

Thiazolidinediones and the Promise of Insulin Sensitization in Type 2 Diabetes

Raymond E. Soccio,¹ Eric R. Chen,¹ and Mitchell A. Lazar^{1,*}

¹Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Department of Genetics, and The Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

*Correspondence: lazar@mail.med.upenn.edu

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Type 2 diabetes is caused by insulin resistance coupled with an inability to produce enough insulin to control blood glucose, and thiazolidinediones (TZDs) are the only current antidiabetic agents that function primarily by increasing insulin sensitivity. However, despite clear benefits in glycemic control, this class of drugs has recently fallen into disuse due to concerns over side effects and adverse events. Here we review the clinical data and attempt to balance the benefits and risks of TZD therapy. We also examine potential mechanisms of action for the beneficial and harmful effects of TZDs, mainly via agonism of the nuclear receptor PPAR γ . Based on critical appraisal of both preclinical and clinical studies, we discuss the prospect of harnessing the insulin sensitizing effects of PPAR γ for more effective, safe, and potentially personalized treatments of type 2 diabetes.

Type 2 diabetes (T2DM) is characterized by insulin resistance and β cell failure, and thiazolidinedione drugs (TZDs) are the only current antidiabetic agents that function primarily by increasing insulin sensitivity. However, this class of drugs has recently fallen into disuse due to concerns over side effects and adverse events. The rise and fall of TZDs is demonstrated by their use in ambulatory diabetes visits: from 6% in 1997 to 41% in 2005 and down to 16% by 2012 (Turner et al., 2014). Anecdotal evidence suggests an even steeper decline since then, as even diabetes specialists now deploy TZDs sparingly, given the proliferation of other treatment options. With this decline in clinical use, research publications on TZDs have also decreased, though not to the same extent (Figure 1), reflecting the continuing promise of insulin sensitization to treat and prevent T2DM and cardiometabolic disease. This review summarizes the beneficial and adverse effects of TZDs, focusing on potential mechanisms for each, and highlights recent clinical, basic, and translational studies from the past several years that have sent the field into new and unexpected directions.

TZDs were first reported as insulin-sensitizing drugs in the early 1980s by the pharmaceutical company Takeda (Fujita et al., 1983), but their mechanism remained a mystery until the mid-1990s, when they were found to be ligands for the nuclear receptor transcription factor PPAR γ (Lehmann et al., 1995). PPAR γ is expressed at high levels in adipose tissue, where it functions as a master regulator of adipocyte differentiation, and at much lower levels in other tissues (Tontonoz and Spiegelman, 2008). The simplest model for TZD function involves PPAR γ agonism in adipose tissue, but recent studies described below suggest alternatives and additions to this model.

TZDs Are Potent Insulin Sensitizers which Treat and Prevent T2DM

Three TZDs have been FDA approved for diabetes: troglitazone (Rezulin), rosiglitazone (Avandia), and pioglitazone (Actos)

(Figure 2 describes various PPAR γ agonist drugs that are discussed in the text). Troglitazone was introduced in 1997 but withdrawn from the market in 2000 due to increased risk of liver failure from fulminant hepatitis (Kohlroser et al., 2000). Some studies suggest the cause is hepatotoxic reactive metabolites of troglitazone (Yokoi, 2010), while others indicate that troglitazone activates the pregnane X receptor in humans, but not rodents (Jones et al., 2000). Though the exact mechanism is still uncertain, PPAR γ activation is not thought to be involved, and hepatotoxicity is not a TZD class effect but an idiosyncratic effect of troglitazone. Rosiglitazone and pioglitazone were both FDA approved in 1999, but pioglitazone has become the TZD of choice for reasons described below.

TZDs lower hemoglobin A1c potentially by \sim 1% as monotherapy in T2DM, where they notably do not cause hypoglycemia like insulin or insulin secretagogues (i.e., sulfonylureas), and they can be used in combination with other antidiabetic agents (reviewed in Yau et al., 2013). The first-line drug metformin is often described as an insulin sensitizer, but its primary effect is suppression of hepatic glucose production, while its effects on peripheral insulin sensitivity are quite small, variable across studies, and absent in a meta-analysis (Natali and Ferrannini, 2006). In the same analysis, TZDs have large and consistent effects improving insulin sensitivity. Furthermore, the ADOPT randomized controlled trial (RCT) showed that rosiglitazone provided more durable glycemic control than metformin or a sulfonylurea (Kahn et al., 2006; Table 1 describes the key clinical studies that are discussed in the text).

Insulin sensitization also appears to be the mechanism whereby TZDs prevent or delay development of T2DM in individuals with prediabetes. The ACT NOW RCT involved 602 patients with impaired glucose tolerance (IGT), and pioglitazone decreased progression to T2DM by 74% over 2.4 years (DeFronzo et al., 2011). Earlier studies of patients with prediabetes showed that troglitazone (Knowler et al., 2005) or rosiglitazone (Gerstein et al., 2006) similarly decreased progression to diabetes.

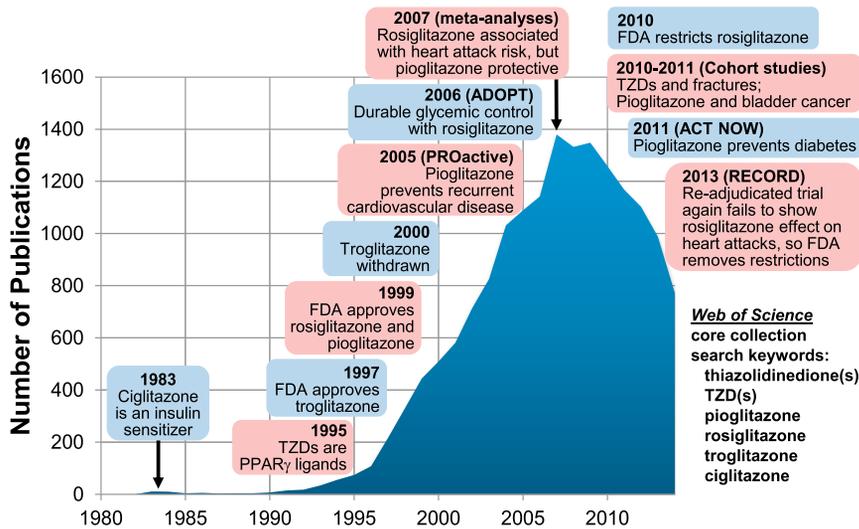


Figure 1. The History of TZDs

The graph shows the annual number of TZD-related publications, with boxes indicating key events in the rise and fall of this drug class. For 2014, publications through May were adjusted to a full year.

TZDs and Insulin Sensitization: Beyond Diabetes

Nonalcoholic fatty liver disease (NAFLD) is now the most common chronic liver disease in the U.S., associated with obesity, insulin resistance, dyslipidemia, and hypertension, as part of the metabolic syndrome (Lomonaco et al., 2013). The mildest form of NAFLD is simple hepatic steatosis, which can progress to nonalcoholic steatohepatitis (NASH), leading to cirrhosis and hepatocellular carcinoma. Metformin acts primarily in liver, yet shows no benefit in NASH (Chalasanani et al., 2012). Dysfunctional adipose tissue plays a key role in NAFLD (Lomonaco et al., 2012), so targeting adipose tissue with TZDs is an attractive treatment option. In a small RCT of 55 patients with IGT or T2DM and NASH, pioglitazone was quite effective at decreasing histological liver fat, inflammation, and fibrosis (Belfort et al., 2006). The subsequent PIVENS RCT with 247 nondiabetic patients showed that pioglitazone improved all secondary NASH endpoints (Sanyal et al., 2010). Excellent reviews of NAFLD have been published recently (Lomonaco et al., 2013), and current practice guidelines endorse the use of pioglitazone for biopsy proven NASH (Chalasanani et al., 2012). Notably, based on the FLIRT trial, rosiglitazone does not appear as effective as pioglitazone in NASH (Ratziu et al., 2010), and gene expression changes in liver biopsies from this trial even suggested proinflammatory changes (Lemoine et al., 2013).

In women with polycystic ovarian syndrome (PCOS), guidelines recommend metformin or TZDs to decrease androgen levels, enhance ovulation, and improve glucose tolerance, though not for first line use in treating hirsutism or infertility (ACOG Committee on Practice Bulletins Gynecology, 2009). In practice, metformin is commonly used in PCOS while pioglitazone is used sparingly, most likely due to concerns over the side effects of pioglitazone and metformin's established safety in pregnancy.

Fluid Retention, Edema, and Congestive Heart Failure Due to TZDs

Water retention due to TZDs was noted early, with about 5% of patients developing peripheral lower extremity edema on TZD monotherapy and even more in combination with other

drugs—up to 18% when combined with insulin (Nesto et al., 2004). Excess fluid can also lead to exacerbations of congestive heart failure (CHF); thus TZDs are contraindicated in patients with symptomatic heart failure (New York Heart Association class III or IV). While TZDs clearly increase CHF events, these are normally responsive to diuretic therapy, and TZDs do not appear to increase mortality from CHF (Lago et al., 2007).

Even without clinical signs of edema or heart failure, TZD-treated patients retain water, typically evidenced by hemodilution. Even this is controversial, as some methods to assess total body water indicate hemodilution actually does not account for the decreased hematocrit on pioglitazone (Berria et al., 2007). Nonetheless, mouse models show that the apparent volume expansion requires PPAR γ expression in the renal collecting duct (Guan et al., 2005; Zhang et al., 2005), and may involve the epithelial sodium transporter as well as other pathways in the renal tubule (reviewed in Beltowski et al., 2013). Regarding CHF, direct effects of TZDs on cardiac muscle are possible, as mouse models with either cardiomyocyte-selective overexpression (Son et al., 2007) or deletion (Duan et al., 2005) of PPAR γ have both reported cardiac dysfunction. The cardiotoxic effects of high doses of rosiglitazone were recently shown to be largely PPAR γ independent and involve mitochondrial dysfunction (He et al., 2014). In human studies, TZDs are associated with lipid accumulation in cardiomyocytes (Marfella et al., 2009), but pioglitazone showed no effect or even beneficial effects on echocardiographic measures of cardiac function (Horio et al., 2005; Sambanis et al., 2008). TZDs are not generally thought to cause cardiomyopathy directly, but rather to exacerbate heart failure via fluid retention in susceptible patients. Recently, some but not all studies have shown an association between TZD use and diabetic macular edema (Idris et al., 2012). The mechanisms for this association may involve the systemic effects of overall volume expansion as well as local effects in the retina.

Weight Gain on TZDs: Both Fluid Retention and Adipose Tissue

Many studies of TZDs show a typical reported weight gain of ~5 kg over 3–5 years. While there is potential for added weight due to fluid retention, there is clearly also expansion of adipose tissue. Studies differ as to the relative contribution of fluid versus adipose tissue: even on the same 45 mg dose of pioglitazone, some measure 75% of the added weight due to water retention (Basu et al., 2006), while others propose 89% due to adipose tissue mass (Berria et al., 2007). Multiple imaging studies consistently show a greater increase in peripheral subcutaneous than

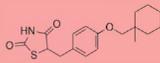
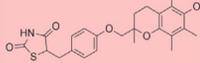
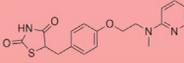
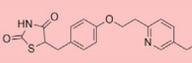
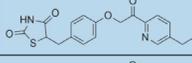
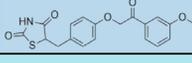
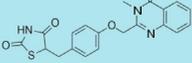
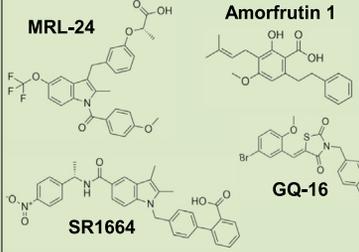
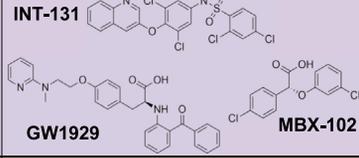
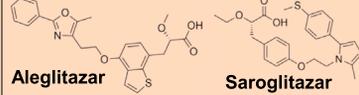
Class	Drugs	Description
Classic PPAR _γ Agonist TZDs	Ciglitazone 	Prototypical first insulin-sensitizing TZD developed in 1983, never used clinically
	Troglitazone (Rezulin) 	First FDA-approved TZD in 1997, withdrawn in 2000 due to idiosyncratic hepatotoxicity
	Rosiglitazone (Avandia) 	Widely used from 1999 until a 2007 meta-analysis suggested association with heart attacks
	Pioglitazone (Actos) 	Current TZD treatment of choice, though use has declined due to risk of side effects and adverse events
mTOT modulator TZDs	MSDC-0160 	Pioglitazone derivatives with less classical PPAR _γ agonism but preserved effects on mitochondria, currently in clinical development
	MSDC-0602 	
Selective PPAR _γ modulators (SSPARM)	Balaglitazone 	Partial agonist, preserved insulin sensitization and trend towards fewer side effects in clinical studies
Partial Agonists Affecting PPAR _γ Ser273	MRL-24 , Amorfrutin 1 , SR1664 , GQ-16 	Inhibit PPAR _γ Ser273 phosphorylation with decreased classical agonism, may preserve insulin sensitization with fewer side effects based on preclinical studies
Non-TZD PPAR _γ agonists	INT-131 , GW1929 , MBX-102 	Non-TZD PPAR _γ agonists with antidiabetic effects and potential for fewer side effects based on preclinical and clinical studies
Dual PPAR Agonists	Aleglitazar , Saroglitazar 	Activate both PPAR _γ and PPAR _α , none approved in US or Europe, aleglitazar investigation halted in phase 2 trials due to safety concerns

Figure 2. PPAR_γ Agonist Drugs

TZDs like pioglitazone and rosiglitazone are potent PPAR_γ agonists (red), but other weaker or partial agonists share the TZD structure with different side chains (blue). There are additional structurally diverse non-TZD PPAR_γ agonists (green and purple), including dual agonists of PPAR_α and PPAR_γ (orange). See text for details.

mouse models showing that rosiglitazone markedly inhibits atherosclerosis (Li et al., 2000). Subsequent meta-analyses also showed increased MI risk, though without increased mortality (Nissen and Wolski, 2010; Singh et al., 2007). Based on such studies, in 2010 the FDA placed restrictions on rosiglitazone through its risk evaluation and mitigation strategies (REMS) program, requiring patient registration and special pharmacies. Only one RCT, an open-label noninferiority study called RECORD, was specifically designed to assess cardiovascular outcomes on rosiglitazone compared to metformin and a sulfonylurea. The interim results were published early in 2007 (Home et al., 2007) and the final results in 2009 (Home et al., 2009), and these were rejudicated by independent investigators at the FDA's request in 2013 (Mahaffey et al., 2013). None of these analyses showed any increased risk of heart attack or death.

Despite RECORD enrolling 4,447 patients, the authors note that it was still underpowered, as a ~60% risk or ~20% benefit of rosiglitazone could not be excluded. Nonetheless, given this new information, in November 2013 the FDA followed advice of an expert panel and removed restrictions on rosiglitazone (FDA, 2013). This decision was supported

visceral fat (reviewed in Bray et al., 2013), and a recent study even found a decrease in visceral fat (Punthakee et al., 2014). Rather than an unwanted effect, this adipose tissue weight gain may be integral to the mechanism of action of TZDs (see below). Indeed, a positive correlation has been reported between the degree of weight gain on rosiglitazone and the improvement in insulin sensitivity (Miyazaki et al., 2005), and between weight gain on pioglitazone and improved cardiovascular outcomes (Doehner et al., 2012).

Cardiovascular Disease: Different Effects of Rosiglitazone and Pioglitazone?

A widely cited meta-analysis in 2007 raised concerns that rosiglitazone was associated with a significant 43% increased risk of myocardial infarction (MI), with a 64% increase in cardiovascular mortality that did not reach statistical significance ($p = 0.06$) (Nissen and Wolski, 2007). Increased MI risk was surprising, given

by a post hoc analysis of the BARI 2D trial, in which 992 subjects on rosiglitazone had no significant change in MI with a trend toward benefit, along with decreased risk of stroke or the composite cardiovascular endpoint (Bach et al., 2013). Furthermore, a recent reassessment of the observational data linking rosiglitazone and MI found many deficiencies and potential for confounding (Rawson, 2014). After the storm of controversy and bad press, it is unlikely this rosiglitazone will ever be widely used again, as there is no unique benefit for this drug compared to pioglitazone—except perhaps for bladder cancer risk (see below). Furthermore, a similar meta-analysis for pioglitazone showed no excess cardiovascular mortality, and in fact an 18% decrease in cardiovascular endpoints (Lincoff et al., 2007). A large observational cohort of U.S. Medicare patients showed that rosiglitazone has cardiovascular harm compared to pioglitazone (Graham et al., 2010), while a UK cohort study showed that pioglitazone but not rosiglitazone reduced all-cause

Table 1. Selected Key Clinical Studies of TZDs and Related Drugs

Trial/Publication	Type	Design	Details	Outcome	Result	Comment
ADOPT: A Diabetes Outcome Progression Trial (Kahn et al., 2006)	RCT	Patients with newly diagnosed T2DM randomized to rosiglitazone, metformin, or glyburide	4,360 patients, median 4 years	Time to monotherapy failure	Only 15% failure at 5 years for rosiglitazone, lower than 21% for metformin and 34% for glyburide	Supports notion that reducing insulin resistance is beneficial in diabetes pathophysiology
ACT NOW: Actos Now for the prevention of diabetes (DeFronzo et al., 2011)	RCT	Patients with prediabetes randomized to pioglitazone or placebo	602 patients, median 2.4 years	Conversion to diabetes	Pioglitazone decreased conversion to diabetes by 72%	Similar studies previously showed diabetes prevention by troglitazone and rosiglitazone
PIVENS: Pioglitazone or Vitamin E for Nonalcoholic Steatohepatitis (NASH) (Sanyal et al., 2010)	RCT	Patients with NASH and without diabetes randomized to pioglitazone, vitamin E, or placebo	247 patients, 96 weeks of treatment	Improvement in histologic features of NASH	Vitamin E, but not pioglitazone, significantly improved primary histological composite endpoint	Pioglitazone improved all secondary endpoints: liver fat, inflammation, and serum aminotransferase levels
Nissen and Wolski, 2007	meta-analysis	Data combined by fixed effects model	42 studies met inclusion criteria	MI or cardiovascular death	Rosiglitazone's odds ratio for MI 1.43 (1.03 to 1.98; p = 0.03), for death 1.64 (0.98 to 2.74; p = 0.06)	Despite many subsequent studies, the association between rosiglitazone and MI remains controversial (see text for details)
RECORD: Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of glycaemia in Diabetes (Home et al., 2007)	RCT	Patients with T2DM on metformin or sulfonylurea monotherapy randomized to addition of rosiglitazone	4,447 patients, mean 5.5 years	Cardiovascular hospitalization or death	No significant increase in cardiovascular mortality with rosiglitazone	Readjudicated results (Mahaffey et al., 2013) support original conclusions, though study was likely underpowered
BARI 2D: Bypass Angioplasty Revascularization Investigation in Type 2 Diabetes (Bach et al., 2013)	post-hoc analysis	In original RCT, patients with T2DM and stable coronary disease were randomized to several interventions	992 on rosiglitazone, mean 4.5 years	Mortality, MI, stroke	No increase in MI on rosiglitazone, with significant decrease in composite endpoint	Agrees with RECORD, though trial not originally designed to study effects of rosiglitazone
TIDE: Thiazolidinedione Intervention with vitamin D Evaluation (Punthakee et al., 2012)	RCT	Patients with T2DM randomized to rosiglitazone, pioglitazone, vitamin D, or placebo	2,553 patients, mean 162 days	MI, stroke, or cardiovascular death	Study initially required by FDA but terminated in 2013 when deemed no longer feasible or necessary	Would have been the only trial comparing rosiglitazone and pioglitazone head to head
CHICAGO: Carotid Intima-Media Thickness (CIMT) in Atherosclerosis using Pioglitazone (Mazzone et al., 2006)	RCT	Patients with T2DM randomized to pioglitazone or glimepiride	462 patients, mean 7.7 years	Change from baseline in CIMT	Pioglitazone slowed progression of CIMT compared to glimepiride	Evidence that pioglitazone is beneficial in atherosclerosis, slowing plaque progression

(Continued on next page)

Table 1. Continued

Trial/Publication	Type	Design	Details	Outcome	Result	Comment
PERISCOPE: Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (Nissen et al., 2008)	RCT	Patients with coronary disease and T2DM randomized to pioglitazone or glimeperide	543 patients, duration 18 months	Change from baseline in atheroma	Coronary atheroma volume decreased on pioglitazone, but progressively increased on glimeperide	Evidence that pioglitazone is beneficial in atherosclerosis, even causing plaque regression
PROactive: PROspective pioglitazone Clinical Trial In macroVascular Events (Dormandy et al., 2005)	RCT	Patients with T2DM and evidence of macrovascular disease randomized to pioglitazone or placebo	5,238 patients, mean 2.9 years	Composite of mortality, MI, stroke, and leg artery revascularization or amputation	Pioglitazone did not affect primary composite endpoint (–10%, $p = 0.09$) but reduced predefined secondary endpoint (mortality, MI, and stroke; –16%, $p = 0.03$)	Pioglitazone is effective in secondary prevention of cardiovascular disease; follow-up analyses showed even more impressive effects in subgroups with prior MI or stroke
Colhoun et al., 2012	database cohort	Scottish national database of prescriptions, hospitalizations, and deaths	37,479 patients exposed to TZD	Hospitalization for hip fracture	Pioglitazone and rosiglitazone increased risk of hip fracture by 15%–20% in men and women	Prior studies had shown mainly an association of TZDs with distal extremity fractures in women
Neumann et al., 2012	database cohort	French national databases	155,535 patients exposed to pioglitazone	Incident cases of bladder cancer	Pioglitazone significantly increased bladder cancer by 22%, with dose- and duration-dependent effects	Other studies support a small but significant increase in bladder cancer on pioglitazone, but not rosiglitazone
Colmers et al., 2012b	meta-analysis	4 RCTs, 7 cohort studies, and 9 nested case control studies were pooled	data on 2.5 million patients	Incidence of cancers at various sites	TZDs confer a small (5%–10%) but significant decreased risk of lung, colorectal, and breast cancers	Compared to bladder cancer, cancers that TZDs may prevent are more common and have greater morbidity and mortality
AleCardio: a study with Aleglitazar in patients with a recent acute Coronary syndrome and type 2 diabetes mellitus (Lincoff et al., 2014)	RCT	Patients with T2DM and hospitalized with acute coronary syndrome were randomized to aleglitazar versus placebo	7,226 patients, stopped early after median 2 years	Recurrent heart attack, stroke, or cardiovascular death	Aleglitazar did not affect the primary endpoint despite having the expected effects on lipids and glucose	This trial was stopped early due to futility, as this dual PPAR agonist showed no benefit and the suggestion of serious adverse events

Fourteen key clinical studies are summarized, in the order they are presented in the text. RCT, randomized controlled trial.

mortality relative to metformin (Tzoulaki et al., 2009). Based on such findings, pioglitazone essentially became the TZD of choice, and prescribing data from the U.S. and UK clearly show a switch from rosiglitazone to pioglitazone between 2007 and 2009 (Hampp et al., 2014; Leal et al., 2013). A large FDA-required RCT called TIDE was designed to compare rosiglitazone and pioglitazone head to head for cardiovascular endpoints (Punthakee et al., 2012), but it has been terminated, as the FDA deemed it no longer feasible or necessary after the readjudication of RECORD.

The potential difference in MI risk between pioglitazone and rosiglitazone may lie in their distinct effects on lipoproteins, with pioglitazone showing a more favorable effect (triglycerides decrease ~15%, HDL cholesterol increase ~10%, with no effect on LDL or total cholesterol) than rosiglitazone (no effect on triglycerides, HDL cholesterol increase ~10%, but 5%–10% increases in LDL and total cholesterol) (Chiquette et al., 2004). This difference in lipid effects may reflect weak PPAR α agonism by pioglitazone (Sakamoto et al., 2000). Subsequent RCT data validated the favorable effects of pioglitazone versus rosiglitazone on lipoprotein particle concentration and size (Deeg et al., 2007). These and other markers of cardiovascular risk favor pioglitazone, and two studies directly measuring atherosclerotic plaques in patients with T2DM showed benefits of pioglitazone compared to the sulfonylurea glimepiride. In the CHICAGO study, pioglitazone slowed progression of carotid intima media thickness (CIMT) (Mazzone et al., 2006), while in PERISCOPE pioglitazone actually led to regression in coronary atheroma volume assessed by intravascular ultrasound (Nissen et al., 2008). In the ACT NOW study of patients with prediabetes, pioglitazone likewise decreased progression of CIMT, and this was interestingly independent of effects on glycemia, insulin resistance, lipids, or inflammatory markers (Saremi et al., 2013). Direct beneficial effects of pioglitazone in the vascular wall are proposed, and many basic studies have explored effects of TZDs and PPAR γ in vascular smooth muscle cells, macrophages, and endothelial cells (Tontonoz and Spiegelman, 2008).

PROactive was a landmark RCT assessing the effect of pioglitazone on secondary prevention of cardiovascular disease in patients with diabetes, enrolling 5,238 patients for an average of 2.85 years (Dormandy et al., 2005). While pioglitazone resulted in a nonsignificant 10% reduction in the primary composite endpoint (all-cause mortality, nonfatal MI, acute coronary syndrome, stroke, revascularization of coronary or leg arteries, and amputation above the ankle), there was a significant 16% reduction in the main prespecified secondary composite endpoint (all-cause mortality, nonfatal MI, and stroke). In effect, pioglitazone reduced all cardiovascular endpoints except for peripheral vascular revascularization. Posthoc subgroup analyses showed that those 2,445 patients with previous MI had a significant 28% decrease in recurrent MI on pioglitazone (Erdmann et al., 2007), while those 984 patients with previous strokes had a significant 47% reduction in recurrent stroke (Wilcox et al., 2007). Despite these impressive cardiovascular benefits to pioglitazone, its use has also declined markedly, potentially due to “guilt by association” with rosiglitazone and the description of new risks for fractures and bladder cancer.

TZDs and Skeletal Fractures

The first large RCT to suggest this association was ADOPT, in which pre- and postmenopausal women (but not men) randomized to rosiglitazone had significantly more fractures (2.74 per 100 patient years) than those receiving metformin or glyburide (1.54 or 1.29 per 100 patient years) (Kahn et al., 2008). Interestingly, the fractures were not at typical osteoporotic sites of spine and hip but instead distal fractures of the upper and lower extremities. RCTs of pioglitazone have showed similar risk, and multiple observational studies of both rosiglitazone and pioglitazone have shown heterogeneity but generally supported this association with distal fractures in women but not men (reviewed in Yau et al., 2013). These findings have led to recommendations against using TZDs in those at risk for osteoporosis and fracture, such as postmenopausal women. These concerns were amplified after a recent national database cohort study in Scotland showed in a large population that risk of hip fracture increased in both women and men, risk was cumulative (increased by 18% for each year of TZD exposure), and the 90-day mortality from hip fractures was expectedly high at ~15% with or without TZD (Colhoun et al., 2012). Consistent with this risk, pioglitazone reduces bone mineral density and content in multiple sites in men and women (Bray et al., 2013). Of the potential adverse effects of TZD, fracture risk may be the most convincing reason to curtail long-term TZD use for treatment and prevention of T2DM, particularly if increased hip fractures are validated.

The mechanism for the skeletal effects of TZDs remains uncertain. One model involves PPAR γ activation driving mesenchymal precursor cells to adipogenesis rather than osteogenesis, and a recent translational study with human cells from bone marrow biopsies supported aspects of this model (Beck et al., 2013). Also consistent with this model, some mice develop more bone marrow fat on rosiglitazone, though this effect was not observed in humans (Harsløf et al., 2011). The molecular mechanisms for TZD effects on bone are complex and involve both decreased osteoblast function and increased osteoclast function (Grey, 2009). For instance, rosiglitazone was very recently shown to alter expression in cell culture of a novel micro-RNA involved in osteoclastogenesis (Krzyszinski et al., 2014), and to increase the number of circulating osteoclast precursors in postmenopausal women (Rubin et al., 2014). Studies of TZD skeletal effects are also complicated by the effects of diabetes itself on bone, as patients with diabetes have a higher risk of fracture independent of bone density, and poor glycemic control may itself worsen fracture risk (Oei et al., 2013).

Pioglitazone and Bladder Cancer

In pharmacological carcinogenicity studies, pioglitazone increased urothelial bladder cancer in male rats, but not in female rats or mice (reviewed in Tseng and Tseng, 2012), leading to several studies in humans indicating that pioglitazone increases bladder cancer risk. Adverse event reporting to the FDA first suggested the risk in 2011 (Piccinni et al., 2011). A longitudinal cohort from Kaiser Permanente Northern California (KPNC) of ~200,000 diabetic patients with ~30,000 on pioglitazone showed a nonsignificant 20% increased risk of bladder cancer overall, but a significant 20% increase in the subgroup with >24 months of drug exposure (Lewis et al., 2011). A French retrospective cohort study of ~1.5 million

patients with ~150,000 exposed to pioglitazone used similar methods to reach similar conclusions: overall pioglitazone significantly but slightly increased risk by 22%, with dose- and duration-dependent further increases up to 75% with cumulative exposure to >28,000 mg (Neumann et al., 2012). Bladder cancer overall was eight times more common in men in this study, and consequently it was underpowered to find effects in women. Nonetheless, all of the excess risk of pioglitazone was in males, and a lack of effect in females would mirror the apparent sex selectivity in rats. Analysis of UK general practice databases confirmed increased bladder cancer risk on pioglitazone (Azoulay et al., 2012), though similar population cohorts in Taiwan, Japan, and Korea failed to show this association (reviewed in Yau et al., 2013). Two meta-analyses have included these studies and others to find risk increases of 22%–23%, though both perform detailed assessments and find moderate overall risk of bias (Colmers et al., 2012a; Ferwana et al., 2013). Recent efforts have tried to identify and eliminate potential confounding sources of bias in these observational studies (Lewis et al., 2014).

RCTs would be the gold standard to show increased bladder cancer risk, but the relative rarity of bladder cancer has limited these efforts. The PROactive study in 2005 initially reported 14 bladder tumors with pioglitazone versus 6 with placebo ($p = 0.069$), but subsequent elimination of a benign bladder mass from the placebo group gave significant risk at $p = 0.04$ (Hillaire-Buys et al., 2011). However, the recently published 6 year interim analysis showed that this imbalance was likely due to chance, as it did not persist into the follow-up period despite randomized exposure to high cumulative doses of pioglitazone and the long latency to development of bladder cancer (Erdmann et al., 2014).

Rat studies have attempted to elucidate pioglitazone's mechanism of bladder carcinogenesis. In addition to pioglitazone, a number of dual PPAR γ /PPAR α agonists (see below) have also shown bladder carcinogenicity in rats (Tseng and Tseng, 2012). Some studies support a "crystalluria hypothesis" with rat-specific formation of urinary solids leading to mucosal irritation and tumors, which is theorized not to occur in mice and humans (Sato et al., 2011; Suzuki et al., 2010). However, other mechanisms are possible, and PPAR γ -dependent effects have not been ruled out.

Overall, the weight of current evidence supports a small but real increase in bladder cancer risk with pioglitazone therapy (Faillie et al., 2013), while no study has seen increased risk with rosiglitazone. It is important to note that the absolute risk increase is very small. The French cohort showed that pioglitazone is associated with an increase from 42.8 to 49.4 cases of bladder cancer per 100,000 person years (Neumann et al., 2012), meaning an individual's annual risk goes from 0.043% to 0.049%, or a 0.006% increase in absolute risk. Similarly, a number needed to harm calculation showed that over 20,000 patients would need to be treated with pioglitazone to cause one additional case of bladder cancer (Ferwana et al., 2013).

TZD Effects on Other Cancers

Despite the attention to bladder cancer, the effects of TZDs on other cancers remain uncertain, and if anything there may be protective effects. In a population of almost 90,000 veterans

with diabetes, TZD use had no significant effect on prostate and colon cancer, but there was a significant 33% decrease in lung cancer (Govindarajan et al., 2007), consistent with pioglitazone's protective effect in a mouse model of lung cancer (Li et al., 2012). In the study populations that showed increased bladder cancer risk, the KPNC cohort showed no significant effects of pioglitazone on the ten most common cancers (Ferrara et al., 2011), but in the French cohort pioglitazone and rosiglitazone actually had significant protective effects for several other cancers (breast, colon, lung, and head and neck) (Neumann et al., 2012).

While many individual cohorts showed a neutral effect of TZDs on cancers (for example, Koro et al., 2007), the largest meta-analysis to date with data on 2.5 million people supports the idea that TZDs confer a small (5%–10%) but significant decreased risk of lung, colorectal, and breast cancers (Colmers et al., 2012b). Given that these cancers are far more common than bladder cancer, these decreased risks assuage the increased risk of bladder cancer. This risk balancing may be particularly relevant, as the great majority of bladder cancers on pioglitazone were non-muscle invasive and managed with transurethral resection (Ferwana et al., 2013), while TZDs could protect against more common cancers with greater morbidity and mortality. There are many proposed mechanisms for the effects of PPAR γ and TZDs in various cancers, many involving increased apoptosis, and this extensive literature with much conflicting data has been reviewed recently (Robbins and Nie, 2012).

Balancing Pioglitazone Clinical Risks and Benefits

The relative risks and benefits of pioglitazone can be inferred based on the abundance of trial data summarized above and published mortality statistics in the U.S. (Figure 3) (CDC, 2010; National Program of Cancer Registries United States Cancer Statistics [USCS], 2006–2010; Yoon and Yi, 2012). Heart disease, stroke, and diabetes are the first, fourth, and seventh leading causes of death, and based on the PROactive trial and improved glycemic control, pioglitazone might decrease these deaths by ~20%—thus 52 fewer deaths per 100,000. Assuming that one-third of cirrhosis and liver cancer (9.4 and 5.6 deaths per 100,000) is attributable to NAFLD, and that pioglitazone might decrease these by ~20%, this means another 1 fewer death per 100,000. This number alone is enough to mitigate the increased risk of bladder cancer: 4.4 deaths per 100,000 increased by ~20% is only 0.9 additional deaths. Given that pioglitazone may protect ~10% against more common cancers, this means ~9 fewer deaths, so pioglitazone would prevent almost 10-fold more cancer deaths than it causes. Hip fracture mortality is more complicated. Based on a landmark analysis of Medicare data, the incidence of hip fractures is 957 and 414 per 100,000 in women and women over 65, with respective 1 year mortalities of 22% and 32% (Brauer et al., 2009), thus an average mortality of 172 per 100,000. If pioglitazone truly increases hip fractures by 20%, then there are ~34 additional deaths per 100,000 people over 65—similar to the estimate of 21 by Colhoun et al. Thus, fractures due to pioglitazone carry a much greater mortality than bladder cancer. Considering all the numbers above, pioglitazone would prevent ~60 deaths and cause only ~30 per 100,000, favoring benefits over risks

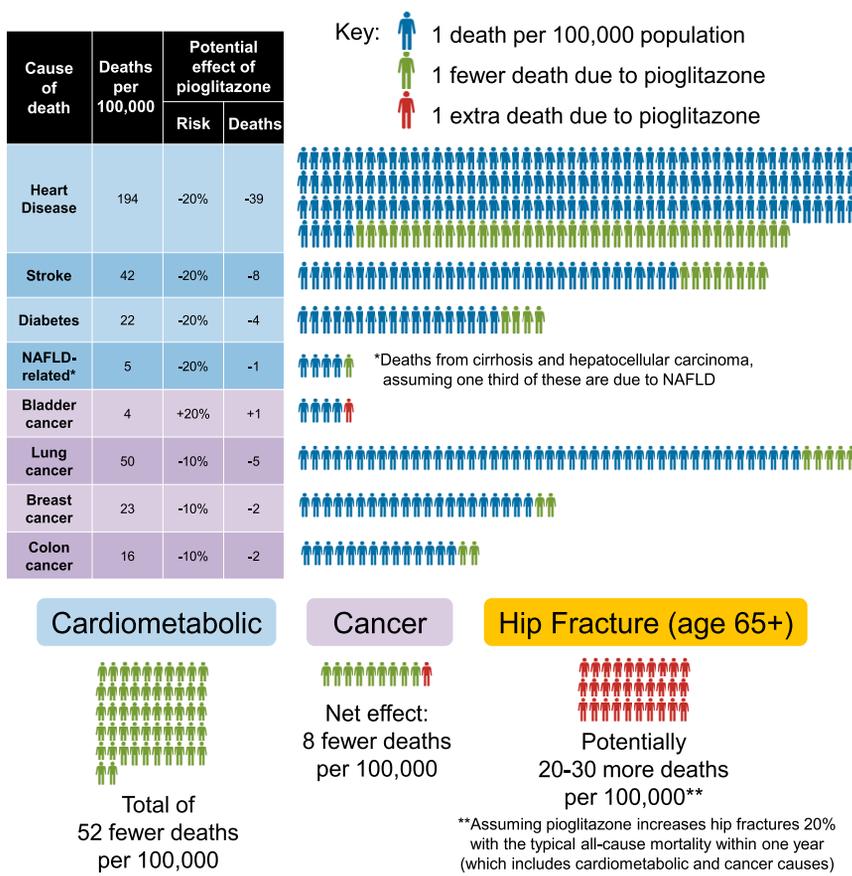


Figure 3. Weighing Mortality Risks and Benefits of Pioglitazone

Age-adjusted mortality rates for the entire U.S. population were derived from published government statistics for 2010-2011. Potential effects of pioglitazone on mortality from cardiometabolic causes or certain cancers were approximated from published analyses. The mortality from hip fracture is more complex to estimate, as it is not a proximal cause of death but clearly carries mortality risk in the elderly. See text for details.

Since TZDs affect transcriptional activity of PPAR γ , their mechanisms may be elucidated by identifying in an unbiased manner: (1) the transcriptional changes induced by TZDs or (2) the genomic binding sites for PPAR γ . Multiple microarray analyses of TZD-regulated gene expression have been performed in cells and animals, and these have revealed many candidate genes involved in lipid and glucose metabolism. The genes activated by TZDs are largely consistent with increased lipid storage capacity in adipocytes but do not truly explain their efficacy (Moore, 2005). With the recognition of brown-like adipocytes in white adipose tissue (“beige cells”), it has become clear that TZDs increase this signature of browning (Vernochet et al., 2009), perhaps by a mechanisms involving the

by 2-fold. These are crude approximations for many reasons (i.e., the mortality statistics are from the overall population, not those with T2DM), but they are nonetheless informative.

Basic Science: How Do TZDs Improve Insulin Sensitivity?

PPAR γ is the master regulator of adipose tissue development and function (Tontonoz and Spiegelman, 2008) and is more abundant in adipocytes than in any other cell type (Chawla et al., 1994; Tontonoz et al., 1994). Indeed, TZDs have important effects on adipose biology. TZDs stimulate progenitor stem cells to differentiate in adipocytes (Tang et al., 2011), and they also affect mature adipocytes. The “lipid steal” model proposes that adipose tissue is the metabolically safe place to store fat (Kim et al., 2003; Ye et al., 2004). In obesity, adipocytes become overwhelmed and dysfunctional, resulting in elevated serum free fatty acids and ectopic lipid deposition in liver and muscle, leading to insulin resistance (Samuel et al., 2010). In this model, TZDs improve the function of fat to safely store lipid, resulting in decreased serum free fatty acids, decreased ectopic lipids, and less insulin resistance. While not fully proven, this model explains two otherwise paradoxical observations relating obesity to insulin resistance: (1) TZDs improve insulin sensitivity despite causing weight gain, and (2) lack of adequate fat (lipodystrophy) causes extreme insulin resistance (Fiorenza et al., 2011). TZDs are also effective in treating some patients with partial lipodystrophy, including HIV-infected individuals (Hadigan et al., 2004).

brown fat transcription factor PRDM16 (Ohno et al., 2012; Qiang et al., 2012), and thus TZDs may also increase fatty acid oxidation and energy expenditure in fat.

Recent advances in DNA sequencing technology have allowed more detailed probing of TZD effects. Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) has revealed tens of thousands of binding sites for PPAR γ throughout the genome, with enrichment near the expected adipocyte metabolic genes (reviewed in Lefterova et al., 2014). In cultured mouse adipocytes, TZD treatment did not induce new PPAR γ binding sites and had only small effects on increasing PPAR γ occupancy at pre-existing sites (Haakonsson et al., 2013). However, TZDs do have genome-wide effects increasing recruitment of coactivators to PPAR γ sites (Haakonsson et al., 2013; Step et al., 2014), thus providing a mechanism for TZD gene activation. Notably, TZD treatment results in repression of similar numbers of genes as are activated, and the mechanism of nuclear receptor ligand-mediated repression is unknown. However, a recent analysis of TZD-repressed transcription at enhancers (eRNAs) has shown that these sites lack PPAR γ , such that redistribution of coactivators from these sites to those with strong PPAR γ binding may account for repression (Step et al., 2014).

Some targets affected by TZDs and PPAR γ are hormones or cytokines secreted by adipose tissue (adipokines), which communicate with other tissues to affect whole-body metabolism (Halberg et al., 2008). Serum adiponectin levels correlate

with insulin sensitivity and are increased by TZDs (Riera-Guardia and Rothenbacher, 2008). Clinically, in the ACT NOW study, increases in adiponectin levels correlated with improved insulin sensitivity on pioglitazone (Tripathy et al., 2014). Consistent with a causal role of adiponectin in TZD effects, mice lacking adiponectin show decreased response to TZD but still improve insulin sensitivity (Nawrocki et al., 2006). Conversely, TZD treatment may decrease levels of other adipose-derived signaling molecules which are linked to insulin resistance, such as TNF- α , RBP4, and resistin (reviewed in Ahmadian et al., 2013).

Recent reports have also implicated two other secreted proteins in the fibroblast growth factor (FGF) family in PPAR γ and TZD effects, though acting locally in fat rather than as hormones. TZDs increase adipose tissue expression of FGF21, which acts in an autocrine or paracrine manner to increase PPAR γ transcriptional activity (via suppression of Lys107 SUMOylation, see below), such that diet-induced obese mice lacking FGF21 showed decreased response to TZDs in insulin sensitization, weight gain, and even fluid retention (Dutchak et al., 2012). However, another report found that FGF21 null mice responded normally to TZDs (Adams et al., 2013), so further study will be necessary to resolve this discrepancy. FGF21 may also mediate TZD effects on bone, as FGF21 gain of function decreases bone mass like TZDs, and FGF21 loss of function actually prevents bone loss due to rosiglitazone (Wei et al., 2012). TZDs also increase expression of FGF1 via PPAR γ activation, and mice lacking FGF1 show insulin resistance upon high-fat diet and failure to remodel adipose tissue upon withdrawal of this diet (Jonker et al., 2012). While response to TZDs has not been reported in FGF1 null mice, pharmacological administration of FGF1 was recently shown to be insulin sensitizing in mice (Suh et al., 2014).

TZDs are potent synthetic PPAR γ ligands, but the endogenous ligand remains uncertain despite a number of candidates (e.g., certain unsaturated fatty acids, prostaglandins, oxidized lipids, and serotonin metabolites) which generally have much lower affinity for PPAR γ and uncertain physiological relevance (Schupp and Lazar, 2010). Assuming that endogenous compounds do modulate PPAR γ activity, it is also unknown whether TZDs have potent effects because they are simply stronger agonists or, alternatively, that TZDs have actions that are qualitatively different than those of the endogenous regulators.

What Tissue(s) Is Most Important for TZD Function?

Over the past 15 years, many mouse models have been reported that elucidate the tissue-specific effects of PPAR γ and TZDs (Table 2). PPAR γ is by far most abundant in adipose tissue, and adipose tissue is necessary for insulin-sensitizing effects of TZDs (Chao et al., 2000). Furthermore, PPAR γ gain of function in adipose tissue is sufficient to cause whole-body insulin sensitization (Sugii et al., 2009). A study of mice with deletion of PPAR γ in fat demonstrated loss of some but not all TZD effects, although these mice had a surprisingly mild lipodystrophic phenotype (He et al., 2003). This differs from a more recent deletion model which described severe lipodystrophic diabetes, though TZD effects were not examined in this case (Wang et al., 2013).

PPAR γ deletion in liver and muscle also causes insulin resistance, though to a much lesser degree than deletion in fat, and with variable effects in response to TZDs. Two groups have

deleted PPAR γ selectively in skeletal muscle, with one reporting resistance to insulin sensitization by TZDs (Hevener et al., 2003), yet the other showing normal response to TZDs (Norris et al., 2003). The reasons for this discrepancy remain unclear. Mice lacking PPAR γ in liver respond to TZDs normally unless adipose tissue is also defective (Gavrilova et al., 2003). PPAR γ and TZDs have also been implicated in pancreatic β cells. While TZDs enhance insulin secretion from isolated islets in a PPAR γ -dependent manner, mice lacking islet PPAR γ have normal glucose homeostasis and response to TZDs (Rosen et al., 2003).

In addition to the classic metabolic tissues (fat, liver, muscle, and endocrine pancreas), metabolic phenotypes have also been found in mice lacking PPAR γ in immune cells, particularly those cells resident in adipose tissue and altered in obesity. PPAR γ is expressed in macrophages and affects their phenotype, as PPAR γ (Odegaard et al., 2007) and TZDs (Bouhidel et al., 2007) lead to alternative M2 polarization, as opposed to classic proinflammatory M1 polarization. Rather than a direct effect on macrophages, one study proposes that TZDs affect adipose resident macrophage polarization indirectly by lipid partitioning (Priour et al., 2011), similar to “lipid steal” between tissues. Nonetheless, mice lacking macrophage PPAR γ show whole-body insulin resistance, though this is still improved by rosiglitazone (Hevener et al., 2007).

More recently, it was reported that a subset of regulatory T (T_{reg}) cells express high levels of PPAR γ and accumulate in visceral fat of lean mice, but they decrease in diet-induced obesity and increase \sim 4-fold upon pioglitazone treatment. Remarkably, diet-induced obese mice with PPAR γ ablation in T_{reg} cells showed no improvement in glucose tolerance or measures of insulin sensitivity in response to pioglitazone (Cipolletta et al., 2012), though the effects were small in the control group. Beyond macrophages and T_{reg} cells, PPAR γ and TZDs have also been proposed to have effects in other immune cells like dendritic cells (Szatmari et al., 2006). It should be noted that while inhibition of atherosclerosis by TZDs in mouse models may involve their anti-inflammatory effects in macrophages (Li et al., 2000), the ability of pioglitazone to decrease atherosclerotic lesions is lost in mice lacking PPAR γ in smooth muscle cells (Chang et al., 2012; Hamblin et al., 2011).

Two recent rodent studies support a role for PPAR γ and TZDs in the central nervous system (Lu et al., 2011; Ryan et al., 2011). Both showed that rosiglitazone increases food intake and weight gain, and this was also seen with administration at low doses into the third cerebral ventricle to avoid systemic effects (Ryan et al., 2011). The hyperphagic effects of oral rosiglitazone were lost upon blocking brain PPAR γ activity (in mice by neuron-specific PPAR γ knockout or in rats by CNS treatment with a PPAR γ antagonist or siRNA). Furthermore, excess brain PPAR γ activity resulted in weight gain, while attenuation of brain PPAR γ activity reduced weight gain on a high-fat diet. Rosiglitazone still improved whole-body (but not hepatic) insulin sensitivity in mice lacking PPAR γ in the brain (Lu et al., 2011), indicating that CNS effects may account for some but not all the metabolic effects of TZDs. PPAR γ mRNA and immunoreactive protein can be detected in certain brain regions (Sarruf et al., 2009), but it remains uncertain whether functional levels of PPAR γ protein are present in neurons, and if so which genes are TZD regulated.

Table 2. Tissue-Specific Effects of TZDs and PPAR γ in Mouse Models

Tissue	TZD Effect in Tissue or Cell Type	Tissue-Specific PPAR γ Knockout		Note
		Overall Effect	TZD Effect	
White adipose	Increased adipogenesis Increased lipid storage Increased browning Increased insulin sensitivity	Severe lipoatrophy with marked insulin resistance (Wang et al., 2013)	Not tested, but absent in another model of lipoatrophy (Chao et al., 2000)	In another knockout model with milder lipodystrophy, the TZD effect was diminished but not lost (He et al., 2003)
Liver	Decreased hepatic steatosis Increased insulin sensitivity	Excess adiposity and whole-body insulin resistance (Gavrilova et al., 2003)	Normal response	Less steatosis is likely via “lipid steal” to adipose, as TZD effect on isolated hepatocytes is lipogenic
Skeletal muscle	Increased insulin sensitivity Decreased ectopic lipids (likely indirect effects via “lipid steal” to adipose)	Excess adiposity and whole-body insulin resistance (Norris et al., 2003)	Normal response	Another knockout model had conflicting results, with no response to TZDs (Hevener et al., 2003)
Pancreatic β cells	Increased insulin secretion	Altered islet mass but normal glucose homeostasis (Rosen et al., 2003)	Normal response	Improved beta cell function on TZDs is also due to lower glucose and less insulin demand
Macrophage	Less M1 pro-inflammatory polarization More M2 polarization	Whole-body insulin resistance (Hevener et al., 2007)	Partial response	Macrophages reside in adipose tissue as well as atherosclerotic lesions
Regulatory T cell (T _{reg})	Increased number of T _{reg} cells in obese visceral fat	Decreased adipose T _{reg} (Cippolletta et al., 2012)	No longer significant response	Effects of TZDs on isolated T _{reg} cells have not yet been reported
Brain	Increased food intake	Less weight gain on high-fat diet (Lu et al., 2011)	Normal but no longer increase food intake	In brain knockout mice, TZDs restored whole-body but not hepatic insulin sensitivity
Kidney	Fluid retention	No whole-body effect reported (Guan et al., 2005)	No longer retain fluid	Same result in a different knockout model (Zhang et al., 2005)
Bone	Increased osteoblasts Decreased osteoclasts Increased adipocytes	Not known	Not known	FGF21 deletion eliminates TZDs effects on bone (Wei et al., 2012)
Cardiac muscle	Cardiac hypertrophy (mice) Increased lipid storage	Hypertrophy with normal cardiac function (Duan et al., 2005)	Still induce further cardiac hypertrophy	There is evidence for PPAR γ -independent effects of TZDs on cardiomyocytes
Vascular smooth muscle	Reduced atherosclerotic lesions (may also be due to effects on macrophages or endothelial cells)	Perivascular adipose tissue lost (Chang et al., 2012)	No longer reduces atheromas (Hamblin et al., 2011)	Both rosiglitazone and pioglitazone protect against atherosclerosis in mouse models

While PPAR γ and TZD effects in adipose tissue are best validated, they have been investigated in other tissues and cell types. Many TZD effects are reported in isolated cells or in tissues of a whole organism, and gene-targeted “knockout” mice have been generated and studied that lack PPAR γ in various tissues.

Taken together, it is remarkable that the insulin-sensitizing effects of TZDs dissipate not only when PPAR γ is removed in adipose tissue but also in several different nonadipose tissues. The evidence for adipose tissue is very robust, validated in multiple independent studies with complementary lines of evidence. The effects in other tissues, however, are typically quite small and based on one or two studies, without independent validation. Future studies are necessary to determine conclusively which of these effects are most important, which are additive, and whether they are coordinated in the context of the whole organism.

Targeting PPAR γ through Posttranslational Modifications

The now-classic model of nuclear receptor function posits that ligand binding causes a conformational change, resulting in release of corepressors and recruitment of coactivators, thus transcriptional activation of target genes (Lehrke and Lazar, 2005). Ligands are thus often defined by “classical agonism” in *in vitro* assays of transcriptional activation or nuclear receptor association with coregulatory peptides (for example, Hughes et al., 2014). However, challenges to this model have emerged, as TZDs have other effects on PPAR γ , including posttranslational modifications.

The two best-studied phosphorylation sites on PPAR γ are Ser112 and Ser273 (numbering is for the PPAR γ 2 isoform). Other phosphorylation sites have been proposed (Ser46 and Ser51) which may affect PPAR γ subcellular localization (von Knethen et al., 2010), but these have not been studied to the same extent. Phosphorylation of PPAR γ at Ser112 is inhibitory, decreasing affinity for TZDs (Hu et al., 1996; Shao et al., 1998). In mice, mutation of Ser112 to Ala mimicked the effect of TZDs, with preserved insulin sensitivity on high-fat diet despite similar weight gain to controls (Rangwala et al., 2003). Ser112 phosphorylation is thought to occur via growth-factor stimulated MAP kinases like MEK1, and several phosphatases have been proposed, most recently PPM1B (Tasdelen et al., 2013). Paradoxically, it has also been reported that phosphorylation of Ser112 by the kinase Cdk9 stimulates rather than represses PPAR γ activity (Iankova et al., 2006). Notably, no reports have indicated that TZDs affect Ser112 phosphorylation. In contrast, the more recently discovered phosphorylation at Ser273 by Cdk5 is blocked by TZDs (Choi et al., 2010). Interestingly, mutation of Ser273 to Ala did not affect overall activity of PPAR γ but led to selective activation of a subset of PPAR γ target genes including adiponectin, suggesting that phosphorylation normally suppresses expression of these beneficial genes—and that TZDs activate them. Furthermore, even partial agonists with weak classical agonism of PPAR γ (like MRL-24) could inhibit Ser273 phosphorylation, similar to full agonists like rosiglitazone. This has led to the hope that drugs decreasing Ser273 phosphorylation of PPAR γ with minimal agonist activity might confer the benefits of TZDs without the adverse events. Indeed, two compounds called SR1664 (Choi et al., 2011) and GQ-16 (Amato et al., 2012) are reported to confer equal insulin sensitization to rosiglitazone in mice, yet remarkably without weight gain or edema. Similarly, natural legume-derived compounds called amorfrutins are also weak PPAR γ ligands that inhibit Ser273 phosphorylation and have surprisingly potent insulin-sensitizing effects (Weidner

et al., 2012). Ser273 phosphorylation was also reduced in mice with adipose tissue deletion of the corepressor NCoR, another model in which increased PPAR γ activity mimics TZD treatment (Li et al., 2011).

PPAR γ can also be covalently attached to ubiquitin or small ubiquitin-like modifier (SUMO) proteins. Like the nearby Ser112 phosphorylation, SUMOylation of PPAR γ at Lys107 also represses its transcriptional activity (Floyd and Stephens, 2004; Ohshima et al., 2004; Yamashita et al., 2004), and TZDs are not reported to affect this. In contrast, TZDs are reported to induce SUMOylation at Lys395, which is central to the “transrepression” model whereby PPAR γ in macrophages stabilizes corepressors at inflammatory gene promoters (Pascual et al., 2005). TZDs also cause the ubiquitination and degradation of PPAR γ (Hauser et al., 2000), and the ubiquitin ligase Siah2 has been implicated (Kilroy et al., 2012). Ubiquitination occurs in the ligand binding domain, though the exact site is unknown. While proteasomal degradation of PPAR γ would clearly decrease transcriptional activation, there is also evidence that PPAR γ ubiquitination is necessary for its activity (Kilroy et al., 2009).

Most recently, glycosylation and acetylation of PPAR γ have been reported. Glycosylation of PPAR γ (O-GlcNAc at Thr84) was shown in cultured mouse adipocytes, and this decreased basal and TZD-stimulated reporter activity (Ji et al., 2012), though it was not reported whether TZDs affected glycosylation. Rosiglitazone was shown to decrease acetylation of overexpressed PPAR γ at Lys268 and Lys293, while other acetylation sites (Lys98, Lys107, and Lys218) were not affected by TZD (Qiang et al., 2012). These authors propose a model whereby ligand-mediated PPAR γ interaction with the deacetylase SirT1 results in deacetylation and alterations in the PPAR γ gene activation profile favoring a beige adipocyte phenotype. Another recent study suggests a conflicting model based on an observation that pioglitazone instead increases acetylation of endogenous PPAR γ in cultured adipocytes (Jiang et al., 2014).

Given that many modifications of PPAR γ are in close proximity based on its crystal structure, there are potential interactions among these phosphorylated, SUMOylated, ubiquitinated, glycosylated, and acetylated residues. However, it must be noted most studies of PPAR γ posttranslational modifications have been performed in cells with limited independent validation. Only Ser112 has been shown to affect insulin sensitivity in a whole-animal model (Rangwala et al., 2003). While other modifications are potentially attractive targets for new drug development, enthusiasm should be tempered until there is rigorous *in vivo* validation of their relevance in physiology and disease.

Non-PPAR γ Targets of TZDs?

TZDs have rapid nontranscriptional effects that appear to be independent of PPAR γ . One such effect is activation of AMP-kinase (LeBrasseur et al., 2006), which would be predicted to have insulin-sensitizing effects (Hardie, 2014). TZDs have also been reported to bind mitochondrial membranes (Feinstein et al., 2005), and the pyruvate carriers MCP1 and MCP2 have recently been strongly implicated as the mitochondrial targets of TZDs (mTOTs) (Colca et al., 2013a; Divakaruni et al., 2013). MSDC-0160 (aka PNU-91325) is considered the prototype

mTOT modulator, and though it is described as “PPAR γ -sparing,” it is important to note that it is a TZD which still activates PPAR γ in reporter assays, though \sim 20-fold less potently than pioglitazone (Bolten et al., 2007). A phase 2 randomized clinical trial of MSDC-0160 was recently reported, and doses of 100–150 mg gave similar HbA1c lowering as 45 mg of pioglitazone at 12 weeks (Colca et al., 2013b). Notably, however, the typical signs of PPAR γ agonism were still present at these doses: there was significant fluid retention (based on hemoglobin decrease), weight gain, and elevations in adiponectin compared to placebo, though each was only \sim 50% as much as pioglitazone. MSDC-0160 is currently being developed for neurodegenerative conditions rather than T2DM, but the similar drug MSDC-0602 was an effective insulin sensitizer in rodents (Chen et al., 2012) and in an unpublished phase 2 trial for diabetes (NCT01280695). Given that both compounds are pioglitazone derivatives with some PPAR γ activation (Figure 2), a better test of mTOT as a drug target would require a non-TZD mTOT inhibitor completely devoid of PPAR γ agonism.

It has even been suggested that beneficial effects of TZDs on insulin sensitivity stem primarily from the mitochondrial pathway, while PPAR γ mediates undesired effects (Colca et al., 2013b). However, PPAR γ is clearly implicated in insulin sensitivity by strong and unbiased evidence from human genetics. Rare families with mutations in the ligand binding domain of PPAR γ show autosomal dominant inheritance of a syndrome of lipodystrophy and insulin resistance (Barroso et al., 1999). Furthermore, common polymorphisms in the *PPARG* gene locus are associated with risk of type 2 diabetes in genome-wide association studies (Gouda et al., 2010). The causative risk allele was long thought to be a coding Pro12Ala polymorphism in PPAR γ 2; however, a recent study has indicated that the true causal polymorphism lies upstream of PPAR γ 2 and affects its gene regulation (Claussnitzer et al., 2014). Regardless, PPAR γ is clearly associated with insulin sensitivity and diabetes, so it would be very surprising if PPAR γ agonism was only incidental to the antidiabetic effects of its potent TZD ligands. Another strong argument favoring PPAR γ as the target of the insulin sensitizing effects of TZDs is the observation that several PPAR γ agonists lack the TZD structure, and thus presumably the non-PPAR γ effects of TZDs, yet are potent insulin sensitizers (Figure 2, see below).

More New Drug Development: Selective, Partial, and Dual Agonists

There has long been hope that, analogous to selective estrogen receptor modifiers (SERMs), selective PPAR γ modulators (SPARMs, either TZD or non-TZD) with reduced or partial agonist activity may retain glucose-lowering benefits with decreased risk of adverse effects (Rangwala and Lazar, 2002). Such drugs that affect mTOTs or PPAR γ Ser273 phosphorylation are described above. The TZD balaglitazone has partial agonist activity with fewer side effects in rat models (Henriksen et al., 2009), and a human trial showed similar glycemic efficacy to pioglitazone with trends toward fewer side effects (Henriksen et al., 2011). However, in this trial, balaglitazone at 10 or 20 mg clearly caused fluid retention, edema, and increased body fat compared to placebo, and only the lower dose—which was less effective at lowering glucose—showed differences in these side effects compared to 45 mg pioglitazone. Bone loss was not significant in any

group, though a trend toward decrease was present for pioglitazone. The diarylsulfonamide non-TZD class of PPAR γ partial agonists, which includes the drug INT-131, is structurally distinct from TZDs with different binding properties (Bajare et al., 2012). In mouse models, INT-131 failed to cause volume expansion and actually increased bone density, and a recent 24-week trial of 362 patients with T2DM showed equal glucose lowering efficacy to 45 mg pioglitazone with evidence for less fluid retention (DePaoli et al., 2014). MBX-102 and GW1929 are two other structurally distinct non-TZD agonists with promising results in animal and cell models (Brown et al., 1999; Gregoire et al., 2009). However, despite this promise, further development of SSPARMs appears to have mostly halted.

Dual agonists of PPAR γ and the related nuclear receptor PPAR α also hold promise for treating insulin resistance and dyslipidemia in metabolic syndrome, as they may combine the beneficial effects of TZDs and fibrates (hypolipidemic PPAR α agonists) with fewer side effects. The dual agonist saroglitazar is approved in India, and a phase 3 study of 302 patients with diabetic dyslipidemia despite statin therapy showed that addition of saroglitazar improved triglycerides and fasting glucose (Jani et al., 2014). AleCardio was a large international multicenter phase 3 trial of 7,226 patients with T2DM hospitalized for acute coronary syndrome, randomized to the dual agonist aleglitazar versus placebo (Lincoff et al., 2014). While aleglitazar had the expected effects on glycemia and lipoproteins, the trial was stopped early at a median of 2 years due to futility, as the primary endpoint of recurrent heart attack, stroke, or cardiovascular death was not altered, yet there was evidence for increased serious adverse events. Smaller trials of other dual agonists (aleglitazar and tesaglitazar) have also been unsuccessful. The failure of the large AleCardio RCT may spell the end of drug development for dual PPAR agonists, and unfortunately even dampens enthusiasm for developing new drugs that target PPAR γ .

Why Does It Matter? The Case for Reducing Insulin Levels

Diabetes continues to increase in prevalence, affecting 29 million people in the United States (9.3%) in 2012, up 3 million from 2010 (CDC, 2014). Insulin resistance is the sine qua non of T2DM, and hence only therapies that improve insulin sensitivity address the basic pathophysiology of this condition (DeFronzo, 2004). Furthermore, there is a school of thought that the increased concentrations of insulin that are prevalent in T2DM are a major contributor to the comorbidities, particularly macrovascular disease (Després et al., 1996). This would explain why even insulin-resistant patients without diabetes are prone to the same vascular complications (Facchini et al., 2001), and thus it is of great concern that one-third of Americans (86 million) have prediabetes (CDC, 2014). Reaven has suggested that hyperinsulinemia is an etiologic component of other dysmetabolic parameters associated with metabolic syndrome and cardiovascular risk, including hypertension, low HDL, and hypertriglyceridemia (Reaven, 1988). Even further, while hyperinsulinemia certainly results from obesity and insulin resistance, Ludwig and Friedman have promoted the model whereby insulin positively feeds back to cause overeating and adiposity in a vicious cycle (Ludwig and Friedman, 2014). Indeed, a mouse model

with decreased insulin gene dosage shows remarkable resistance to diet-induced hyperinsulinemia and weight gain (Mehran et al., 2012).

Even beyond cardiometabolic disease, hyperinsulinemia is also thought to be central to the elevated cancer risk associated with diabetes and obesity (Gallagher and LeRoith, 2013). However, many therapies for T2DM focus on overcoming insulin resistance by increasing insulin levels, either by stimulating secretion of endogenous insulin (e.g., glucagon-like peptide 1 [GLP-1] receptor agonists, DPP4 inhibitors, sulfonylureas) or by providing insulin exogenously. A very recent retrospective cohort study of VA patients (Roumie et al., 2014) supports the idea that high insulin levels are harmful. In patients with diabetes started on metformin from 2001 to 2008, those for whom insulin was added as the second agent had a significant 44% higher all-cause mortality (mainly due to cancer) versus those adding a sulfonylurea. Note that patients receiving any other antidiabetic medications, including TZDs, were excluded from the study, and thus the likely effect of TZDs to reduce insulin requirements was not evaluated. Another very recent industry-funded retrospective cohort study did compare TZD to insulin and found that patients started on pioglitazone from 2000 to 2010 had a remarkably significant 67% lower all-cause mortality than those started on insulin (Yang et al., 2014), though this study was unable to adjust for glycemic control.

Since hyperinsulinemia is a plausible contributor to the comorbidities of type 2 diabetes, it is prudent to develop novel therapies that address this underlying problem. Treatment of obesity could address this, and while bariatric surgery is effective, clinical experience with lifestyle medication and pharmacotherapy for obesity has been disappointing to date, particularly in terms of weight regain (Hainer et al., 2008). Novel therapeutics directed at activating brown or beige adipocytes have promise for treating obesity as well as diabetes, but none are currently available for clinical use. Since TZDs are the most potent known insulin sensitizers, by definition patients on TZDs will require lower levels of endogenous and exogenous insulin to maintain euglycemia. Indeed, patients using insulin who are started on TZDs typically reduce their insulin dose or even discontinue insulin injections (Yau et al., 2013). Therefore, understanding how TZDs work and effectively harnessing the underlying mechanisms with fewer side effects would be a welcome advance in the arsenal of antidiabetic drugs (Kahn and McGraw, 2010).

There is great hope that recent studies highlighted here may translate to new drug development. There is promise of selective, partial, and dual agonism, as well as specifically targeting post-translational modifications of PPAR γ . In addition to the canonical PPAR γ ligand binding domain, recent studies have identified an alternate site is occupied potentially by several non-TZD ligands but not TZDs, adding additional complexity to agonist pharmacology (Hughes et al., 2014). Furthermore, rather than PPAR γ per se, novel drugs may also achieve insulin sensitization by targeting other TZD-related systems such as AMP kinase, mitochondrial transporters, or FGFs. Tissue selectivity is also a key issue. For instance, a PPAR γ agonist that failed to reach the brain, kidneys, or skeleton might be expected to eliminate side effects of weight gain, fluid retention, and bone loss, respectively. The future may involve targeting small molecule drugs to relevant tissues—such as TZDs to adipose tissue—by conjugation with

selective peptides, as estrogen has been conjugated to GLP-1 to target tissues that express the GLP-1 receptor and treat metabolic syndrome in mice (Finan et al., 2012). Another way to achieve tissue selectivity might be an orally administered PPAR γ agonist that is inactivated by first-pass liver metabolism, thus having selective effects on visceral fat without reaching other tissues. Targeting of TZDs selectively to immune cells, both in adipose tissue and the vascular wall, may likewise be beneficial.

Another way forward would be to identify those patients most likely to benefit from TZDs or novel insulin sensitizers with minimal risks. It has been noted that about a quarter of patients with T2DM are “nonresponders” who do not improve insulin sensitivity on TZDs, while an equal number have large responses (Sears et al., 2009). The umbrella of T2DM encompasses many heterogeneous phenotypes (Gale, 2013), and further investigation may identify a distinct subset of patients in which TZDs may be most effective. Genetic predispositions, such as polymorphisms affecting PPAR γ genomic occupancy, may also modulate response to the insulin-sensitizing or harmful effects of TZDs. Together, new drug development and more personalized pharmacotherapy may fulfill the promise of insulin sensitization in T2DM.

Conclusions

Despite the benefits of insulin sensitization by TZDs, this once widely used class of drugs has fallen into disrepute and disuse. The goal of diabetes therapy is not only glucose lowering, but also protection from its comorbidities, and in this aspect, TZDs carry benefits and risks that must be weighed, as for any pharmacological treatment. In the case of TZDs, public attention has been focused more on the potential harms of these drugs than on their benefits. For instance, the meta-analysis showing cardiovascular risk for rosiglitazone had drastic effects on policy and prescribing, while other meta-analyses showing beneficial effects—like cancer protection by TZDs—are largely dismissed. Even randomized studies consistently indicating cardiovascular benefits of pioglitazone are dismissed for various reasons (i.e., PROactive did not meet the primary endpoint, PERISCOPE looked at a surrogate endpoint, etc.). Bladder cancer risk based on observational studies is now widely cited as a reason to abandon pioglitazone, yet the absolute risk increase is extremely small, and fracture risk—with better clinical and mechanistic evidence and likely greater harm—is a more convincing reason to reconsider this drug.

The data may warrant a more balanced view weighing the potential risks and benefits for TZDs, particularly pioglitazone, which might be used more often in selected patients. For instance, there may be a role in younger patients with prediabetes or early T2DM, when insulin sensitization may do the most to reduce hyperinsulinemia and preserve β cell function—but fracture risk is much less than in the elderly. This is consistent with the current evidence-based trend to individualize T2DM management, as early glycemic control may have long-term cardiovascular benefits via “metabolic memory,” yet tight glycemic control in older patients with long-standing diabetes does not carry these benefits and may even cause harm (American Diabetes Association, 2014).

Whatever the balance between benefits and risks of current TZD therapies, the promise of insulin sensitization for treatment

of metabolic syndrome and diabetes should not be abandoned. Basic mechanistic studies continue to unravel the complex biology underlying the beneficial and adverse effects of TZDs, and better understanding of their salient as well as their harmful effects has great potential to pave the way for the next generation of therapeutics.

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