Insulin resistance is a characteristic feature of most patients with type 2 diabetes mellitus and is almost a universal finding in type 2 diabetic patients who are overweight (1–3). The presence of insulin resistance leads to increased β-cell insulin secretion with compensatory hyperinsulinemia (1–3). As long as the hyperinsulinemia is adequate to overcome the insulin resistance, glucose tolerance remains normal. In patients destined to develop type 2 diabetes, the β-cell compensatory response declines, and relative, or absolute, insulin insufficiency develops. At this point, insulin secretion cannot keep pace with the underlying insulin resistance, and glucose intolerance and eventually frank type 2 diabetes occur. Based on these observations it is evident that, except for very unusual patients, type 2 diabetes only develops in the context of insulin resistance plus β-cell dysfunction. Although there is still some debate as to whether the insulin resistance or the β-cell defect comes first, most epidemiologic studies have indicated that in the early, prediabetic state, insulin resistance is the antecedent abnormality (4, 5). Since type 2 diabetes only develops in insulin-resistant patients with a concomitant β-cell defect, it follows that there are many subject groups with insulin resistance who do not have diabetes. Some of the major categories of nondiabetic insulin resistance include simple obesity, polycystic ovarian syndrome (PCOS), and aging. There are a number […]
Treatment of insulin resistance with peroxisome proliferator–activated receptor γ agonists

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Insulin resistance is a characteristic feature of most patients with type 2 diabetes mellitus and is almost a universal finding in type 2 diabetic patients who are overweight (1–3). The presence of insulin resistance leads to increased β-cell insulin secretion with compensatory hyperinsulinemia (1–3). As long as the hyperinsulinemia is adequate to overcome the insulin resistance, glucose tolerance remains normal. In patients destined to develop type 2 diabetes, the β-cell compensatory response declines, and relative, or absolute, insulin insufficiency develops. At this point, insulin secretion cannot keep pace with the underlying insulin resistance, and glucose intolerance and eventually frank type 2 diabetes occur. Based on these observations it is evident that, except for very unusual patients, type 2 diabetes only develops in the context of insulin resistance plus β-cell dysfunction. Although there is still some debate as to whether the insulin resistance or the β-cell defect comes first, most epidemiologic studies have indicated that in the early, prediabetic state, insulin resistance is the antecedent abnormality (4, 5).

Since type 2 diabetes only develops in insulin-resistant patients with a concomitant β-cell defect, it follows that there are many subject groups with insulin resistance who do not have diabetes. Some of the major categories of nondiabetic insulin resistance include simple obesity, polycystic ovarian syndrome (PCOS), and aging. There are a number of other abnormalities that are associated with insulin resistance, and Reaven has collected this constellation of abnormalities under the term “syndrome X” (2). Syndrome X refers to patients who are insulin-resistant and hyperinsulinemic and have dyslipidemias (usually elevated triglyceride and decreased HDL levels). Frequently these subjects can have hypertension, elevated uric acid levels, and increased plasminogen-activator inhibitor-1 (PAI-1) levels (5). Much epidemiologic evidence has accumulated indicating that patients with syndrome X (also called the metabolic syndrome or the insulin resistance syndrome) have enhanced propensity to develop cardiovascular disease. Thus, while it is well established that treatment of insulin resistance has beneficial effects in patients with type 2 diabetes mellitus, it is also possible that enhancing insulin sensitivity will be therapeutically important for nondiabetic individuals, such as patients with syndrome X or PCOS. In recent years, these speculations have moved from theoretical research concepts to practical reality. Thus, the introduction of thiazolidinediones (TZD) insulin-sensitizing drugs has allowed us to move pharmacologic treatment of insulin resistance from the bench to the bedside. TZDs are a class of compounds that improve insulin action in vivo and have recently been introduced as therapeutic agents for the treatment of type 2 diabetes (6–8). A great deal of information is available on TZDs with respect to animal studies, clinical research, and in vitro mechanisms of action.

Animal studies with TZDs
TZDs have been administered to a variety of insulin-resistant obese and diabetic animal models (9–13). These drugs have uniformly been shown to reduce plasma glucose levels in insulin-resistant diabetic mice and rats and concomitantly to lower insulinemia. This combination of reduced glucose and insulin levels indicated that these agents improved insulin resistance, and this has been directly borne out by formal studies of insulin sensitivity in TZD-treated animals. Thus, using the euglycemic glucose clamp technique, treatment with a variety of TZDs has been shown to improve insulin-stimulated glucose disposal, as well as insulin inhibition of hepatic glucose production in Zucker fatty rats (9), Zucker diabetic fatty rats (10), fructose-fed insulin resistant rats (11), TNF-α-treated insulin resistant rats (12), glucosamine-treated insulin-resistant rats (13), as well as fat-fed rats (14). All of these represent standard models of genetic or acquired insulin resistance, some associated with obesity and some not, and, taken together, clearly demonstrate that TZDs can improve insulin action across a wide spectrum of insulin-resistant states, regardless of the underlying mechanisms. It is important to note that in these rodent models, the TZDs are remarkably efficacious. In many instances, glucose levels are reduced to normal in diabetic animal models, and insulin resistance can be completely reversed or prevented. This has led to some interesting models; e.g., animals can be obese but not insulin-resistant, which can allow one to study the effects of a particular perturbation (obesity, TNF-α treatment, lipid infusions, etc.) without the concomitant variable of insulin resistance.

TZDs and human insulin resistance
Based on the uniformly positive and encouraging results in animals, many studies have now been completed using TZD treatment in various diabetic and
insulin-resistant study groups in man. In terms of human studies, TZDs have been most extensively evaluated in patients with type 2 diabetes. Initial clinical research studies with TZDs clearly demonstrated that treatment of type 2 diabetic patients with these agents lowered both fasting and postprandial glucose levels, as well as circulating insulin levels (15, 16). This combination of findings is consistent with the presumed action of these compounds as insulin-sensitizing agents, as was directly demonstrated by performing glucose clamps in type 2 diabetic patients before and after a period of TZD treatment. These studies showed that essentially all patients exhibited an improvement in insulin-stimulated glucose disposal after the drug treatment period, and on average this amounted to a 30% increase in this action of insulin (15). Elevated hepatic glucose production rates are a characteristic feature of type 2 diabetic patients with fasting hyperglycemia (1), and this is also a manifestation of insulin resistance. The effects of TZD treatment on basal hepatic glucose production rates in type 2 diabetic patients have been somewhat variable. Some studies have shown striking decreases in this abnormality (15), while others have shown either no effect (17) or effects only at higher doses of TZDs (18). Furthermore, it is not clear whether the effects of TZDs to lower hepatic glucose production rates represent direct actions on the liver, or indirect beneficial effects secondary to some other aspect of the improved metabolic environment produced by these drugs. For example, TZD treatment reduces FFA levels, which may secondarily lower hepatic glucose output.

TZDs have also been used in the treatment of nondiabetic human insulin-resistant states. For example, treatment of obese nondiabetic subjects, subjects with impaired glucose tolerance (IGT), and women with PCOS have all demonstrated an improvement in insulin sensitivity (19–21). The mechanisms of insulin resistance are almost certainly heterogeneous across all these human conditions. Thus, these results are consistent with what has been learned from animal studies in that TZDs are effective at ameliorating insulin resistance regardless of the diverse underlying genetic and acquired mechanisms. It is also important to point out that, unlike in the animal studies, the effects of TZDs result in only partial improvement in insulin sensitivity. Thus, TZD treatment leads to anywhere from a 20–40% improvement in insulin-stimulated glucose disposal in human insulin-resistant states, compared with near normalization in insulin-resistant animals. Therefore, even after effective TZD treatment, patients with type 2 diabetes, obesity, IGT, and PCOS still remain moderately insulin-resistant.

Treatment of patients with TZDs seems to have beneficial effects on most, if not all, of the components of syndrome X (see Ginsberg, this Perspective series, ref. 22). For example, not only does TZD treatment improve insulin sensitivity, but it also leads to a reduction in circulating triglyceride levels, modest increases in HDL levels, decreased blood pressure, and reductions in PAI-1 levels (6, 19, 23). Since treatment with the insulin-sensitizing TZDs can improve most of the manifestations of syndrome X, this provides pharmacologic evidence that insulin resistance is the core abnormality in these patients and that the associated abnormalities are, in some way, mechanistically related to the impairment of insulin action.

The emergence of TZDs as insulin-sensitizing agents in animals and, more importantly, in humans, has generated enormous interest in studying the molecular mechanisms to explain the pharmacologic actions of these drugs. Despite this intense interest, these mechanisms remained relatively obscure until Ibrahim et al. (24) discovered that these agents could behave as agonists for the peroxisome proliferator–activated receptor γ (PPARγ) nuclear receptor (see refs. 6, 7 for reviews on the molecular and biochemical details of TZD-PPARγ interactions). PPARγ is a member of the PPAR family of nuclear receptors, which includes PPARα, PPARδ, and PPARβ and PPARγ (25). PPARγ2 is a splice variant of PPARγ containing 30 additional amino-terminal amino acids, and it is expressed primarily in fat (26). Various lipid or prostaglandin molecules have been proposed as natural PPARγ agonists (27, 28). The current concept is that PPARγ receptors exist as heterodimers with retinoid X receptors (RXRs) and bind to PPAR response elements (PPREs) within the promoter domains of target genes (Figure 1).

In the unliganded state, the PPARγ RXR heterodimer is associated with a multiprotein corepressor complex that contains histone deacetylase activity. The deacetylated state of histone tends to keep the nucleosome in a state in which transcription is inhibited. Once a PPARγ ligand binds to the receptor, the corepressor complex dissociates and a coactivator complex, containing histone acetylase activity, is recruited to the PPARγ RXR heterodimer. Acetylation of histone is one factor in chromatin remodeling which facilitates active gene transcription (29). PPARγ activation promotes differentiation of adipocytes, as well as other cell types, and this is associated with induction of lipogenic enzymes as well as glucoregulatory proteins. It is assumed that normal PPARγ interactions with its endogenous ligands help to maintain the proper level of key glucoregulatory molecules so that tissues exhibit a state of normal insulin sensitivity. TZDs, then, activate PPARγ receptors leading to the induction of glucoregulatory molecules and enhanced insulin sensitivity.

**Targets and mechanisms of TZD action**

An important but unresolved question concerns the tissue site of action of the TZDs. Do these agents produce in vivo insulin-sensitizing effects by altering expression of fat-cell genes, which, in turn, convey some signal (metabolic or otherwise) to skeletal muscle, leading to improved insulin sensitivity; or can these agents exert direct effects...
Lessons from animal and human PPARγ mutations

To define the roles of PPARγ receptors in glucose homeostasis and insulin resistance, several groups have generated knockout mice lacking the receptor gene (see Kadowaki, this Perspective series, ref. 38). Homozygous PPARγ null animals are not viable, but heterozygous PPARγ+/– mice have allowed these researchers to examine the effect of a 50% reduction in the dosage of the PPARγ receptor on relevant physiologic pathways (39, 40). Entering these studies, the notion was that these animals, missing one PPARγ allele, would be insulin-resistant and possibly diabetic, but on a normal chow diet, these animals displayed normal growth and development, normal body weight, and normal adipose tissue mass (40), as well as normal basal glucose and FFA levels. During oral glucose tolerance tests, glucose levels were comparable to wild-type controls, but decreased insulin levels were observed in PPARγ–/– mice (40). Normal glucose tolerance in the face of reduced insulinemia suggests a state of enhanced insulin sensitivity. This suggestion was confirmed in studies of GDR in animals maintained under euglycemic conditions. These studies demonstrated a 35% increase in insulin-stimulated GDR and a 60% augmentation of insulin inhibition of hepatic glucose production in PPARγ–/– mice as compared with wild-type littermates. Thus, contrary to the expected results, these studies show that a 50% reduction in PPARγ receptors led to enhanced insulin sensitivity in the peripheral tissues, as measured by increased GDR, as well as in the liver, as seen in the exaggerated suppression of hepatic glucose production.

In this case, the enhanced insulin sensitivity of PPARγ–/– mice runs counter to what would have been expected based on the biology of PPARγ receptors and the effects of TZD (see Kadowaki, this Perspective series, ref. 38). The phenotypes of PPARγ–/– mice raise the intriguing possibility that inhibition of PPARγ could render animals, or people, less susceptible to endogenous and exogenous causes of insulin resistance. Whether the effects of obesity, aging, or other insults that impair insulin action could be prevented or ameliorated by inhibition of PPARγ function remains to be determined. These results have led to the hypothesis that the normal role of PPARγ receptors and their natural ligands might be to dampen insulin action, thereby promoting a state of insulin resistance (40). In this event, decreased expression of PPARγ receptors, as seen in PPARγ–/– mice, would partially alleviate this dampening effect, leading to heightened insulin sensitivity.

Reports of mutations in the PPARγ gene in humans are beginning to emerge, and although some of these reports are consistent with the observations on PPARγ–/– mice, they present a somewhat confusing picture. For example, Deeb et al. (41) have shown that subjects with an inactivating mutation (pro 12 ala) in the gene for PPARγ2 receptor have decreased insulin levels, enhanced insulin sensitivity, and improvements in other syndrome X features. However, these individuals also had a somewhat lower body mass index, which could possibly confound the results, and other population-based studies have
The proposed ability of endogenous PPARγ receptors to suppress insulin action could offset exaggerated effects on glucose metabolism that occur when PPARγ stimulation leads to unrestrained activation of the differentiation program. At least two molecular mechanisms could account for this effect. The first builds on the observation that many synthetic PPARγ ligands are not pure PPARγ activators but, rather, partial agonists and, therefore, partial antagonists. As such, these molecules can displace endogenous ligands, which are presumed to act as pure agonists. Therefore, TZD-induced insulin sensitization may reflect the ability of these compounds to inhibit the dampening effect on insulin action of endogenous PPARγ ligand(s).

Another possibility, which is consistent with both pharmacologic data and the PPARγ/− findings, concerns the nature of transcriptional activation by PPARγ receptors. Although endogenous PPARγ receptors may simply rotate between a transcriptional activator and inactivator mode, other nuclear receptors, such as the retinoic acid and thyroid hormone receptors, have been shown to actively repress expression of specific target genes when ligand is absent (45). PPARγ receptors contain the functional modules involved in transcription repression, and the PPARγ receptor readily interacts with corepressors in its ligand-free state. Thus, PPARγ-induced transcriptional repression of key glucoregulatory genes may be responsible for dampening of insulin action, with insulin sensitivity restored by genetically reducing the level of the PPARγ receptor or by reversing this repression with a TZD partial agonist.

The concept of TZDs as partial agonists/antagonists has been supported by recent work from Leff and colleagues (46), who used a promoter/reporter assay in 293 cells and found that rosiglitazone and pioglitazone behave as full agonists, albeit with different binding affinities for the PPARγ receptor. In their system, troglitazone was a partial agonist and its antagonistic properties were clearly evident by the fact that this compound inhibited rosiglitazone activation of the promoter/reporter construct. On the other hand, when they examined the induction of the endogenous gene CAP in 3T3-L1 adipocytes, they found that troglitazone was a full agonist. These researchers also showed that different TZDs induced overlapping, but substantially different, sets of genes. For example, it has been shown that there is a set of genes induced (or repressed) by troglitazone, a set of genes induced (or repressed) by rosiglitazone, a set of genes induced (or repressed) by pioglitazone, and a set of genes induced (or repressed) by all three.

Since all of the TZDs readily bind to PPARγ receptors, how can we explain the absence of an effect of a given TZD on a gene whose expression is altered by another such drug? Each TZD has a different binding affinity, with somewhat different residence times on the receptor, and, therefore, various TZDs interact with the bind-
ing pocket in slightly different ways. Hence, as depicted in Figure 2, the three-dimensional conformation of the TZD•PPARγ complex will be different with different ligands; a given TZD•PPARγ complex may interact with a PPRE differently, and it may recruit somewhat different sets of coactivators or corepressors or do so with altered kinetics. These differences in the assembly of the transcriptional complex will be specific to the context of a given gene and its promoter. For example, the degree of histone acetylation in the vicinity of a given promoter might be different, synergistic interactions with other transcription factors might be different, or the effects of coactivators on subsets of genes might differ. In the unliganded state, nuclear receptors repress transcription of target genes by associating with corepressor complexes that contain histone deacetylase activity (47). Ligand binding causes release of the corepressors with subsequent recruitment of a series of coactivators to the transcription complex. Many of these coactivators contain histone acetylase activity, and acetylation serves to facilitate transcription from that gene. Thus, different three-dimensional conformations of the ligand receptor complex may lead to differences in the balance between acetylation and deacetylation, altering transcriptional regulation.

Even if a given PPARγ ligand appears to behave as a full agonist in an vitro promoter/reporter system, this does not mean that it behaves as a full agonist with respect to a given endogenous gene. In fact, the finding that different sets of overlapping genes are induced by different TZDs indicates that these compounds cannot be full agonists at all genes. Thus, if PPARγ ligand A induces gene X, and PPARγ ligand B does not, then it follows that ligand B must be an antagonist to ligand A with respect to gene X. One model, shown in Figure 2, is that unliganded PPARγ receptors, with their RXR heterodimer partners, are associated with PPREs as well as a corepressor complex. PPARγ ligands then bind to the binding domain of the PPARγ receptor. Whether transcription then initiates will depend on the context of the specific promoter and its immediate environment. Since it is assumed that a PPARγ ligand will have access to, and bind to, all PPARγ receptors, if a given PPARγ ligand does not induce transcription (or repression) of a given gene, then it must behave as a partial antagonist to PPARγ ligands that activate or repress that gene. Hence, the ability of a PPARγ ligand to behave as a full or partial agonist or antagonist is specific to the context of a particular promoter. A similar concept has already been proposed for another nuclear receptor, namely the estrogen receptor: the selective estrogen receptor modulator (SERM) idea (48). The SERM concept proposes that different estrogen receptor ligands can have different agonist or antagonist properties depending on the cell context and the specific target gene in question. For the PPARγ receptor, this idea can be called the selective PPAR modulator, or SPPARM, model (49). This model greatly expands the signaling repertoire of a specific nuclear receptor, since it would allow a single receptor to respond to a given endogenous ligand in a way that is gene context–specific. Thus, different endogenous ligands, working through the same nuclear receptor, could lead to different biologic responses.

Prospects

Although much remains to be learned about PPARγ receptors and TZD action, the advent of TZD insulinsensitizing agents has had an enormous impact on our understanding of pathophysiology and clinical medicine, since they represent the first direct means to treat insulin resistance. TZDs provide the proof of principle that pharmacologic treatment of insulin resistance can be of enormous clinical benefit. The goal of uncovering the roles of PPARγ receptors in insulin signaling and the precise mechanisms of TZD action will undoubtedly
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continue to inspire major efforts, which should lead to better treatments for insulin resistance. In addition, the great potential of insulin resistance therapy illuminated by the TZDs will continue to catalyze research in this area directed toward the discovery of new insulin-sensitizing agents that work through other mechanisms.


