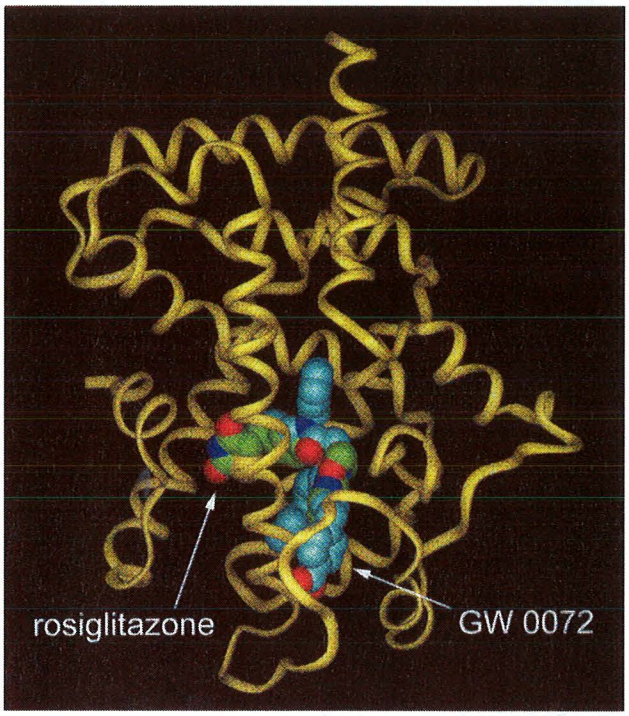
Thiazolidinediones:

Cardiovascular Friend or Foe



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Introduction

The global epidemic of diabetes mellitus is upon us. The World Health Organization (WHO) estimates that there will be 300 million people with diabetes worldwide by the year 2025, more than doubling the number in 1995.[1] Moreover, as many as one-half of affected patients remain undiagnosed.[2,3]

Cardiovascular disease (CVD), including atherosclerotic vascular disease and congestive heart failure, is the most common complication of type 2 diabetes mellitus, accounting for approximately 80% of deaths.[4] In the context of clinical decision-making as well as therapeutic drug development, the high prevalence of CVD among diabetic patients is important for 2 principal reasons. First and foremost, therapies that have a beneficial effect on the development, progression, and clinical manifestations of CVD should be expected to materially improve clinical outcomes among diabetic patients. Second, therapies that increment the risk of CVD complications could prove particularly deleterious among this high-risk population of patients. Therefore, it is imperative to consider cardiovascular risk modification as a primary focus for the treatment of the patient with diabetes. Existing and emerging therapies for diabetes should be rigorously evaluated in adequately powered large-scale randomized clinical trials, not only with respect to their glycometabolic effects, but also with regard to their cardiovascular consequences.

A number of therapies have been shown to favorably affect the cardiovascular risk of patients with diabetes. Lifestyle modification is recommended for all patients with diabetes, and should include dietary modification, weight management, regular physical activity, and for the patients with diabetes who smoke, cessation counseling.[5,6] In addition, several medical therapies have proved to be especially beneficial among the high-risk population of patients with diabetes with regard to primary and secondary cardiovascular risk modification. These include antiplatelet therapy with aspirin and clopidogrel;[7-9] management of hypertension with thiazide diuretics, ACE inhibitors, beta-blockers, and angiotensin receptor blockers;[10-12] treatment with ACE inhibitors independent of blood pressure control;[13] and lipid lowering therapy, especially with HMG Co-A reductase inhibitors (statins) and also gemfibrozil.[14-16] Therefore, based on the existing evidence, the diagnosis of type 2 diabetes should prompt consideration of each of these therapies-aspirin (or clopidogrel), statins (or gemfibrozil), ACE inhibitors, and anti-hypertensive treatment- in the absence of contraindications, especially among the population of patients with concomitant cardiovascular risk or known CVD.

Glycemic control

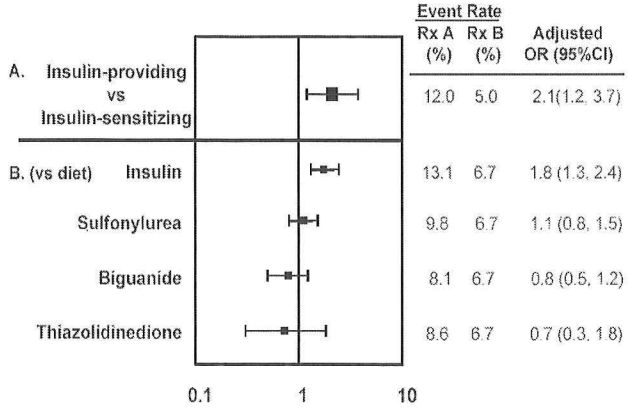
Intensive glycemic control improves microvascular complications of diabetes,[17-19] but there is little evidence regarding the effect of glycemic control on macrovascular complications. The lack of cardiovascular outcomes data in this area is largely a product of the regulatory approach applied to endocrine and metabolic pharmacologic therapies. In the US, and around the world, therapies for glycemic control have been developed, approved, and accepted into clinical practice based on "proof of principle" evidence (i.e. that they lower blood glucose) in the absence of "safety signals" (e.g. mortality, abnormal liver function, etc.).

Publications from a series of controlled clinical trials of glycemic control have reported cardiovascular event rates, but these trials have all been dramatically

underpowered to definitively address the question and their findings have been inconsistent. The trials have reported an adverse effect of intensive control with tolbutamide and neutral effect of insulin (University Group Diabetes Project (UGDP)),[20,21] a trend toward improved cardiovascular outcomes using insulin montherapy,[19] and a trend of similar magnitude toward worse outcomes associated with intensification of glycemic control using a tiered therapeutic strategy.[22] Most recently, results from the United Kingdom Prospective Diabetes Study (UKPDS) provided support for the benefit of intensive glycemic control on a broad spectrum of adverse clinical events over a 10-year follow-up period.[18] The UKPDS randomized 3867 patients with newly diagnosed diabetes to a policy of conventional glycemic control versus intensive glycemic control with sulfonylurea therapy or insulin. Intensive glycemic control, achieving an absolute difference of 0.9% HgbA1c (7.9% vs. 7.0%), was found to be the superior strategy. However, the effect was a modest 12% relative risk reduction for a composite clinical endpoint comprised of 13 individual components. Like the prior studies reported, the UKPDS analyses of cardiovascular effects of glycemic control were underpowered. Although there was a trend toward a decreased relative risk (16%) for myocardial infarction associated with intensive control, the difference failed to achieve statistical significance and was countered in part by a trend of similar magnitude toward increased stroke risk (11%). Therefore, the influence of glycemic control on cardiovascular outcomes remains to be defined, and the relative effects of the various therapies available are unknown.

Presently, there are 6 classes of medications available to manage hyperglycemia associated with type 2 diabetes. These include sulfonylureas (e.g. glyburide; glipizide), biguanides (metformin), meglitinides (repaglanide; nateglinide), alpha-glucosidase inhibitors (acarbose), insulin, and the thiazolidinediones (rosiglitazone; pioglitazone). Among these, the biguanides and thiazolidinediones hold particular promise due to their ability to improve insulin sensitivity, and both classes of drugs appear to have favorable "extra-glycemic" (pleiotropic) effects on cardiovascular pathophysiology.

An important substudy of the UKPDS that included metformin in the randomization strategy among a subset of obese patients demonstrated a significant improvement in cardiovascular outcomes associated with metformin compared with sulfonylureas or insulin.[23] Similarly, using a clinical trial database comprising almost 16,000 patients with an acute coronary syndrome episode (including almost 4000 with diabetes), outcomes among diabetic patients were compared between those treated exclusively with insulin-providing therapy (insulin and/or sulfonylurea) versus insulin-sensitizing therapy (metformin and/or thiazolidinedione).[24] After multivariable statistical adjustment, there was more than a 2-fold increased



Odds ratio plot for Death/MI/Recurrent Ischemia following an acute coronary syndrome according to diabetes status.(McGuire, 2001)

risk over the ensuing 90-days of major adverse cardiovascular events associated with insulin-providing therapy. In summary, available evidence raises the possibility that a strategy using insulin-sensitizing drugs as the foundation for the treatment of hyperglycemia associated with type 2 diabetes may have particular advantage with regard to cardiovascular outcomes.

Thiazolidinediones

The thiazolidinedione (TZD) class of drugs, which includes troglitazone (recently removed from the US market due to hepatotoxicity), rosiglitazone, and pioglitazone, has emerged as a safe and effective treatment of hyperglycemia associated with type 2 diabetes, alone or in combination with other oral hypoglycemic medications, and with insulin (pioglitazone).

Table 1. Thiazolidinedione medications approved by the FDA for clinical use.

	Indications	**Dosing**	**Cautions**	**Notes**
Troglitazone (Rezulin®)		N/A		Withdrawn from Market 3/2000 due to hepatotoxicity
Rosiglitazone (Avandia®)	Monotherapy; Combination with sulfonylurea or metformin	4mg-8mg; single or (preferred) divided	Observe for heart failure; Not recommended for NYHA Class 3-4 CHF	LFT's at baseline and q2 months X 1 year
Pioglitazone (Actos™)	Monotherapy; Combination with sulfonylurea, metformin, or insulin	15mg-45mg once daily	Observe for heart failure; Not recommended for NYHA Class 3-4 CHF	LFT's at baseline and q2 months X 1 year; With insulin, consider decreasing insulin dose 10-25%

Peroxisome Proliferator-Activated Receptors (PPARs)

Although in clinical development for over 15 years, the mechanism of action of the TZDs has only recently been elucidated. In the mid 1990's, the TZDs were found to be potent and selective agonists for one of the Peroxisome Proliferator-Activated Receptors (PPARs),[25] a class of nuclear receptors that have been cloned and characterized over the past decade. The PPARs, which include PPARα, PPARγ, and PPARδ (sometimes referred to as PPARβ), are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, which also includes the retinoic acid receptor (RXR), the steroid hormone receptors, and thyroid hormone receptors.[26] A number of naturally occurring compounds, such as modified fatty acids, members of the prostanoid family (e.g. 15-deoxy-prostaglandin J_2), and linoleic acid derivatives have been demonstrated to bind to and activate PPARs.[27,28] Interaction of the PPARs with agonist ligands induces the formation of PPAR-RXR heterodimers that bind to DNA response elements (PPAR-response element; PPRE). This interaction regulates transcription of a number of genes that contain in their promoter region a PPRE, a process known as "transactivation". Among their many effects, PPAR-regulated gene products function as key regulators of adipogenesis and glucose and lipid metabolism.[26]

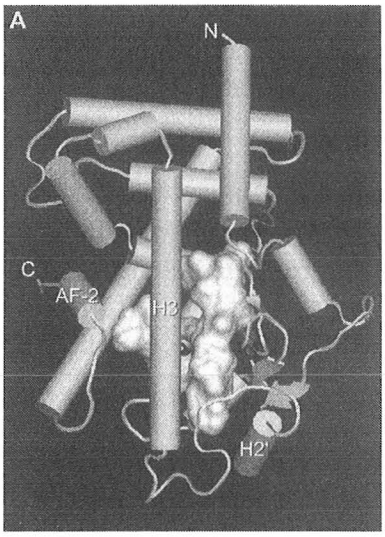
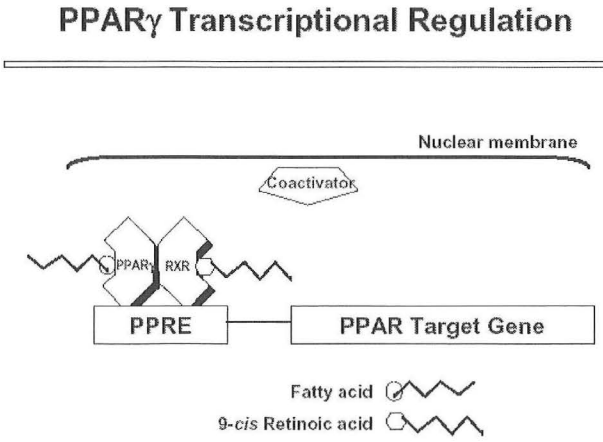


Figure 0. Crystal structure of the PPARγ ligand binding domain. The ligand-binding site is depicted by the white shaded surface.(Willson, 2000)



Although a number of natural ligands to the PPARs have been described, it remains unclear which of these are responsible for the biologic effects that have become the target of therapeutic drug development, such as lipid lowering and glycometabolic effects. Synthetic agonists of the PPAR family of receptors have been developed to treat a number of clinical disease states. For example, PPARα is predominantly expressed in the liver and plays a key role in lipid metabolism. PPARα is the target receptor for the fibrate-derivative class of drugs, which includes gemfibrozil, fenofibrate, clofibrate and bezafibrate. Although they are relatively weak agonists of PPARα, drugs in this class are effective at lowering triglycerides and also to a lesser extent, increasing HDL-cholesterol. PPARδ appears to selectively regulate reverse cholesterol transport and high-density lipoprotein metabolism; no agonists to this receptor have been approved for clinical use.

The thiazolidinedione medications were developed by chemists as fibric acid derivatives in a compound screening program for lipid lowering agents. In animal models, it was observed that these compounds had unexpected glucose lowering effects. It was later determined that the modifications that resulted in the TZD compounds had created selective agonists to PPARγ with loss of agonist activity to the PPARα. These agents subsequently underwent pre-clinical development and ultimately clinical approval, despite little information known regarding their mechanism of action beyond activation of PPARγ.

PPARγ is the most extensively studied of the PPAR subtypes, with 3 isoforms identified to date (PPARγ1; -γ2; -γ3). It is primarily expressed in adipose tissue, but also identified in liver, skeletal muscle, cardiac muscle, renal cortex, colonic epithelium, vascular endothelial cells, and macrophages.[29] Although there is fairly consistent tissue expression observed across species, some differences have been observed between the distribution in animals and humans,[30] making it imperative that effects observed in animal models be verified in humans. PPARγ primarily regulates glucose and lipid homeostasis and adipogenesis.[31] Presently, there are 2 thiazolidinedione PPARγ agonists available for use for the treatment of type 2 diabetes, rosiglitazone and pioglitazone.

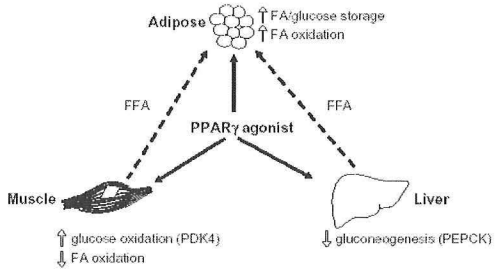
Table 2. Summary of PPAR distribution in humans.

	PPARα	PPARδ	PPARγ
Tissue Expression Profile	Liver, kidney, skeletal, muscle, brown adipose tissue	Ubiquitous	Adipose tissues, skeletal muscle, heart, liver, kidney, gut, macrophages, vascular smooth muscle cells
Isoforms	α	δ	γ_1, γ_2, γ_3
Pharmacologic Activators	Fibrates	Hypolipidemics	Thiazolidinediones

Hypoglycemic effects of TZDs

Although the exact mechanism by which TZDs affect carbohydrate metabolism is incompletely understood, these drugs most likely improve insulin sensitivity through PPARγ-mediated direct or indirect effects on skeletal muscle, the principal organ of glucose disposal, and to a lesser degree, decreased hepatic gluconeogenesis.[32,33] Potential indirect mechanisms of enhanced insulin sensitivity include adipocyte expression of intermediate signals (e.g. leptin, tumor necrosis factor (TNF)-α, adiponectin, and resistin),[34,35] or decreased intra-myocyte free fatty acid accumulation leading to enhanced insulin signaling and glucose transport.[36,37] Although PPARγ is expressed at low levels in skeletal muscle, the enhanced insulin sensitivity associated with TZDs may be due to a direct effect on skeletal muscle. After Zucker rats were exposed to a TZD, expression of skeletal muscle pyruvate dehydrogenase kinase (PDK)-4, an inhibitor of oxidative glucose metabolism, was significantly decreased.[38]

Model of PPARγ Glycometabolic Effects



Regardless of the mechanism responsible, the TZDs are effective at improving glycemic control acutely and chronically, and are well tolerated. Across the trials reported, compared with placebo, TZDs were associated with absolute reductions in HgbA1c of 0.8-1.7%, whether used as monotherapy or added to other hypoglycemic medications. However, the published data are limited due to excessive study drug discontinuation due to inadequate glycemic control, with rates of protocol non-compliance ranging from 11-51%. Throughout these studies, few serious side effects were reported and rarely required discontinuation of study treatment. The rate of clinically important hypoglycemia was similar to placebo, except for the studies that combined TZD use with insulin where a slight increase in hypoglycemic events were reported with the addition of a TZD.[39-41] Presently, only pioglitazone is approved for combination therapy with insulin, and consideration for decreasing insulin dose by 10-25% is recommended when initiating pioglitazone. Other reported side effects commonly reported, which were dose-dependent, included weight gain (0.3-3.5 kg) due largely to increased adiposity, and small decreases in hematocrit (0.2-3%, absolute) most likely due to intravascular volume expansion. As discussed below, peripheral edema was also a common dose-dependent side effect reported in a minority of patients (2.5-10.7%) treated exclusively with oral agents, with significantly higher rates reported in the studies combining TZDs with insulin (13.1-16.2%).

Table 3. Summary of randomized clinical trials enrolling >100 patients evaluating the glucose-lowering effects of thiazolidinediones.

	Study	TZD	Control	Size	Length	Baseline HbA1c (%)	ΔHbA1c Vs. Control (%)
Monotherapy							
	Fonseca, 1998[42]	Tro	Placebo	402	6 mos	8.7	-1.1
	Horton, 1998[43]	Tro	Glyburide	552	1 yr	9.5	No Δ
	Lebovitz, 2001[44]	Rosi	Placebo	493	26 wks	8.9	-1.5
	Phillips, 2001[45]	Rosi	Placebo	959	26 wks	8.9	-1.5
	Aronoff, 2000[46]	Pio	Placebo	408	26 wks	10.2	-1.6
Combination Therapy							
Sulfonylurea	Kipnes, 2001[47]	Pio (15mg; 30mg)	Placebo	560	16 wks	9.9	-0.9; -1.3
	Wolffenbuttel, 2000[48]	Rosi (2mg; 4mg)	Placebo	574	26 wks	9.2	-0.6; -1.0
Metformin	Einhorn, 2000[49]	Pio	Placebo	328	16 wks	9.8	-0.8
	Fonseca, 2000[50]	Rosi (4mg; 8mg)	Placebo	348	26 wks	8.8	-1.0; -1.2
	Gomez-Perez, 2002[51]	Rosi (4mg; 8mg)	Placebo	116	26 wks	9.9	-1.0; -1.5
Sulfonylurea and Metformin	Yale, 2001[52]	Tro	Placebo	200	24 wks	9.6	-1.4
Meglitinide	Raskin, 2000[53]	Tro (-/+ repaglinide)	Repaglinide	256	22 wks	8.7	+0.4; -0.9
Insulin	Buse, 1998[40]	Tro (200mg; 400mg)	Placebo	222	26 wks	9.2	-0.04; -0.3
	Rosenstock, 2002[39]	Pio (15mg; 30mg)	Placebo	566	16 wks	9.8	-1.0; -1.3
	Raskin, 2001[41]	Rosi (4mg; 8mg)	Placebo	319	26 wks	9.0	-0.6; -1.2

TZD-thiazolidinedione; HbA1c-hemoglobin A1c; Tro-troglitazone; Rosi-rosiglitazone; Pio-pioglitazone

Based on these data, troglitazone was approved for clinical use in the US by the FDA in 1997 for the treatment of type 2 diabetes, but was withdrawn from the market in March, 2000 due to hepatotoxicity observed during post-marketing surveillance. Rosiglitazone was approved for clinical use in the US in May 1999 as monotherapy or in combination with other available oral hypoglycemic medications. Shortly thereafter, pioglitazone was approved in August 1999 with the same indications, and in addition was approved for use at low and medium doses in combination with insulin therapy. In the few years that the 2 presently available TZDs have been on the market, they have quickly become among the most widely prescribed medications for the treatment of hyperglycemia associated with diabetes, presently accounting for about 20% of prescriptions for oral hypoglycemic medications. In the setting of this prevalent use and judicious serial monitoring of liver function tests in the wake of the troglitazone experience, no excess of hepatotoxicity has been observed with either rosiglitazone or pioglitazone.

Effects on Cardiovascular Risk Factors

Given the substantial cardiovascular risk associated with diabetes, and the inability to demonstrate marked improvements in cardiovascular outcomes with glycemic control strategies, much recent interest has focused on the pleiotropic effects of the various hypoglycemic therapies available. In that light, data are rapidly emerging that demonstrate myriad favorable effects of the TZDs on a number of intermediate biomarkers and risk factors associated with cardiovascular disease.[54] These include effects on conventional cardiovascular risk factors such as dyslipidemia and hypertension, as well as modulation of endothelial reactivity, the inflammatory cascade, and vascular smooth muscle cell migration and proliferation. These effects lend great promise for long-term coronary and peripheral atherosclerotic disease risk modification.

Table 4. Pleiotropic effects of thiazolidinediones (TZDs) on cardiovascular parameters.

Lipids	**Inflammation**
↓/-Triglycerides	↓ IL-6, TNF-α, CRP, MMP-9
↑/- HDL-c and LDL-c	↓ I-CAM; V-CAM
↑/? Buoyancy of LDL particles	↓ MCP-1
Coagulation	**Ventricular Performance**
↓ PAI-1	↑/- Systolic Function
↓ Fibrinogen	↑/- Cardiac Output
↓ Platelet aggregation	↓/- LVH
	↑ Diastolic Function
Vascular Effects	**Fat Distribution**
↓ Blood Pressure	
↓ Intimal-media thickness	↓ Visceral Fat
↑ Brachial Artery Reactivity	↑ Subcutaneous Fat
↑ Coronary Flow Reserve	
	Other
↑ Vascular Permeability	↑ Intravascular Volume
↓ Smooth Muscle Cell Migration & Proliferation	↓ Microalbuminuria

Adipogenesis

PPARγ is a critical transcription factor in the regulation of adipocyte differentiation. Through their effects on the PPARγ, TZDs modulate adipocyte differentiation, increase the number of insulin-sensitive small adipocytes,[55] and lead to a transition of fat distribution from visceral to subcutaneous depots-a pattern that has been associated with lower cardiovascular risk.[56-60] Thiazolidinediones also (directly or indirectly) inhibit lipolysis and up-regulate expression of free fatty acid transporters in adipocytes (fatty acid transport protein (FATP)-1; CD36),[61] with resultant decreases in circulating free-fatty acids to a degree commensurate with the associated improvement in insulin sensitivity.[57,62,63] Therefore, the net effect of PPARγ activation is increased mass of small, insulin-sensitive adipocytes in the subcutaneous compartment that serves as a reservoir for free-fatty acid deposition, and weight gain associated with these drugs therefore is a manifestation of continued "over nutrition". Despite increased fat mass, TZDs improve insulin sensitivity, and due to the transition from visceral to subcutaneous depots, may also be associated with overall improvement in cardiovascular risk.

Lipid metabolism

Dyslipidemia is common among patients with diabetes, characterized by high triglycerides, low HDL cholesterol, and modest elevations of LDL cholesterol with increased fractions of small, dense LDL particles. TZDs modestly affect circulating lipoprotein concentrations among patients with diabetes, although the effects appear to differ somewhat between the available agents. TZDs also appear to shift the LDL particle profile from a preponderance of small, dense (atherogenic) particles to larger, more buoyant particles.[64]

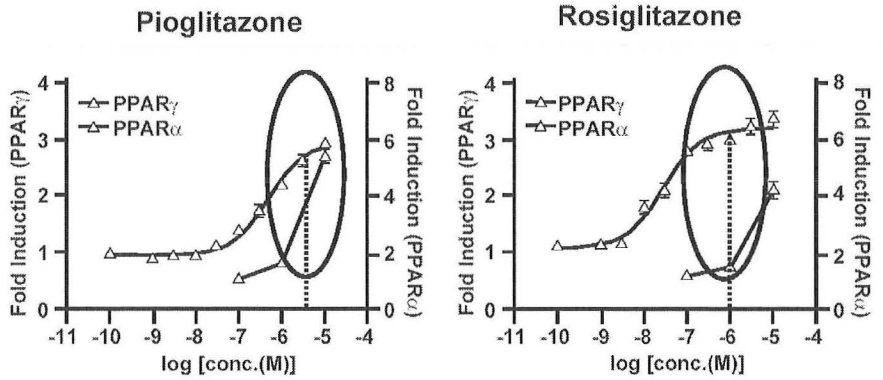
Table 5. Summary of lipid effects abstracted from reports of randomized clinical trials of pioglitazone and rosiglitazone presented in Table 3.

	Rosiglitazone	**Pioglitazone**
Triglycerides	Neutral-may ↑35%	↓9-27%
HDL-cholesterol	↑ up to 19%	↑ up to 19%
LDL-cholesterol	↑ 5-19%	No Change
LDL:HDL ratio	No Change	Improved
Small, dense LDL	↓	↓

Whether the reported changes in lipoprotein concentrations are an indirect result of improved glycemic control, a direct effect of the TZDs, or a combination remains unclear. There are a number of limitations to the available data on the lipid effects of the TZDs, especially when comparing results across studies or between drugs. The studies have included patients with a broad spectrum of baseline lipid abnormalities and have tended to exclude patients with marked dyslipidemia; study treatment periods are highly variable as are the concomitant medications used that may influence lipid responses;

there are no published data on the lipid effects of these drugs among non-diabetic individuals; and most importantly, no clinical outcomes data are available to determine whether the reported changes in lipoprotein parameters are clinically relevant.

Although the mechanisms responsible for the observed differential treatment effects on lipoprotein parameters remains unclear, they may be related to PPARα agonism. Although the activity of rosiglitazone and pioglitazone on PPARγ is comparable, pioglitazone also activates PPARα at high therapeutic concentrations,[65] which may explain its fibrate-like effects on lipoprotein profiles. Whether these differences will translate into clinical advantage remains to be determined.



PPARγ and PPARα induction of pioglitazone and rosiglitazone. Dashed lined reflects mean peak serum concentration at highest approved dose. (Sakamoto, 2000)

Hypertension

Thiazolidinediones attenuate the development of hypertension in a number of animal models, including obese Zucker fatty rats;[66] Sprague-Dawley rats fed high-carbohydrate or high-fat diets;[67] and Dahl salt-sensitive rats.[68] In addition, TZDs have been shown to attenuate the pressor response to norepinephrine and angiotensin II.[69] The mechanism of blood pressure reduction remains unclear, but appears to independent of insulin sensitization, and may be related to direct or indirect effects blocking voltage-gated (L-type) calcium channels, a property of all TZDs that is similar to the dihydropyridine class of calcium-channel blocking medications.[69-71] TZDs have also been demonstrated to increase expression of vascular endothelial growth factor (VEGF) and inhibit expression of endothelin (ET)-1, both of which likely contribute to the favorable influence on hypertension. In patients with diabetes and hypertension, treatment with TZDs reduces blood pressure among hypertensive and non-hypertensive patients,[72-75] although the absolute changes in blood pressure tend to be modest (2-3mmHg) and affect primarily diastolic blood pressure.

Vascular Effects

The vascular endothelium comprises a complex organ with myriad biologic functions, and at the critical interface between the blood and the vascular wall, functions

as a mediator of atherosclerosis.[76] Once considered a passive "lining", understanding of the complex physiology of the vascular endothelium continues to evolve. In response to mechanical and hemodynamic stress, as well as to autocrine, paracrine, endocrine, and inflammatory mediators, the endothelium regulates vasomotor tone and acts as a transducer of atherosclerotic stimuli. Many of the endothelial responses affecting atherosclerosis are propagated through transcriptional regulation, and with the recent demonstration of PPARγ in vascular endothelial cells,[77] the TZD medications have been the focus of increasing interest in the area of vascular pathobiology.[76,78]

Endothelial Function

TZDs have been shown to favorably affect endothelial dysfunction, a common abnormality among patients with diabetes detectable even before atherosclerotic disease develops. One proposed mechanism by which TZD therapy may improve endothelial function involves augmentation of insulin-dependent endothelial nitric-oxide release resulting in enhanced endothelium-dependent vasodilation.[79,80] This theory is supported by the observation that pioglitazone augments insulin-dependent vasodilation in an *ex vivo* rat aorta preparation.[81] In controlled human studies, troglitazone increased forearm blood flow following ischemic insult,[82] and rosiglitazone improved coronary flow reserve in response to cold-pressor stimulus.[83] The effect of TZDs on endothelial reactivity likely contributes to the antihypertensive effects observed with this class of medications described above.

Inflammation

Data continue to accumulate that suggest a causal relationship between inflammation and atherosclerotic vascular disease development and progression.[84] At the level of vascular pathology and injury, endothelial cells release chemokines (e.g. interferon-inducible protein of 10kDa (IP-10), monokine induced by interferonγ (MIG), interferon-inducible T cell α-chemoattractant (I-TAC)) that attract T-cells and affect local inflammatory cell attraction and activation.[76] The activated T-cells in turn release inflammatory mediators such as interferon (IFN)-α, tumor necrosis factor (TNF)-α, interleukin (IL)-2, and IL-6 that serve to propagate the inflammatory insult. This cascade of events is thought be associated with and possibly causal of early atherosclerotic vascular disease. TZDs have been shown to effectively inhibit this cascade of events in *ex vivo* preparations of human endothelial cells.[85,86]

Likewise, endothelial cells express intra-cellular adhesion molecules (e.g. intracellular adhesion molecule (ICAM)-1; vascular cellular adhesion molecule (VCAM)-1) that facilitate leukocyte and monocyte/macrophage homing to area of vascular injury, including atherosclerotic plaque.[87] In human endothelial cell culture, TZDs inhibit the expression of these cellular adhesion molecules.[88] TZDs reduce circulating levels of monocyte chemoattractant protein (MCP)-1, the primary chemokine responsible for recruiting monocytes into the developing atheroma.[89] TZDs also exhibit direct effects on the monocytes/macrophages, including reduction of expression of inflammatory cytokines, such as TNF-a, IL-1, IL-6.[89,90] In contrast, TZDs upregulate expression of the macrophage free fatty acid scavenger receptor CD36, which increases oxidized LDL uptake and may facilitate formation and growth of foam cells that are directly implicated in plaque formation and progression.[26,27] However, this latter effect may be countered by

up-regulation of ABCA-1, a transporter that facilitates ApoA1-mediated cholesterol efflux from macrophages.[91] Therefore, TZDs appear to directly influence the local inflammatory process of early atherosclerotic disease, and the clinical relevance of these observations remains to be defined.

In the clinical arena, markers of subclinical inflammation are powerful predictors of cardiovascular outcomes,[84] but a definitive causal link between inflammation and CVD and the role of pharmacologic modulation of this cascade remain unproven. In addition to the established markers of C-reactive protein (CRP) and interleukin (IL)-6, an emerging inflammatory marker of interest is matrix metalloproteinase (MMP)-9.[92] The family of metalloproteinases degrades collagen, and in the setting of an atherosclerotic plaque, these enzymes may destabilize the fibrous cap and precipitate unstable ischemic events. To evaluate the effect of TZDs on these markers of subclinical inflammation, Haffner *et al* analyzed serum samples from 357 patients who had participated in a 26-week randomized blinded study evaluating rosiglitazone versus placebo. They found that treatment with rosiglitazone was associated with significant reductions in circulating CRP, MMP-9, and white blood cell counts (but not IL-6). Although the clinical relevance of these observations remains uncertain, the apparent anti-inflammatory effect of rosiglitazone suggests that TZDs may play a role in preventing atherosclerotic disease development and progression.

Atherosclerosis Development and Progression

Several studies have demonstrated favorable effects of TZDs on parameters of atherosclerotic disease development and progression. These include *ex vivo* preparations of vascular endothelial and vascular smooth muscle cells, animal models of atherosclerosis, and a few human studies evaluating surrogate markers of atherosclerotic disease.

The development and progression of intraluminal atherosclerotic plaque is dependent upon migration and proliferation of vascular smooth muscle and endothelial cells. In addition to propagation of native disease, this process has been implicated in restenosis following percutaneous intervention. In *ex vivo* preparations of animal and human vascular smooth muscle and endothelial cells, TZDs are potent inhibitors of migration and proliferation.[93-96]

Animal studies have also suggested a role for TZDs in the inhibition of atherosclerosis. In 2 different mouse models of atherosclerosis (apolipoprotein (apo)-E knockout; low-density lipoprotein receptor deficient ($LDLR^{-/-}$)), treatment with TZD therapy results in a dose-dependent inhibition of atherosclerotic lesion development. Using *in vivo* rat models, TZD therapy attenuates the atherosclerotic response to arterial injury.[95,97,98] In addition to attenuation of arterial pathology in these models, TZDs were also demonstrated to favorably modify lipid parameters and markers of inflammation, and to inhibit smooth muscle cell proliferation.

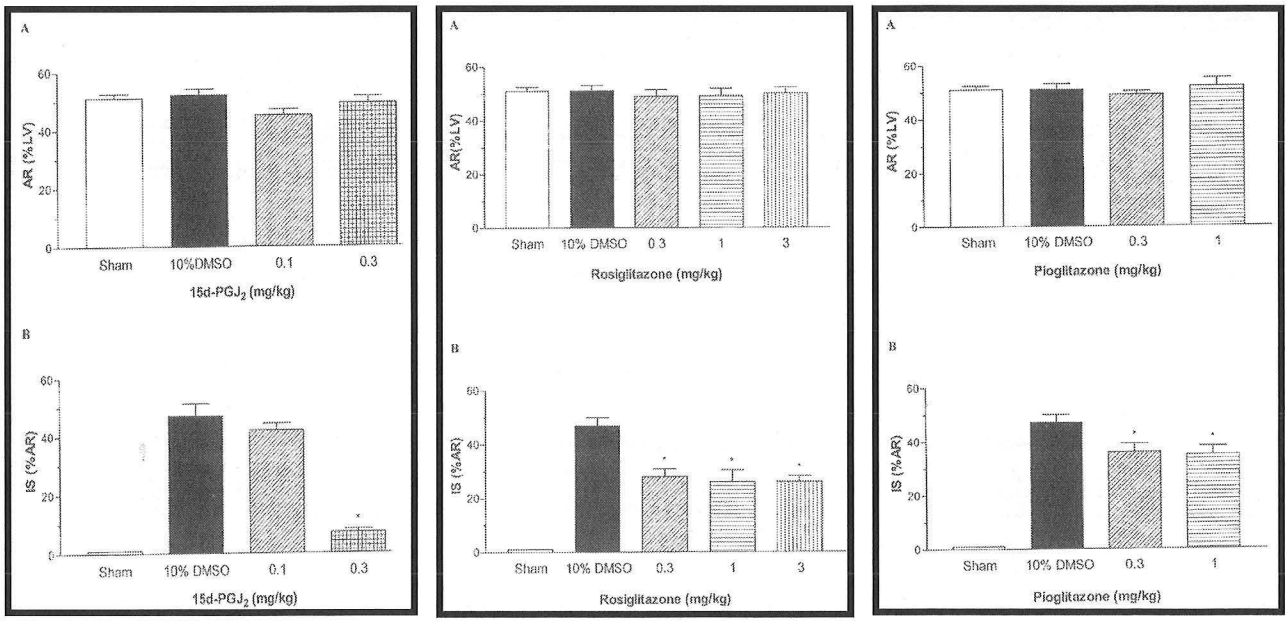
In the clinical setting, several studies have demonstrated favorable effects of the TZDs on intermediate markers of atherosclerotic disease progression. Epidemiologic data have demonstrated a clear association between common carotid intimal-media thickness (IMT) determined by ultrasound and long-term cardiovascular risk,[99] raising the possibility that IMT is a marker of underlying systemic atherosclerosis. In 2 separate placebo-controlled studies, 6 months of treatment with either troglitazone or pioglitazone

was associated with significant decreases in carotid IMT, suggesting a favorable impact on atherosclerotic disease progression.[100,101] In 2 other controlled studies, the magnitude of angiographic restenosis following percutaneous coronary intervention was decreased with troglitazone versus placebo over a 6-month treatment period.[102,103] Therefore, the accumulated data from animal and human investigations provide strong support for a possible role of the TZDs in cardiovascular risk modification among patients with type 2 diabetes.

Acute coronary syndromes

Acute coronary ischemic events, including unstable angina, non-ST elevation myocardial infarction, and ST-elevation myocardial infarction occur as the consequence of atherosclerotic plaque rupture and resultant intraluminal thrombosis. Once atherosclerotic lesions have formed, a fibrous cap comprised of vascular smooth muscle cells and extracellular matrix develops, and this cap is thought to effectively stabilize the plaque from rupture and subsequent atherothrombotic ischemic complications. However, the integrity of the fibrous cap may be compromised by a number of local mechanisms, including degradation via matrix metalloproteinase (MMP) activity, as discussed above. One of the most important of these enzymes is MMP-9, because if functions as a downstream effector in the MMP cascade. Since the expression of MMP-9 appears to be inhibited by TZDs,[90,104] the possibility exists that even in the setting of advanced atherosclerosis, TZD therapy may stabilize the disease process and therefore decrease clinical risk of acute ischemic complications.

Once an acute coronary ischemic event occurs, TZDs may influence the ischemic complications through a number of plausible mechanisms. In several rat models of myocardial ischemia/reperfusion, PPARγ agonism with either natural ligand (15d PGJ_2) or with TZDs result in dose-dependent decreases in myocardial infarct size and improved parameters of cardiac performance.[105-108]



Myocardial area at risk and infarct size in rats subjected to ischemia (25 minutes) followed by reperfusion (2 hrs), treated with vehicle or different doses of PPARg agonists.(Wayman, 2002)

In one study evaluating the effect of TZDs on infarct size, rosiglitazone inhibited activation of the mitogen-activated protein (MAP) kinase, Jun NH_2-terminal kinase (JNK), which plays a key role in cardiac myocyte apoptosis. This observation implies that the decrease in infarct size may at least in part be due to decrease apoptosis in response to injury. Other potential mechanisms by which infarct size may be reduced with TZDs include improved coronary flow reserve (discussed above);[83] decreased circulating free-fatty acids, which are associated with adverse ischemic outcomes;[109] or possibly through the mechanism of ischemic preconditioning,[110] although no data are available regarding the latter possibility.

Diabetes and impaired glucose tolerance are associated with a relative hypercoagulable state,[111] perhaps due to increased expression of plasminogen activator inhibitor (PAI)-1,[112] which inhibits the fibrinolytic activity of tissue plasminogen activator (tPA). Natural ligands of PPARγ increase expression of PAI-1 in *ex vivo* human endothelial cell preparations,[77] an effect that could exacerbate the hypercoagulable state and worsen the consequences of acute coronary ischemic events. However, the TZDs do not appear to have the same effect as the natural PPARγ ligands on PAI-1 expression. For example, in human endothelial cell culture, troglitazone and pioglitazone blocked TNF-α stimulation of PAI-1 expression.[113] Likewise, in the clinical setting, treatment with troglitazone was associated with decreased plasma levels of PAI-1 in nondiabetic obese patients,[89] as well as in patients with polycystic ovarian syndrome.[114] In summary, several studies suggest the possibility of benefit associated with TZDs in the setting of acute coronary ischemic events, but the net influence of these effects on clinical outcomes remains to be defined.

Congestive Heart Failure

Both pioglitazone and rosiglitazone have been associated with an increased risk for peripheral edema and rare cases of congestive heart failure, especially when used in combination with insulin or in elderly patients. These observations prompted the FDA recently to require a modification in the product labeling for both available TZDs advising physicians to monitor patients on TZDs for rapid increases in weight, development of edema, shortness of breath, or other CHF symptoms, and to discontinue drug therapy if these symptoms develop. The FDA also cautions against treating patients with New York Heart Association (NYHA) Class III and IV heart failure with either rosiglitazone or pioglitazone, because these patient groups have not been represented in clinical investigations.

Despite the FDA warnings and labeling modifications, the clinical importance of the observed risk for peripheral edema and CHF, and the mechanisms responsible remain undefined. In the absence of sufficient data to accurately determine long-term CVD risk and benefit of TZDs, the clinical relevance of such reported events is not clear. For example, even if the edema represents a new diagnosis of heart failure, the apparent small increment in CHF risk may be countered by other benefits. To place these observations and the FDA warnings into context, a review of the literature regarding the peripheral vascular, hemodynamic, and myocardial effects of the TZDs is necessary.

Vascular permeability

Peripheral edema is one of the most common adverse effects associated with the TZDs, with a reported incidence of 3.5-16%. However, it remains unclear if the peripheral edema represents "cardiac" edema (i.e. due to elevated end-diastolic pressures) or "low pressure" peripheral edema similar to that observed with dihydropyridine calcium-channel blockers and non-steroidal anti-inflammatory medications. Of note, TZDs exhibit some properties of L-type calcium channel antagonism very similar to the dihydropyridine calcium-channel blockers, [69,115] and may cause peripheral edema by similar mechanisms. The TZDs have also been shown to increase expression of vascular endothelial growth factor (VEGF),[116-118] which induces microvascular permeability and may contribute to peripheral edema in the absence of cardiac abnormality.[119]

Although peripheral edema has consistently been associated with all TZDs evaluated, reports of congestive heart failure associated with TZD use have been rare and are limited to isolated case-reports.[120,121] However, the true incidence of CHF has not been reported to date by the companies marketing TZDs, and may be higher than what has been reported. The discordance in the incidence of peripheral edema versus overt congestive heart failure suggests that the peripheral edema is most likely a consequence of vascular permeability rather than impaired cardiac performance. Therefore, if the development of peripheral edema associated with TZDs is a consequence of altered vascular permeability, it unlikely portends the adverse prognosis associated with cardiac edema and therefore may not require discontinuation of the drug.

Volume overload and cardiac performance

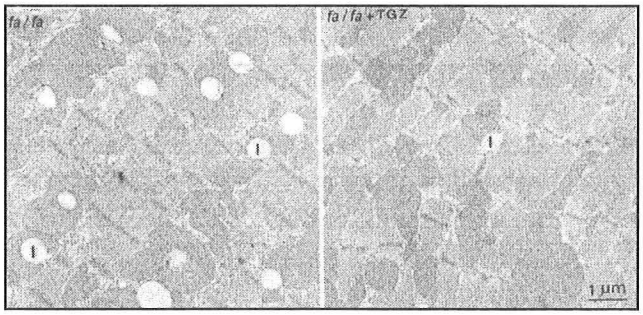
One mechanism by which TZDs could precipitate congestive heart failure involves plasma volume expansion (approximately 6-8%) that occurs subacutely after initiation of TZD therapy.[122-124] While most patients can tolerate modest increases in intravascular volume, patients with impaired cardiovascular reserve of any etiology (including patients with diabetes) may manifest CHF signs and symptoms. In this paradigm, there are no direct adverse cardiovascular effects of TZDs, but instead the incident CHF is simply a manifestation of previously occult cardiovascular disease.

Although it is possible that direct adverse cardiac effects of TZDs could contribute to the development of peripheral edema and CHF, such as impairment in systolic or diastolic function, there are no data yet reported to support this notion. In fact, there is an extensive dataset from *ex vivo* experiments and animal models that suggests the opposite: TZDs inhibit or reverse cardiac hypertrophy and improve parameters of systolic and diastolic performance. In controlled experiments using rat cardiac myocyte cell culture, TZDs inhibited myocyte hypertrophy in response to either angiotensin II exposure or to mechanical strain.[125,126] In intact animal models, PPARγ agonists improve contractility and systolic performance,[107,127-129] enhance diastolic performance,[107,128-130] and decrease cardiac hypertrophy independent of loading conditions.[125,126,131]

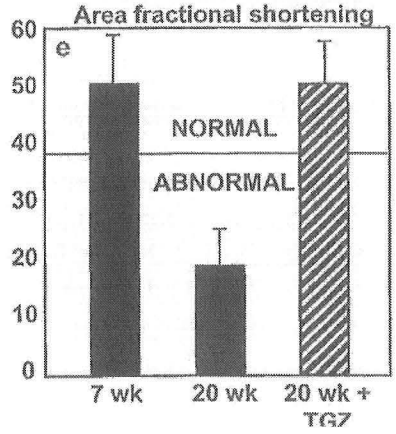
Similarly, human studies have demonstrated no untoward effects in parameters of cardiac performance and some trends toward improved systolic function associated with longer-term TZD therapy.[127,132] For example, a randomized trial evaluating 48 weeks of treatment with troglitazone versus glyburide in 154 patients with type 2 diabetes demonstrated significant improvements from baseline in stroke volume index and cardiac index associated with troglitazone therapy, with no change in left ventricular mass

index.[127] No changes in these parameters were observed in the group treated with glyburide. In a similar randomized blinded clinical trial that included 203 patients, the effects of rosiglitazone versus glyburide on echocardiographic parameters of left ventricular mass and function with type 2 diabetes were evaluated. After 52 weeks on study drug, no difference was observed between the treatment groups in LV mass index, ejection fraction, or left ventricular end-diastolic volume.[132] The incidence of peripheral edema in these studies was 10% and 6.7%, respectively, and no adverse effects on cardiovascular performance were reported among the patients with peripheral edema.

Finally, an emerging area of interest relates to the toxic deposition of lipid in of non-adipose tissues,[37] including cardiac myocytes,[133] a process that has been termed "lipotoxicity".[37] In the absence of obesity, and in the presence of normal leptin concentrations and activity, triglycerides are preferentially stored in adipocytes as a reservoir of energy supply. However, in the setting of obesity or absolute or relative leptin deficiency/resistance, triglycerides may be deposited in non-adipocyte tissues, including cardiac myocytes. This intracellular accumulation of "ectopic" fatty acid results in cellular dysfunction of non-adipose tissues and stimulates nonoxidative fatty acid metabolism that increases traffic through ceramide pathway that ultimately leads to "lipo"-apoptosis.[37] In addition, such accumulation of intra-cardiac lipid may alter the compliance and contractile characteristics of the myocardium leading to impaired diastolic and systolic performance.[134] Troglitazone has been demonstrated in the Zucker rat model to favorably affect intra-cardiomyocyte lipid concentrations and to prevent loss of contractile function.[133] Therefore, the possibility remains that chronic treatment with TZDs could favorably affect cardiac performance and local metabolism by directly reducing intracardiac lipid deposition in obese or leptin deficient individuals.



Reduction of lipid droplets in rat cardiac myocytes after treatment with troglitazone.(Zhou, 2000)



Effect of 13 weeks of troglitazone therapy on Zucker rat LV performance. (Zhou, 2000 #3214)

In summary, peripheral edema is a relatively common adverse effect associated with TZD therapy, and the mechanism and clinical relevance of this observation remains unclear. The most likely explanation for the development of peripheral edema involves a combination of a modest increase in intravascular volume associated with TZDs and

increased peripheral vascular permeability. No studies reported to date have demonstrated adverse effects on either systolic or diastolic cardiac performance, and an accumulating dataset suggests that long-term treatment with TZDs may have favorable effects on these parameters.

Future Developments

The promise of the TZD class of medications and the renewed interest in strategies of insulin sensitization has led to a flurry of activity in drug development, with many of the major pharmaceutical companies now developing agonists to PPARs. In addition to the PPARγ agonists, the pharmaceutical development "pipeline" includes compounds that have activity across the family of nuclear receptors with the hopes of combining the lipid lowering effects of PPARα and –δ agonism with the glycometabolic effects of PPARγ activation.

Table 6. Agonists to nuclear receptor super-family presently in development.

	Drug	Phase	Company
PPARγ			
	MK 0767	III	Merck
	Netoglitazone	II	J&J/Mitsubishi
	JTT 501	II	Pharmacia/Japan Tobacco
	CS 011	II	Pfizer/Sankyo
	NC 2100	Discovery	Nippon Chemipar
	SB 219994	Discovery	GSK
	R 483	Discovery	Roche
PPARα/γ			
	NN 622	III	Novo Nordisk
	BMS 298585	IIb/III	BMS
	KRP 297	II	Merck/Kyorin
	Reglitazar	II	Jaban Tobacco/Pharmacia
	NN 2344	II	Novo Nordisk
	AZ 242	II	AstraZeneca
	FK 614	II	Fujisawa
	GW 409544	I	GSK
	LY 510929	Discovery	Lilly/Ligand
	DRF 4158	Discovery	Novartis
PPARδ			
	GW 501516	Phase I	GSK/Ligand
	L 165041	Discovery	Merck
RXR modulators			
	LG 101506	Discovery	Ligand
	MX 6054	Discovery	J&J/Maxia

Clinical investigations

To further evaluate the effect of TZDs on long-term cardiovascular outcomes, a number of large-scale randomized trials (summarized in the table below) are underway that should be adequately powered to assess the effects of TZD therapy on objective cardiovascular clinical outcomes. However, all of these trials have planned follow up periods of 3-6 years, so the results will not be available for several years.

The Prospective Pioglitazone Clinical Trial in Cardiovascular Events (PROactive) is the most promising of the studies underway to determine the effect of TZD therapy on cardiovascular disease due to the large sample size (5000), the simple placebo-controlled design, the inclusion of high-risk patients, and the evaluation of objective clinical endpoints. Likewise, the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of glycaemia in Diabetes (RECORD) study will compare the cardiovascular effects of rosiglitazone added to either metformin or sulfonylurea compared with an active control arm treated with metformin and sulfonylurea. Compared with PROactive, RECORD is a smaller study with an active-control design and 3 randomized treatment strategies, all of which decrement the statistical power of the study. However, the follow-up planned for RECORD is 50% longer than PROactive, which will offset these limitations. The Bypass Angioplasty Revascularization Intervention-type 2 Diabetes (BARI 2D) study, sponsored jointly by the NHLBI and GSK, is underway and plans to enroll 3000 patients with type 2 diabetes and obstructive coronary artery disease into a trial that will randomize initial glycemic control therapy to insulin-providing therapy (insulin and/or sulfonylurea) versus insulin-sensitizing therapy (metformin and/or TZD). Rosiglitazone will be the TZD of choice for use in the insulin-sensitizing arm. Because use of TZDs within the insulin-sensitizing arm will be at the discretion of the treating physician (and not a randomized assignment), the influence of TZDs on outcomes will only be possible through *post hoc* observational analyses in this trial. Finally, the Diabetes Reduction Assessment with ramipril and rosiglitazone Medication (DREAM) study will enroll 4000 patients with impaired glucose tolerance to evaluate the effect of rosiglitazone versus placebo on the primary outcome of incident diabetes and all-cause mortality over at least 3 years of treatment, with planned secondary analyses of cardiovascular outcomes.

Table 7. Randomized clinical trials underway evaluating the effect of TZDs on cardiovascular outcomes.

Study	Objective	Rx's	Size	Length	1^0 Endpoint	2^0 Endpoints
DREAM	Prevent IGT→DM and Death	Rsg vs plac; Ram vs. plac	4000	3 years	New DM or Death	MACE; Microvascular endpoints
RECORD	Prevent CV events	Met+Rsg; Rsg+SU; Met+SU	4000	6 years	Time to CV event composite	Individual CV events and glycemic parameters
BARI-2D	Prevent Death and CV events	IP (ins +/- SU) vs. IS (TZD +/- Met)	3000	5 years	All-cause mortality	CV events Quality of life
PROactive	Prevent CV events	Pio vs Plac	5000	4 years	MI, CVA, Amputation, Revascularization, CV death	Individual events; CV risk factors

IGT-impaired glucose tolerance; DM-diabetes mellitus; Rsg-rosiglitazone; Plac-placebo; Ram-ramipril; CV-cardiovascular; Met-metformin; SU-sulfonylurea; IP-insulin-providing therapy; IS-insulin-sensitizing therapy; Pio-pioglitazone; Glyb-glyburide;
DREAM-Diabetes reduction assessment with rosiglitazone and ramipril medication
RECORD-Rosiglitazone evaluated for cardiac outcomes and regulation of glycaemia in diabetes
BARI-2D-Bypass angioplasty revascularization intervention in type 2 diabetes
PROactive-Prospective Actos clinical trial in macrovascular events

Conclusions

The thiazolidinedione class of medications has emerged as a safe and effective therapy for the treatment of hyperglycemia associated with type 2 diabetes, as monotherapy or in combination with existing therapies. A number of pleiotropic effects of these medications have fostered optimism regarding their possible effects on improving cardiovascular outcomes among this high-risk population of patients. However, some data suggest a safety concern for patients with or at high risk for congestive heart failure, and the clinical importance of these observations remain to be defined. Additional investigation is required to clarify the CHF risk of these drugs, and most importantly, large-scale clinical investigations to define the effect of the TZDs on important cardiovascular outcomes are underway.

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