theoretical problem is that essentially none of the nuclear receptor coactivators or corepressors identified to date are selective for particular receptors. Hence, it is not clear which cofactors are more important for the function of any particular receptor. It is also not obvious how the tremendous specificity of biological actions of the individual nuclear receptors are generated. It is extremely likely that many more cofactors will be identified, including some that function selectively for individual receptors, including PPAR-γ. Once this issue is clarified, the appropriate cofactor molecules may then serve in the development of assays that could yield greater efficacy in the improvement of insulin sensitivity.

Of course, learning more about the components of the PPAR-γ signaling system—endogenous ligands and the enzymes that produce them, receptor levels and modifications, coactivators and corepressors, downstream transcriptional targets—may also lead to a better understanding of the pathogenesis of NIDDM. A genetic defect in any of these aspects of this receptor system could result in reduced insulin action, and further progress identifying these components will allow scrutiny in diabetic patients.

In summary, the last several years have seen a remarkable improvement in our understanding of the role of PPAR-γ as

a receptor for the TZD drugs and as a key regulator of adipocyte differentiation. Considerable gaps in our knowledge still exist, particularly with regard to PPAR-γ cofactors and the direct tissue targets and downstream-effector genes of these drugs. However, our knowledge has advanced sufficiently to make it likely that the full therapeutic potential of PPAR-γ ligands will be revealed by the combination of modern cell and molecular biology, coupled with the newest techniques of drug development. That improvements beyond the current generation of TZD drugs can be made in potency and/or safety is, in my view, a virtual certainty. It is also highly likely that more efficacious drug regimens that target PPAR-γ and a better understanding of the role of this system in the pathogenesis of NIDDM will also be developed.

ACKNOWLEDGMENTS

I am grateful to several colleagues for critical comments and suggestions on this manuscript, including Drs. Evan Rosen, Stephen Farmer, Timothy Willson, Juergen Lehmann, and Alan Saltiel.

REFERENCES

1. Spiegelman BM, Flier JS: Adipogenesis and obesity: rounding out the big picture. *Cell* 87:377–389, 1996
2. Graves RA, Tontonoz P, Spiegelman BM: Analysis of a tissue-specific enhancer: ARF6 regulates adipogenic gene expression. *Mol Cell Biol* 12:1202–1208, 1992
3. Tontonoz P, Hu E, Graves RA, Budavari AB, Spiegelman BM: mPPAR-γ2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8:1224–1234, 1994
4. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W: Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 68:879–887, 1992
5. Zhu Y, Alvares K, Huang Q, Rao MS, Reddy JK: Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. *J Biol Chem* 268:26817–26820, 1993
6. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM: Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 91:7355–7359, 1994
7. Tontonoz P, Graves RA, Budavari AB, Erdjument-Bromage H, Lui M, Hu E, Tempst P, Spiegelman BM: Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPARγ2 and RXRα. *Nucleic Acids Res* 22:5628–5634, 1994
8. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS: Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99:2416–2422, 1997
9. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM: PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* 15:351–357, 1995
10. Tontonoz P, Hu E, Spiegelman BM: Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell* 79:1147–1156, 1994
11. Brun RP, Kim JB, Hu E, Spiegelman BM: Peroxisome proliferator-activated receptor gamma and the control of adipogenesis. *Curr Opin Lipidol* 8:212–218, 1997
12. MacDougald OA, Lane MD: Transcriptional regulation of gene expression during adipocyte differentiation. *Ann Rev Biochem* 64:345–373, 1995
13. Hu E, Tontonoz P, Spiegelman BM: Transdifferentiation of myoblasts by the adipogenic transcription factors PPARγ and C/EBPα. *Proc Natl Acad Sci USA* 92:9856–9860, 1995
14. Tontonoz P, Kim JB, Graves RA, Spiegelman BM: ADD1: a novel helix-loop-helix protein associated with adipocyte determination and differentiation. *Mol Cell Biol* 13:4753–4759, 1993
15. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL, Brown MS: SREBP-1, a basic helix-loop-helix, loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell* 75:187–197, 1993
16. Kim JB, Spiegelman BM: ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 10:1096–1107, 1996
17. Wu Z, Xie Y, Bucher NLR, Farmer SR: Conditional ectopic expression of C/EBP-β in NIH-3T3 cells induces PPARγ and stimulates adipogenesis. *Genes Dev* 9:2350–2356, 1995
18. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, Reddy JK: Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two PPAR gamma isoforms. *Proc Natl Acad Sci USA* 92:7921–7925, 1995
19. Harris PK, Kletzien RF: Localization of a pioglitazone response element in the adipocyte fatty acid-binding protein gene. *Mol Pharmacol* 45:439–445, 1994
20. Lehmann JM, Morre LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ. *J Biol Chem* 270:12953–12956, 1995
21. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM: 15-deoxy-$\Delta^{12,14}$-Prostagalandin J2 is a ligand for the adipocyte determination factor PPARγ. *Cell* 83:803–812, 1995
22. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, Beck KD, Moore LB, Kliewer SA, Lehmann JM: The structure-activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. *J Med Chem* 39:665–668, 1996
23. Mukherjee R, Davies PJ, Crombi DL, Bischoff ED, Cesario RM, Jow L, Boehm MF, Modon CE, Nadzan AM, Paterniti JR Jr, Heyman RA: Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* 386:407–410, 1997
24. Hallakou S, Doare L, Foufelle F, Kergoat M, Guerre-Millo M, Berthault MF, Dugail I, Morin J, Auwerx J, Ferre P: Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rat. *Diabetes* 46:1393–1399, 1997
25. Henry RR: Thiazolidinediones. *Endocrinol Metab Clin North Am* 26:9553–9573, 1997
26. Gimble JM, Robinson CE, Wu X, Kelly KA, Rodriguez BR, Kliewer SA, Lehmann JM, Morris DC: Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol* 50:1087–1094, 1996
27. Food and Drug Administration: FDA Talk Paper. Washington, DC, U.S. Department of Health and Human Services, December 1, 1997
28. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM: A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 83:813–819, 1995
29. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM: Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci USA* 94:4318–4323, 1997
30. Burant CF, Sreenan S, Hirano K, Tai T-AC, Lohmiller J, Lukens J, Davidson NO, Ross S, Graves RA: Troglitazone action is independent of adipose tissue. *J Clin Invest* 100:2900–2908, 1997
31. Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44:1038–1045, 1995
32. Rebrin K, Steil GM, Mittelman SD, Bergman RN: Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J Clin Invest* 98:741–749, 1996
33. Tai TAC, Jennermann C, Brown KK, Oliver BB, MacGinnitie MA, Wilkison WO, Brown HR, Lehmann JM, Kliewer SA, Morris DC, Graves RA: Activation of the nuclear receptor peroxisome proliferator-activated receptor gamma promotes brown adipocyte differentiation. *J Biol Chem* 271:29909–29914, 1996
34. Foellmi-Adams LA, Wyse BM, Herron D, Nedergaard J, Kletzien RF: Induction of uncoupling protein in brown adipose tissue: synergy between norepinephrine and pioglitazone, an insulin-sensitizing agent. *Biochem Pharm* 52:693–701, 1996
35. Hotamisligil G, Shargill N, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
36. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389:610–614, 1997
37. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor P: Effects of an engineered human anti-TNF-α antibody (CD571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45:881–885, 1996
38. Hofman C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil GS, Spiegelman BM: Altered gene expression for tumor necrosis factor-a and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* 134:264–270, 1994
39. Peraldi P, Xu M, Spiegelman BM: Thiazolidinediones block TNF-α-induced inhibition of insulin signaling. *J Clin Invest* 100:1863–1869, 1997
40. Miles PDG, Romeo OM, Higo K, Cohen A, Rafaat K, Olefsky JM: TNF-α-induced insulin resistance in vivo and its prevention by troglitazone. *Diabetes*

46:1678–1683, 1997
41. Kallen CB, Lazar MA: Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 93:5793–5796, 1996
42. De Vos P, Lefebvre AM, Miller SG, Guerre-Millo M, Wong K, Saladin R, Hamann LG, Staels B, Briggs MR, Auwerx J: Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *J Clin Invest* 98:1004–1009, 1996
43. Muller G, Ertl J, Gerl M, Preibisch G: Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J Biol Chem* 272:10585–10593, 1997
44. Lefebvre AM, Peinado-Onsurbe J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J, Staels B: Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler Thromb Vasc Biol* 17:1756–1764, 1997
45. Hotamisligil GS, Distel RJ, Johnson RS, Papaioannou V, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in the fatty acid binding protein aP2. *Science*, 274:1377–1379, 1996
46. Young PW, Cawthorne MA, Coyle PJ, Holder JC, Holman GD, Kozka IJ, Kirkham DM, Lister CA, Smith SA: Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes: association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. *Diabetes* 44:1087–1092, 1995
47. Wu Z, Xie Y, Bucher NLR, Farmer SR: PPAR(gamma) induces the insulin-dependent glucose transporter, GLUT4, in the absence of C/EBP(alpha) during the conversion of 3T3 fibroblasts into adipocytes. *J Clin Invest* 101:22–32, 1998
48. Hu E, Kim JB, Sarraf P, Spiegelman BM: Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science* 274:2100–2103, 1996
49. Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK: Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. *J Biol Chem* 272:5128–5132, 1997
50. Shibata H, Spencer TE, Onate SA, Jenster G, Tsai SY, Tsai MJ, O'Malley BW: Role of co-activators and co-repressors in the mechanism of steroid thyroid receptor action. *Recent Prog Horm Res* 52:141–164, 1997
51. Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG: The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* 387:677–684, 1997
52. Pazin MJ, Kadonaga JT: What's up and down with histone deacetylation and transcription? *Cell* 89:325–328, 1997

Author Queries (please see Q in margin and underlined text)

Q1: Please spell out ETYA.>
Q2: Please spell out C/EBP.>
Q3: Please spell out ADD1/SREBP1, if possible.>
Q4: Do you mean kilodaltons or K_d? Please specify what KD stands for.>
Q4a: Should this read “expected to result” not “expected in result” as it is now?
Q5: Please clarify what K_d stands for.>

Ref 27: Please add more information for ref. 27. Can the FDA be considered the author of the paper?
What is the title of the paper?
>
Ref 30: Can you update ref. 30 now?>
Ref 40: Please add the journal title to ref. 40.>
Ref 47: Can you update ref. 47 now?>