In Vivo Actions of Peroxisome Proliferator–Activated Receptors

Glycemic control, insulin sensitivity, and insulin secretion

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Peroxisome proliferator–activated receptors (PPARs) form a family of nuclear hormone receptors involved in energy hemostasis and lipid metabolism (1,2) and include three isotypes encoded by different genes: PPARα (chromosome 22q12–13.1), PPARβ/δ (chromosome 6p21.2–21.1), and PPARγ (chromosome 3p25). PPARα was the first discovered and causes cellular peroxisome proliferation in rodent livers (3), giving this receptor family its name. Upon activation, PPARs interact with retinoid X receptor to create heterodimers, which bind to a specific DNA sequence motif termed peroxisome proliferator response element (4). Peroxisome proliferator response element usually appears in promoter regions and is constructed from repeats of nucleotide sequence AGGTCA separated by a single nucleotide.

PPARα is widely expressed in tissues with high fatty acid catabolic activity: brown fat, heart, liver, kidney, and intestine (5). Upon activation by endogenous fatty acids and their derivatives, PPARα mediates fatty acid catabolism, gluconeogenesis, and ketone body synthesis, mainly in liver (6–9). In rodents, PPARα activation also influences immune modulation (10,11) and amino acid metabolism (12), reduces plasma triglyceride, reduces muscle and liver steatosis, and ameliorates insulin resistance (IR) (13,14). Pharmacologic PPARα activation is achieved by fibrates (7) and results in reduced (30–50%) triglyceride and VLDL levels by increasing lipid uptake, lipoprotein lipase–mediated lipolysis, and β-oxidation (15). This is accompanied by a modest increase in HDL cholesterol (5–20%), secondary to transcriptional induction of apolipoprotein A-I/A-II synthesis in liver (15). In man, the primary effect of PPARα is to reduce plasma triglyceride concentration; effects on plasma free fatty acid (FFA) concentration/FFA oxidation, muscle/liver fat content, and muscle/hepatic insulin sensitivity have not been demonstrated with current PPARα agonists such as fenofibrate (16,17). Fibrates are used to treat severe hypertriglyceridemia and combined hyperlipidemia (18–20). Clinical trials to establish a role for PPARα agonists (fenofibrate, gemfibrozil) in primary or secondary cardiovascular prevention in patients with hypertriglyceridemia or diabetes have been disappointing (21,22). Clinically significant effects of fibrates on glucose homeostasis, IR, and insulin secretion in man have not been demonstrated (16,17,23).

PPARβ/δ is expressed ubiquitously, correlating with the level of cellular proliferation exhibited in different tissues (24). In rodents, PPARβ/δ activation exerts metabolic effects in skin, gut, skeletal muscle, adipose tissue, and brain (25,26). Several PPARβ/δ agonists are in clinical trials because of their beneficial effects on dyslipidemia (27,28) and other components of metabolic syndrome (29,30).

PPARγ has two splice variants, PPARγ1 and PPARγ2, differing by 30 amino acids in the N′ terminal end. While PPARγ1 is widely expressed in tissues (skeletal muscle heart, liver) at low levels, both are highly expressed in adipose tissue (31,32). PPARγ is considered the "master" regulator of adipogenesis (33). PPARγ overexpression in cultured fibroblasts transforms them into adipocytes (34), while selective adipose deletion of PPARγ results in lipodystrophy and IR (35–37). Dominant negative PPARγ mutations are associated with lipodystrophy (in the limbs and gluteal region), dyslipidemia, hypertension, and severe IR (38–40). PPARγ polymorphisms (specifically, Pro12Ala) are associated with increased risk of developing type 2 diabetes (T2DM) (41–43). PPARγ agonists, thiazolidinediones (2,44,45), are potent insulin sensitizers, enhance insulin secretion, improve glucose tolerance, and are the focus of this review.

THIAZOLIDINEDIONES: PAST TO PRESENT—Troglitazone was the first thiazolidinedione approved by the U.S. Food and Drug Administration (FDA) and shown to improve insulin sensitivity and β-cell function in T2DM, impaired glucose tolerance (IGT), and nondiabetic individuals (46–50). Troglitazone also was shown to improve endothelial dysfunction in obesity and T2DM (49,51), induce ovulation in PCOS (52), and effectively treat lipodystrophy (53). Troglitazone also caused fat redistribution from visceral to subcutaneous adipose tissue (54,55) and reduced circulating levels of inflammatory adipocytokines and FFAs, while increasing plasma adiponectin levels (2). Thus, troglitazone shares many beneficial effects with pioglitazone and rosiglitazone. However, because of hepatotoxicity troglitazone was removed from the U.S. market by the FDA in 1997 (56). However, the idiosyncratic liver toxicity observed with troglitazone does not appear to be a class effect. In a review of the literature, alanine aminotransferase

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This publication is based on the presentations from the 4th World Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension (CODHy). The Congress and the publication of this supplement were made possible in part by unrestricted educational grants from Abbott, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Ethicon Endo-Surgery, Janssen, Medtronic, Novo Nordisk, Sanofi, and Takeda.
DOI: 10.2337/dcS13-2003
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levels >10 times the upper limit of normal were observed in 0.68% of diabetic patients treated with troglitazone versus no individuals treated with pioglitazone or rosiglitazone (57). (See subsequent discussion on nonalcoholic steatohepatitis [NASH].)

Rosiglitazone shares similar beneficial effects with pioglitazone and troglitazone on insulin sensitivity, β-cell function, glycemic control, endothelial function, and adipocyte metabolism (see subsequent discussion). However, because of concerns about cardiovascular safety rosiglitazone has been severely restricted in the U.S. and has been removed from the market in Europe and many other countries. In 2007, a meta-analysis by Nissen and Wolski (58) suggested an increased incidence of cardiovascular events in diabetic patients treated with rosiglitazone. In 2010, a patient-level analysis by FDA statisticians of data supplied by GlaxoSmithKline gave hazard ratio (HR) 1.4 for composite MACE end point (cardiovascular death, myocardial infarction [MI], stroke) and 1.80 for MI (59), leading to removal of rosiglitazone from the U.S. market for all practical purposes. In a recent literature review, Schernthaner and Chilton found that rosiglitazone consistently was associated with HR >1.0 for cardiovascular events, while pioglitazone was associated with HR <1.0 (60).

In subsequent sections, we will focus on the pleotrophic effect of thiazolidinediones, with emphasis on pioglitazone and rosiglitazone.

Pleotrophic effects of PPARγ agonists

PPARγ agonists exert pleotrophic effects on glucose and lipid metabolism in multiple tissues and have become an important therapeutic agent for treating T2DM (45,61,62).

Glycemic control. Thiazolidinediones are potent insulin sensitizers in liver/muscle/adipocytes (14,61–67), augment/preserve β-cell function (68), and produce durable HbA_{1c} reduction in T2DM. In eight of eight long-term (>1.5 years), double-blind, or active comparator studies (Fig. 1), thiazolidinediones caused durable HbA_{1c} reduction (rev. in 61) lasting up to 5–6 years (69). Their durable effect on glycemic control results from combined action to both augment β-cell function and enhance insulin sensitivity. In T2DM patients with starting HbA_{1c} 8.0–8.5%, one can expect a 1.0–1.5% decrease in HbA_{1c} (70–76). Thiazolidinediones are approved for monotherapy and add-on therapy to all oral hypoglycemic agents, glucagon-like peptide-1 analogs, and insulin (76).

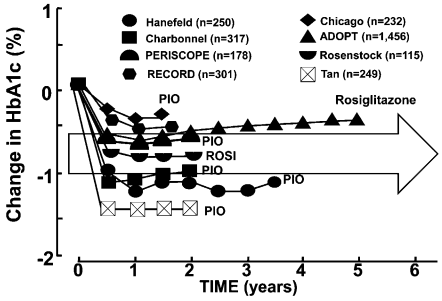

Figure 1—*Thiazolidinediones produce a sustained long-term reduction in HbA_{1c} in eight of eight double-blind or placebo- or active-comparator controlled studies. (See text for a more detailed discussion.) Reprinted with permission from DeFronzo (61).*

Insulin sensitivity in liver and muscle. In liver, thiazolidinediones augment insulin sensitivity and inhibit gluconeogenesis, leading to reduction in fasting plasma glucose concentration (63,64). In muscle, thiazolidinediones are the only true insulin sensitizers, producing a decline in postprandial glucose levels (61,66,67). Metformin is a weak insulin sensitizer in muscle, and it has been difficult to demonstrate a muscle insulin-sensitizing effect in absence of weight loss (77,78). Thiazolidinedione-mediated improvement in insulin sensitivity in T2DM is mediated via multiple mechanisms: PPARγ activation, enhanced insulin signaling, increased glucose transport, enhanced glycogen synthesis, improved mitochondrial function, and fat mobilization out of muscle/liver, i.e., reversal of lipotoxicity (45,62,79–82). Recent studies suggest that metabolic effects of thiazolidinediones are mediated by mitochondrial target of thiazolidinediones, mtot1 and mtot2, which represent the pyruvate transporter (83,84).

For insulin to exert its metabolic effects, it must first bind to and activate insulin receptor by phosphorylating three key tyrosine molecules on β chain (Fig. 2). This causes insulin receptor substrate (IRS)-1 translocation to plasma membrane, where it undergoes tyrosine phosphorylation, leading to phosphatidylinositol 3-kinase (PI3 kinase) and Akt activation. This causes glucose transport into cell, activation of nitric oxide synthase with arterial vasodilation (85–87), and stimulation of multiple intracellular metabolic processes (45).

In humans, we demonstrated that insulin-stimulated tyrosine phosphorylation of IRS-1 in muscle is severely impaired in lean T2DM (81,88,89), in obese normal glucose tolerant (NGT) individuals (89), and in insulin-resistant NGT offspring of two T2DM parents (90,91) (Fig. 2); similar results have been reported by others (92–95). This insulin-signaling defect leads to reduced glucose transport, impaired nitric oxide release (explaining endothelial dysfunction), and multiple defects in intramyocellular glucose metabolism.

In contrast to the defect in IRS-1 activation, the mitogen-activated protein (MAP) kinase pathway, which can be activated by Shc, is normally responsive to insulin (61,62,88,89) (Fig. 2). Stimulation of MAP kinase activates multiple intracellular pathways involved in inflammation, cellular proliferation, and atherogenesis (62,96–98).

The defect in IRS-1 tyrosine phosphorylation impairs glucose transport, and resultant hyperglycemia stimulates fasting/postprandial insulin secretion. Because MAP kinase retains normal sensitivity to insulin (62,88,89,94), hyperinsulinemia causes excessive stimulation of this pathway and activation of multiple intracellular pathways involved in inflammation and atherogenesis. This provides a pathogenic link that, in part, can explain the strong association between IR and atherosclerotic cardiovascular disease in nondiabetic and T2DM individuals (99–102).

Thiazolidinediones are the only antidiabetes drugs that simultaneously augment insulin signaling through IRS-1 and inhibit MAP kinase pathway (61,77,81), providing a molecular mechanism to explain results from CHICAGO (104) and Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) (105) studies, in which pioglitazone reduced progression of carotid intima-media thickness (IMT) and coronary atherosclerosis in T2DM. Consistent with these anatomical studies, pioglitazone in PROactive (106) decreased (P = 0.027) MACE end point (death, MI, stroke) by 16%.

Adipocyte insulin sensitivity. In adipose tissue, thiazolidinediones are potent insulin sensitizers, inhibiting lipolysis and release of inflammatory cytokines, while increasing adiponectin secretion (67,79,80,107–109). In T2DM and obese NGT individuals, adipocytes are resistant to insulin's antilipolytic effect, resulting in

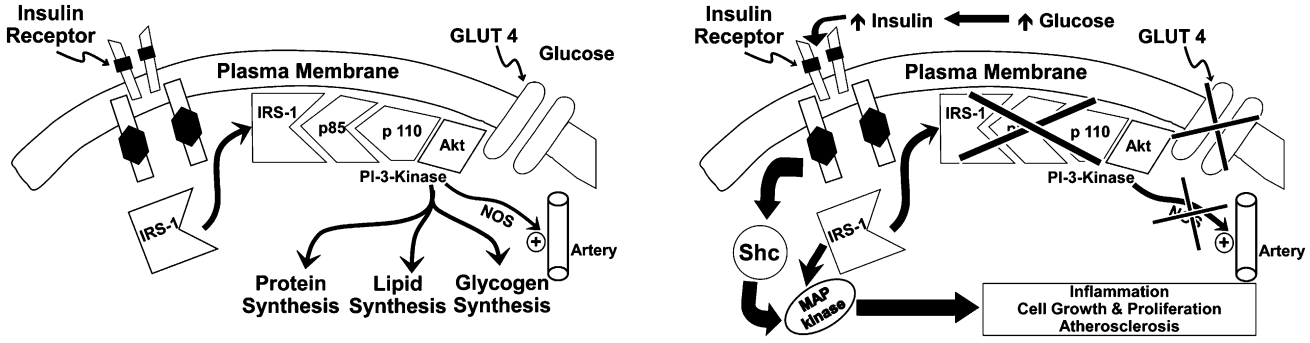

Figure 2—*Insulin signal transduction in healthy nondiabetic* (left panel) *and T2DM* (right panel) *subjects. Thiazolidinediones improve insulin signaling through the PI-3 kinase pathway, while inhibiting insulin signaling through the MAP kinase pathway. Reprinted with permission from DeFronzo (61).*

accelerated triglyceride breakdown with release of FFA. Elevated plasma FFAs enhance FFA flux into cells, leading to accumulation of toxic lipid metabolites (fatty acyl CoAs, diacylglycerol, ceramides), which inhibit insulin action in muscle/liver (62,110–112) and impair β-cell function (113). Thus, these lipotoxic molecules antagonize the core defects that characterize T2DM. By improving insulin sensitivity in adipocytes and inhibiting lipolysis, thiazolidinediones reduce plasma FFA, leading to enhanced insulin sensitivity in muscle/liver and improved β-cell function in T2DM.

In T2DM, adipocytes are in a state of chronic inflammation, as evidenced by monocyte infiltration (114). Inflamed adipocytes release adipocytokines (tumor necrosis factor-α, resistin, angiotensinogen, plasminogen activator inhibitor 1, interleukin-6, and others), which cause IR, impair β-cell function, promote inflammation in distant tissues, augment thrombosis, and accelerate atherogenesis (79,80). Adipocytes from T2DM patients have reduced ability to secrete adiponectin (81,82), a potent vasodilator and antiatherogenic molecule. Thiazolidinediones suppress inflammation in adipose tissue, inhibit release of inflammatory and prothrombotic adipokines, and augment adiponectin secretion.

Thiazolidinediones reverse lipotoxicity

The current diabetes epidemic is being driven by the obesity epidemic. Both obesity and T2DM are characterized by tissue fat overload (Fig. 3). Accumulation of intracellular toxic lipid metabolites causes IR in muscle/liver by inhibiting insulin signaling, glycogen synthesis, and glucose oxidation (rev. in 61,62). Fat accumulation in liver causes nonalcoholic fatty liver disease (NAFLD) and NASH (115), which has become the leading cause of cirrhosis in Westernized countries. Fat accumulation in β-cells impairs insulin secretion and promotes apoptosis (113). Fat deposition in arteries promotes atherogenesis (62), while fat accumulation in visceral depots is associated with coronary arterial disease (116).

Thiazolidinediones reverse lipotoxicity by mobilizing fat out of muscle/liver/β-cells/arteries and relocating fat to subcutaneous adipose depots where it is metabolically "benign" (62,79,80) (Fig. 3). After binding to PPARγ, thiazolidinediones stimulate subcutaneous adipocytes to divide and induce multiple genes involved in lipogenesis (117). Newly formed subcutaneous adipocytes take up FFA, leading to marked reduction in plasma FFA and decreased FFA flux into liver/muscle/β-cells/arteries. Thiazolidinediones also increase expression of PPARγ coactivator (PGC-1), the master regulator of mitochondrial biogenesis (118,119). Increased PGC-1 upregulates multiple mitochondrial oxidative phosphorylation genes, increasing fat oxidation and decreasing levels of intracellular toxic lipid metabolites.

Thiazolidinediones and β-cell function

Thiazolidinediones exert potent effects to improve/preserve β-cell function (68) and demonstrate durability of glycemic control for up to 5–6 years in eight of eight studies (rev. in 61). This is in contrast to sulfonylureas and metformin, which, after initial HbA_{1c} decline, are associated with progressive HbA_{1c} rise, resulting from progressive β-cell failure (120–122).

In addition to studies performed in T2DM, six studies demonstrate that thiazolidinediones prevent IGT progression

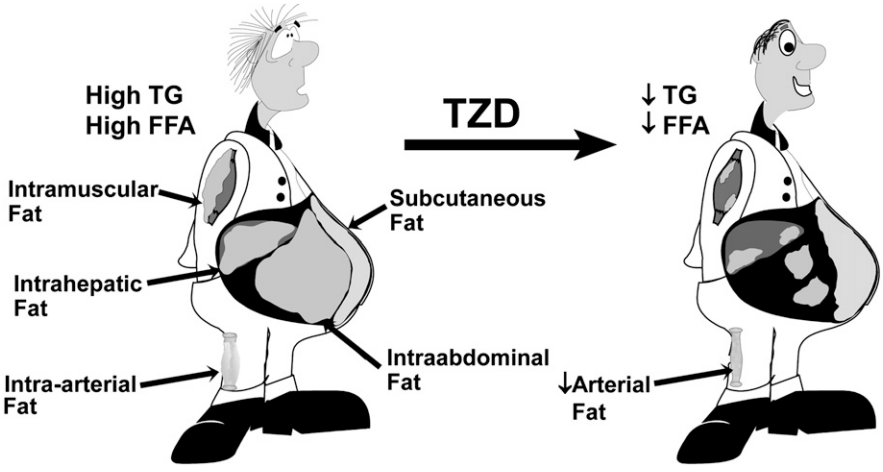

Figure 3—*Body fat distribution in T2DM patients and its redistribution with thiazolidinediones (TZD). (See text for a detailed discussion.) TG, triglyceride. Reprinted with permission from DeFronzo and colleagues (79).*

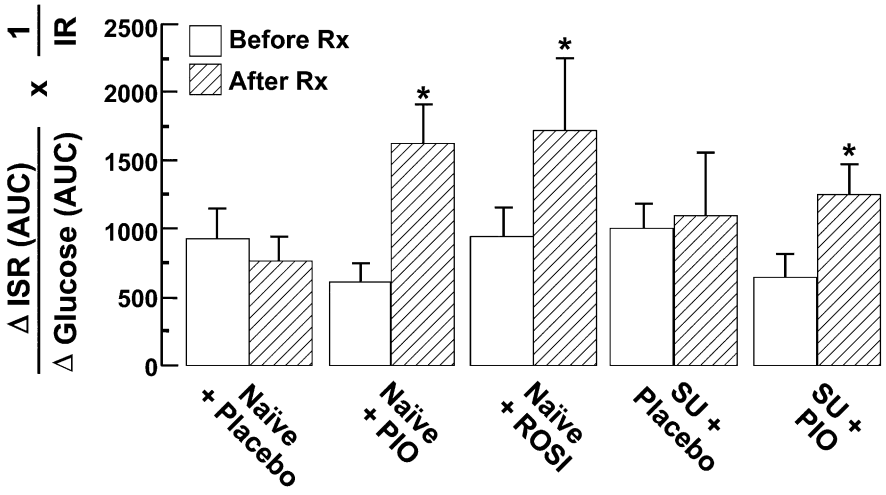

Figure 4—*Thiazolidinediones enhance β-cell function (insulin secretion/IR index) in new-onset, drug-naïve T2DM patients and in long-standing, sulfonylurea-treated T2DM individuals (69). *P < 0.01.*

to T2DM (123–128). In Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM), T2DM was reduced by 62% with rosiglitazone (124), while in Actos Now for the prevention of diabetes (ACT NOW) (127) pioglitazone decreased IGT conversion to T2DM by 72%. All six studies demonstrated that, in addition to their insulin-sensitizing effect, thiazolidinediones preserved β-cell function. β-Cells respond to increased plasma glucose levels with an increase in insulin secretion, and $\Delta I/\Delta G$ is modulated by severity of IR (128). The insulin secretion/IR index ($\Delta I/\Delta G \div$ IR) represents the gold standard for β-cell function and should not be equated with plasma insulin response. In ACT NOW, improvement in insulin secretion/IR index was the strongest predictor of diabetes prevention in IGT subjects and reversion to NGT. Similar results have been demonstrated in TRoglitazone In the Prevention Of Diabetes (TRIPOD) and Pioglitazone In Prevention Of Diabetes (PIPOD) (123,126), in which development of diabetes in Hispanic women with GDM was decreased by 52 and 62%. In Canadian Normoglycemia Outcomes Evaluation (CANOE) (128), low-dose rosiglitazone (4 mg/day), combined with low-dose metformin (1,000 mg/day), reduced IGT conversion to T2DM by 70%. In vivo and in vitro studies with human/rodent islets demonstrate that thiazolidinediones exert protective effects on β-cell function (129–131). Studies from our group using insulin secretion/IR index have shown that thiazolidinediones markedly augment β-cell function in T2DM patients (68) (Fig. 4).

Improved β-cell function with thiazolidinediones results from *1*) stimulatory effect of PPARγ to increase GLUT2, glucokinase (132), and Pdx (133) in β-cells; *2*) reduced intracellular levels of toxic lipid metabolites (129,132,134,135); *3*) muscle/liver insulin-sensitizing effect of thiazolidinediones, which reduce insulin and, therefore, amylin secretion (amylin degradation products are toxic to β-cells [136,137]; the ability of thiazolidinediones to protect human islets from amylin toxicity is mediated via PI3 kinase–dependent pathway [138]); and *4*) studies in β-cell insulin receptor knockout (BIRKO) mice suggest that defective insulin signaling through IRS-1/PI3 kinase impairs insulin secretion (139) and that thiazolidinediones correct this insulin signaling defect (129), resulting in enhanced insulin secretion.

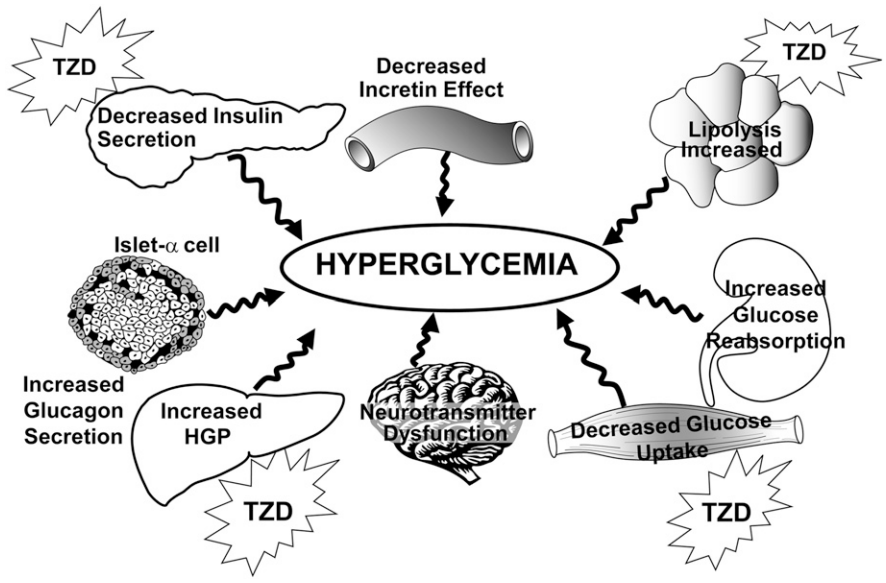

Figure 5—*Pioglitazone corrects four of the eight pathophysiologic components of the Ominous Octet. Modified with permission from DeFronzo (61). TZD, thiazolidinediones.*

Summary

Thiazolidinediones improve multiple defects (IR in liver/muscle/adipocytes and β-cell dysfunction) that comprise the Ominous Octet (61) (Fig. 5), cause durable HbA_{1c} reduction, and can be used as monotherapy or in combination with any other antidiabetes agent. Pioglitazone and rosiglitazone similarly reduce HbA_{1c}, improve insulin sensitivity in muscle/liver/adipocytes, and enhance β-cell function.

THIAZOLIDINEDIONES AND IR SYNDROME

—IR (metabolic) syndrome represents a cluster of metabolic and cardiovascular disorders, each of which represents a major cardiovascular risk factor (62). A common thread linking all IR syndrome components is the basic molecular etiology of IR (61,62,81,88,89), which not only promotes inflammation and atherogenesis but also aggravates other components of the syndrome. Pioglitazone and rosiglitazone ameliorate the molecular defect in insulin signaling, enhance muscle/hepatic/adipocyte insulin sensitivity, correct hyperinsulinemia, improve glucose tolerance and endothelial dysfunction, reduce blood pressure, decrease plasma FFA

levels, increase HDL cholesterol, transform small dense LDL particles into larger less atherogenic ones, shift body fat from visceral to subcutaneous depots, mobilize fat out of muscle/liver, reduce plasminogen activator inhibitor 1/tumor necrosis factor-α levels, and increase plasma adiponectin (rev. in 62). Rosiglitazone produces metabolic effects similar to those of pioglitazone with two notable exceptions: rosiglitazone increases both plasma LDL cholesterol and triglycerides (140). Concerns about cardiovascular safety (58) have led to removal of rosiglitazone from U.S. (56) and European markets.

Pioglitazone reduces cardiovascular events

Pioglitazone is the only antidiabetes medication shown, in a large prospective placebo-controlled outcome study, to reduce cardiovascular events. In PROactive, 5,238 T2DM patients with prior cardiovascular event or multiple CVD risk factors were randomized to pioglitazone or placebo plus standard of care for all cardiovascular risk factors (106). Compared with placebo, pioglitazone reduced the second principal MACE end point (cardiovascular mortality, MI, stroke) by 16% ($P < 0.02$) (Fig. 6*A*). Cardiovascular benefit most likely resulted from combined improvements in dyslipidemia (increased HDL cholesterol), endothelial dysfunction, blood pressure, HbA_{1c}, other inflammatory markers that were not measured, and direct effect on arterial wall to inhibit atherogenesis (141). In a subgroup of 2,445 patients with previous MI, pioglitazone reduced (HR 0.72, $P = 0.04$) likelihood of subsequent MI by 16% (142) (Fig. 6*C*). In 984 patients with previous stroke, pioglitazone caused 47% reduction (HR 0.53, $P = 0.008$) in recurrent stroke (3,143) (Fig. 6*D*).

The composite primary end point (mortality, nonfatal MI, silent MI, stroke, acute coronary syndrome, coronary artery bypass grafting/percutaneous coronary intervention, leg amputation, leg revascularization) did not reach significance (HR 0.90, $P = 0.09$) because of increased number of leg revascularization procedures in the pioglitazone group. Leg revascularization is not a MACE end point and typically is excluded from cardiovascular intervention trials, i.e., with statins, because the major risk factors for peripheral vascular disease are gravity (i.e., subject's height) and smoking, which are not influenced by antidiabetes therapy. Subsequent PROactive analyses confirmed that pioglitazone has no beneficial effect on peripheral vascular disease (144). Consistent with PROactive, a meta-analysis of all pioglitazone studies published (excluding PROactive) and reported to the FDA demonstrated a 25% decrease in cardiovascular events (145) (Fig. 6*B*), and a recent review recommended that pioglitazone should be considered in diabetic patients with cardiovascular disease (146).

Two additional studies demonstrated that pioglitazone slows anatomical progression of atherosclerotic cardiovascular disease. In PERISCOPE (105), T2DM patients with established coronary

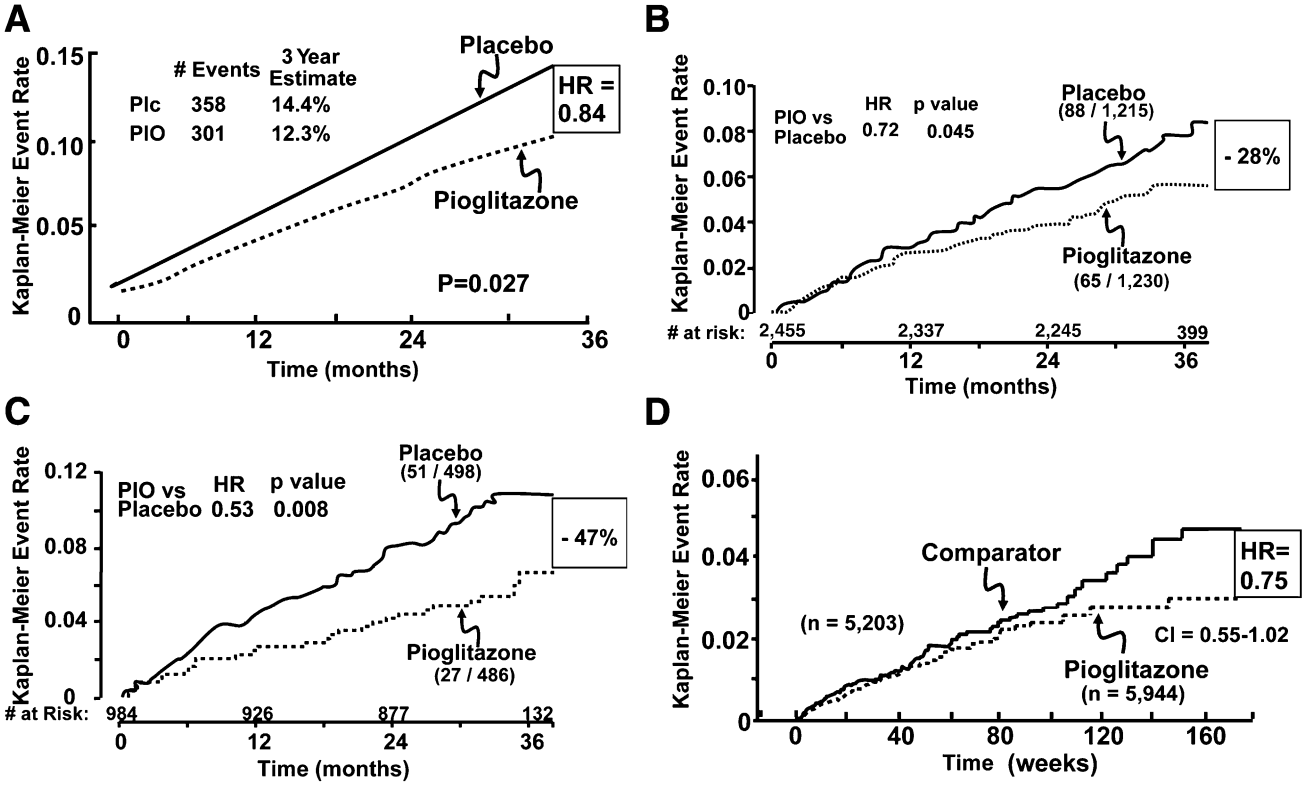

Figure 6—A: *Kaplan-Meier plot of time to MACE end point (mortality, MI, stroke) in T2DM patients treated with pioglitazone (PIO) or placebo (Plc) in PROactive. Redrawn with permission from Dormandy et al. (106).* B: *Pioglitazone reduces recurrent MI in diabetic patients with a previous MI in PROactive. Redrawn with permission from Erdmann et al. (142).* C: *Pioglitazone reduces recurrent stroke in diabetic patients with a previous stroke or PROactive. Redrawn with permission from Wilcox et al. (143).* D: *Meta-analysis of all published studies (excluding PROactive) in which the effect of pioglitazone versus placebo or active comparator on cardiovascular events is examined. Redrawn with permission from Lincoff et al. (145).*

artery disease were randomized to pioglitazone or glimepiride for 1.5 years. In the glimeperide-treated group, percent atheroma volume progressed, while percent atheroma volume regressed in the pioglitazone-treated group. In CHICAGO, pioglitazone halted progression of carotid IMT, whereas carotid IMT progressed in the glimepiride-treated group ($P = 0.008$) (104). Results of these two anatomical trials (104,105), when viewed in concert with cardiovascular outcome trials (106,145), strongly suggest that pioglitazone provides cardiovascular protection, especially in individuals with established cardiovascular disease.

The different effects of pioglitazone and rosiglitazone on cardiovascular outcomes remains unexplained. One obvious explanation is rise in plasma LDL cholesterol and triglyceride observed with rosiglitazone (140). Another explanation involves differential regulation of gene expression by rosiglitazone and pioglitazone. In muscle (147) and adipocytes (148), multiple genes are differentially stimulated or inhibited by the two thiazolidinediones, and the function of these genes is largely unknown.

Thiazolidinediones prevent T2DM in high-risk individuals

Six large prospective, randomized, double-blind, placebo-controlled studies (TRIPOD [126], PIPOD [123], DPP [125], DREAM [124], CANOE [128], and ACT NOW [127]) have provided conclusive evidence that thiazolidinediones dramatically reduce by 52–72% conversion of prediabetes (IGT and/or IFG) to T2DM. In ACT NOW, IGT conversion to T2DM was reduced by 72% and carotid IMT progression was diminished by >50% versus placebo (127). Increased β-cell function (insulin secretion/IR index) was the strongest predictor of diabetes prevention. In ACT NOW and other prevention trials reductions in HbA_{1c}, blood pressure, triglycerides, inflammatory cytokines, and rise in HDL cholesterol also have been observed (127).

THIAZOLIDINEDIONES AND NASH

—In T2DM hepatic fat accumulation, NAFLD is common and represents a precursor for NASH. NASH is associated with hepatic/muscle IR (115) and accelerated atheogenesis (148). Several large, placebo-controlled studies have demonstrated that pioglitazone mobilizes fat from liver, reduces hepatic injury, and causes histologic improvement in inflammation/fibrosis in NASH (149–151). Pioglitazone also reduces liver fat and improves IR in lipodystrophic patients (152). Studies examining effect of rosiglitazone in NASH have shown an initial beneficial effect on liver histologic parameters with no benefit from prolonged continuous treatment (153).

THIAZOLIDINEDIONES AND KIDNEY

—Diabetic rodents develop renal insufficiency and histologic lesions analogous to those in man, and thiazolidinediones reduce mesangial matrix (hallmark lesion of diabetic nephropathy) volume, decrease urinary protein excretion, and prevent renal failure (154,155). PPARγ is expressed diffusely throughout kidney, and PPARγ agonists inhibit mesangial cell proliferation and reduce mRNA expression of matrix proteins (collagen, fibronectin) and transforming growth factor-β, which has been implicated in glomerular injury (156). In diabetic humans, pioglitazone (157) and rosiglitazone (158) reduce albuminuria, although long-term studies examining effect of thiazolidinediones on GFR have not been performed. Beneficial effect of thiazolidinediones to reduce albuminuria cannot be explained by improved glycemic control and is closely correlated with improved insulin sensitivity (159).

Diabetic individuals with renal insufficiency are at increased risk for cardiovascular disease/mortality (159). In PROactive, pioglitazone significantly reduced MACE end point in patients with and without reduced GFR (160). Thiazolidinediones also reduced all-cause mortality in hemodialysis-treated patients (161).

SAFETY

—Benefits of pioglitazone on glycemic control and prevention of cardiovascular disease are well established. However, physicians must be cognizant of potential side effects to maximize benefit and minimize risk. The majority of pioglitazone's beneficial effects on glucose metabolism, insulin sensitivity, insulin secretion, and cardiovascular risk factors are observed with a dose of 30 mg/day (70,162). At this dose, side effects are mild and manageable. Increasing dose to 45 mg/day provides little more efficacy and substantially increases risk of side effects (70). Therefore, we recommend a starting dose of 7.5–15 mg/day, tritiated to 30 mg/day (163–165). Combined pioglitazone/metformin therapy (166,167) is particularly effective in reducing HbA_{1c}, does not cause hypoglycemia, and minimizes side effects. Moreover, both pioglitazone (106,145) and metformin (121) reduce cardiovascular events, although the number ($n = 344$) of subjects in the metformin arm of the UK Prospective Diabetes Study (UKPDS) was small and would not satisfy current standards for a cardiovascular intervention study.

Fat weight gain

On average, pioglitazone-treated subjects gain ~2–3 kg of fat weight after 1 year (70,76,106,168), which results from PPARγ stimulation of hunger centers in hypothalamus (169). Simultaneously, PPARγ activation redistributes fat from visceral to subcutaneous depots (55,79,170), mobilizes fat out of muscle/liver/β-cells (79,80,149,150,171), inhibits lipolysis/reduces plasma FFA (79,80,109), and stimulates PGC-1/other mitochondrial genes involved in lipid oxidation (118). The net result is a metabolically more favorable fat distribution from visceral to subcutaneous depots where it is metabolically benign (79,80) and depletion of toxic lipid metabolites in muscle/liver/β-cells (62). Of note, the greater the weight gain, the greater the improvements in β-cell function and insulin sensitivity and the greater the reduction in HbA_{1c} (68,170,172). On a short-term basis, i.e., up to 3 years (106), no adverse effects of thiazolidinedione-associated weight gain have been observed. Long-term effects, if any, of thiazolidinedione-associated weight gain remain unknown. Weight gain, if excessive, should be managed with reinforcement of dietary advice and exercise, reduction in pioglitazone dose, or use of pharmacologic agents approved for weight loss.

Bone fractures

T2DM patients treated with thiazolidinediones have increased risk of fracture (173–176), which primarily occurs in distal long bones of upper (forearm, hand, wrist) and lower (foot, ankle, fibula, tibia) limbs and is related to trauma. Excess fracture risk is 0.8 fractures per 100 patient-years (1.9 in pioglitazone treated vs. 1.1 in comparator treated) (173–176). This represents a small but significant risk. Since increased fracture risk primarily occurs in postmenopausal females and not in premenopausal women or men, pioglitazone should be used with caution in postmenopausal women or not at all.

Fluid retention and congestive heart failure

Thiazolidinediones may cause fluid retention, which can exacerbate heart failure in diabetic patients who do not uncommonly have underlying diastolic dysfunction (106). When used as monotherapy, edema occurs in 3–5% of individuals and is dose related (177). Edema most commonly occurs when thiazolidinediones are used with sulfonylureas and especially with insulin (177–180). Fluid retention occurs secondary to peripheral vasodilation (181) and stimulation of ENac (epithelial sodium) channel in collecting duct (182). Sodium retention responds well to distally acting diuretics, spironolactone or triamterene (183). Pedal edema identifies individuals at risk to develop congestive heart failure (CHF) and who should be treated with a diuretic or reduction in pioglitazone dose. In PROactive, incidence of CHF was 6%. However, cases were not adjudicated, and mortality and cardiovascular events tended to be decreased in pioglitazone-treated individuals who developed CHF (106,184). These results suggest that after excess fluid has been diuresed, the cardioprotective effect of pioglitazone becomes evident. Lastly, pioglitazone has no negative impact on cardiac function (185) and improves endothelial dysfunction (186).

THIAZOLIDINEDIONES AND CANCER

—In PROactive (106), incidence of malignancy was similar in pioglitazone (3.7%) and placebo (3.8%) groups. However, two imbalances were noted. There were more cases of bladder cancer in pioglitazone (n = 16) versus placebo (n = 6) groups (P = 0.069). Prior to unblinding, external experts adjudicated that 11 cases could not plausibly be related to treatment. Of the remaining nine case subjects, six were treated with pioglitazone and three with placebo (P = 0.309). The other imbalance was related to breast cancer; there were fewer breast cancers in the pioglitazone versus placebo group (3 vs. 11, P = 0.034). Thus, the nonsignificant increase in bladder cancer was numerically offset by the statistically significant decrease in breast cancer.

In 2003, the FDA requested that a safety study be conducted to assess whether pioglitazone increased bladder cancer risk. After 4 years of a 10-year longitudinal cohort study of 193,099 patients (187), ever use of pioglitazone was not associated with increased bladder cancer risk (HR 1.2 [95% CI 0.9–1.5]). However, in patients receiving pioglitazone for ≥24 months, there was slight increased bladder cancer risk (1.4 [1.03–2.0]); 95% of cancers were detected at an early in situ stage, and authors acknowledged that this could have been attributed to the fact that pioglitazone-treated patients underwent greater surveillance for bladder cancer. Bladder cancer risk increased from 7/10,000 patient-treatment years (no pioglitazone) to 10/10,000 (with pioglitazone)—an increase of 3 cases per 10,000 patient-treatment years. Overall, there was no increase in total cancers in pioglitazone-treated patients (187,188), and risk of some cancers (colon, kidney/renal pelvis, breast) was decreased (188). In a recent 8-year analysis of the same study population, HR for bladder cancer was 0.98 (95% CI 0.81–1.18) (189). If pioglitazone actually increased bladder cancer risk, one would have expected HR to increase—not decrease—after 8 years. These results argue against a putative role for pioglitazone in development of bladder cancer. Further, overall incidence of malignancy has been reported not to increase (106) or decrease in certain cancer types (breast and liver) in pioglitazone-treated patients (188,190–192). Lastly, any increased bladder cancer risk must be viewed in the context of protection against all-cause death, MI, and stroke, i.e., MACE end point in PROactive. It has been estimated that treatment of 10,000 patients with pioglitazone would avoid 210 MIs, stroke, or deaths over 3 years (193) compared with a potential increase of three cases of bladder cancer per 10,000 patients over the same period. Moreover, even this increase of 3/10,000 disappeared after 8 years (189).

Based upon the body of evidence reviewed above (not including 8-year follow-up data reported by Lewis), the FDA recommended that pioglitazone not be used in patients with active bladder cancer or prior bladder cancer history. We recommend that any hematuria be evaluated to exclude bladder cancer before starting pioglitazone.

BENEFIT-RISK ANALYSIS

—As reviewed in preceding sections, the benefit-to-risk ratio for pioglitazone is very favorable. Importantly, if physicians are aware of potential risks associated with thiazolidinediones and if the pioglitazone dose does not exceed 30 mg/day, side effects can be reduced even further (Table 1).

Acknowledgments—R.A.D. is a member of the Advisory Board of Takeda, Bristol-Myers Squibb, Janssen, Boehringer Ingelheim, Novo Nordisk, Lexicon, and Amylin. R.A.D. is a member of the Speakers Bureau of Novo Nordisk, Amylin, Bristol-Myers Squibb, and Janssen. No other potential conflicts of interest relevant to this article were reported.

Table 1—*Benefits and risks associated with thiazolidinedione therapy*

Benefit	Risk
• Potent, durable HbA_{1c} reduction	• Fat weight gain
• Low risk of hypoglycemia	• Fluid retention/heart failure
• Reduces IR	• Bone fractures (distal long bones; trauma-related)
• Improves β-cell function	
• Improves cardiovascular risk factors (↑ HDL, ↓ triglyceride, ↓ blood pressure, ↓ inflammation, ↓ microalbuminuria)	• Bladder cancer (potentially)
• Decreases cardiovascular events in high-risk diabetic patients (PROactive; meta-analysis)	
• Reduces cardiovascular events in diabetic patients with chronic kidney disease	
• Improves endothelial dysfunction	
• Improves liver damage in NASH	
• Prevents IGT progression to T2DM (ACT NOW, TRIPOD, PIPOD, DREAM)	

R.E., R.A.D., and M.A.-G. contributed to writing, revising, and reviewing the manuscript. R.A.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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