DRUG EVALUATION

Diabetes Management

Pioglitazone: lessons and learnings 15 years since launch and beyond

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Practice points

- Thiazolidinediones (TZDs) or glitazones are PPARγ receptor agonists.
- The primary action site for TZDs is adipose tissue due to high PPARγ concentration. During the early development of TZDs, the primary focus of efficacy studies was their lipid effects. Carbohydrate metabolic effects were discovered incidentally during this period.
- The first TZD that came on the market was troglitazone; withdrawn 3 years later due to hepatotoxic effects.
- Binding of TZDs with the PPARγ receptor leads to a cascade of intracellular molecular phenomena. Through transactivation and transrepression mechanisms, several genes' transcription can be either stimulated or inhibited, yielding a varied clinical response.
- Pioglitazone is a TZD with a half-life of approximately 9 h. It has a low distribution volume since it is protein-bound in the blood stream (97%). It is metabolized through the liver CYP450 system.
- Pioglitazone's effect on increasing insulin sensitivity has been shown using various methods, followed by glycosylated hemoglobin AIc reductions that varied from -0.78 to -1.7% as monotherapy.
- Among other efficacy endpoints described for pioglitazone, a reduction in free fatty acids as well as high sensitive C-reactive protein has been documented. Several studies have proven a reduction in triglyceride levels with some increase in high density lipoprotein cholesterol concentrations, improved liver histology in patients with fatty liver disease and clinical benefit in women with polycystic ovary syndrome.
- There is evidence for anti-inflammatory effects of pioglitazone at the endothelial level, as well as a decrease in serum inflammatory markers which may also render some potential benefit from a cardiovascular standpoint, except for those patients who suffer from congestive heart failure, in whom pioglitazone is contraindicated.
- Several safety concerns have accompanied pioglitazone since it was introduced into the market. The most frequent side effects are weight increase and fluid retention that can worsen congestive heart failure. It has been established that bone fracture risk exists only in women with other risk factors for fragility fractures. Supported by a robust body of evidence, bladder cancer risk associated with pioglitazone use is less likely and is currently contraindicated only in patients with previous history of bladder cancer.
- Pioglitazone, a PPARγ agonist, causes significant increments in insulin sensitivity not only in fat and muscle tissue, but also in the liver, resulting in an improvement in glycemic control in patients with Type 2 diabetes.
- Pioglitazone's pleiotropic effects on fatty liver disease and endothelial function make it an attractive therapeutic alternative for patients with Type 2 diabetes and insulin resistance (metabolic syndrome).
- Clinical judgment must be used in finding a balance between gains and side effects while selecting patients to be treated with pioglitazone.

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Pioglitazone is a PPARγ agonist that is widely used as an oral antihyperglycemic agent. Its main effect consists of blunting insulin resistance in tissues such as muscle, liver and adipose tissue. Its strength in terms of glycosylated hemoglobin AIc reduction varies within a range of -1.0 to -1.5%. This article examines the role of pioglitazone in the treatment of Type 2 diabetes mellitus and assesses efficacy data on insulin sensitivity and glycemic control, as well as the effects on hepatic steatosis, endothelial function and cardiovascular risk. Finally, concerns linked to pioglitazone use, such as weight gain, fluid retention, bone fragility, bladder cancer and macular edema, are analyzed finding that pioglitazone use must be balanced between the risks and benefits for each individual patient.

KEYWORDS
• glitazone • insulin resistance • pioglitazone • rosiglitazone • thiazolidinedione • troglitazone • Type 2 diabetes mellitus

Thiazolidinediones

In the context of the pathophysiological complexity of Type 2 diabetes mellitus (T2DM), insulin resistance must be understood as an essential mechanism for the development and maintenance of this disease. Organ dysfunction in T2DM is caused in part by a great amount of free fatty acids (FFAs) in the circulation and other inflammatory substances, originating from intra-abdominal adipose tissue, which is subject to constant lipolysis and turnover. The first PPAR to be described was PPARγ. The principal mediators in lipogenesis, these receptors interact with transcription factors that can stimulate or inhibit the synthesis of certain proteins involved in both the biochemical machinery responsible for the intracellular management of glucose and in the inflammatory pathways at the systemic level.

Thiazolidinediones (TZDs), or glitazones, are molecules that exhibit agonist actions on PPARγ receptors. Initially, their development sought reductive effects on lipids without a clear understanding of their mechanism of action. The first of these drugs, ciglitazone, was never marketed due to several toxicity findings during its clinical research program [1]. The effects of TZDs on carbohydrate metabolism were subsequently described and the first of these drugs to be marketed was troglitazone in 1997. In 2000, it was recalled due to toxic effects on hepatic function.

Pioglitazone

• Pharmacokinetic profile

Pioglitazone administered once daily regardless of food intake is well absorbed and subsequently metabolized through the hepatic CYP450 system. Its absorption is rapid and its concentrations are measurable in serum up to 30 min after dosing [2]. Even though pioglitazone's half-life is approximately 9 h, its effect is prolonged due to the presence of two active metabolites (M-III and M-IV). The mean absolute bioavailability of pioglitazone is 83% and the time to reach maximum plasma concentration (t_{max}) is 1.5 h (range: 0.5–3.0). Food intake may discretely delay t_{max}. The average clearance is 2.4 l/h (range: 1.72–4.17) [2]. After single oral doses between 2 and 60 mg, both the maximum concentration (C_{max}) and the area under the curve (which indicates systemic exposure) show lineal, dose-dependent increases without any changes after repeated dosing of the drug. Its distribution volume is low (0.253 l/kg), most probably because more than 97% of the drug is protein bound [2]. Its metabolism through the liver's CYP450 system's isoenzymes is carried out primarily by oxidation and hydroxylation of methylene groups, resulting in the presence of several metabolites. A minimal proportion of intact pioglitazone may be detected in urine. Its elimination is primarily biliary and through feces. Drug interactions are presumed to be low since the induction or inhibition of P450 enzymes involved in the metabolism of pioglitazone by other drugs has not been observed [2]. Finally, no differences in pioglitazone's pharmacokinetic profile were observed between healthy subjects and patients with T2DM. As regards subjects with renal insufficiency, no pharmacokinetic changes requiring dose adjustments were observed. However, a decrease by around 57% in the C_{max} was observed in patients with liver failure, while the distribution volume was increased **(Figure 1)** [2].

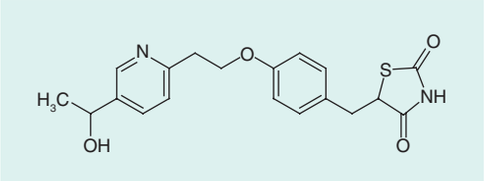

Figure 1. Chemical structure of pioglitazone.

• Clinical efficacy: effects on insulin resistance & glucose metabolism

Through its transactivation and transrepression mechanisms, several effects of pioglitazone on insulin sensitivity, fasting plasma glucose (FPG)

and glycosylated hemoglobin A1c (HbA1c) concentrations have been described, along with its effects on other metabolic parameters, especially lipid profile.

Pioglitazone decreases insulin resistance, as shown in 26 therapy-naive patients with T2DM randomly assigned to receive pioglitazone or intensive nutritional therapy for 6 months. Before and after follow-up, a muscle biopsy was performed (vastus lateralis) as well as an euglycemic–hyperinsulinemic clamp (80 mU m[-2] min[-1]) [3]. Upon completion of the trial, the most notable observations were statistically significant decreases in FFA plasma concentrations, as well as increases in adiponectin levels. Insulin-stimulated uptake of glucose by muscle increased by 30%, as did the activity of AMP kinase and acetyl CoA carboxylase. The activation of several genes involved in mitochondrial function and oxidation of fatty acids by pioglitazone were described, while none of these phenomena were observed in subjects under nutritional therapy alone [3]. A notorious increase in insulin signaling through IRS-1 has also been described [4].

Several research works have determined the effects of pioglitazone on peripheral insulin sensitivity: fasting insulin concentrations [5–8], homeostasis model assessment insulin sensitivity (HOMA-S index) [5,7–9], quantitative insulin sensitivity check index (QUICKI index) [7,10] and adiponectin plasma levels [8]. In general terms, findings with pioglitazone consist of significant decreases in fasting insulin plasma concentrations, notorious increases in the HOMA-S and QUICKI indexes, either as monotherapy or added to other oral agents and increases in adiponectin levels. Tan *et al.* showed pioglitazone's effects on the HOMA-S and QUICKI indexes (primary outcome variable) in an Hispanic-Mexican population (n = 244), consisting of significant improvements in the latter, as compared with glimepiride-based therapy [7]. Alternately, in a trial comparing the effects of a fixed-dose mixture of pioglitazone + metformin with those of metformin monotherapy in Japanese patients, much more marked improvements in HOMA-S index, fasting insulin plasma concentrations and increases in adiponectin levels were shown. In addition, decreases in C reactive protein (CRP) as a systemic inflammation marker with the mixture of pioglitazone + metformin were described, and were not observed with metformin monotherapy [8]. These effects were stronger with 30 and 45 mg doses of pioglitazone.

The observed decrease in systemic inflammation markers such as high sensitivity CRP supports the anti-inflammatory role of TZDs as they inhibit the release of inflammatory mediators by adipose tissue and increase the synthesis and release of adiponectin [11].

The activation of PPARγ receptors by pioglitazone involves the need for a faster FFA uptake by fat cells. This explains the decrease in FFA plasma concentrations, the improvement in insulin sensitivity in muscular and adipose tissue and the decrease in the lipotoxic effect on β cells [12]. Both the decrease of insulin resistance and the lowering of the lipotoxic effect on the β cell might explain the sustained effect on glycemic control described in various trials [6,8–9,13–16]. The protection of the β cell from lipotoxicity might imply, and might have an effect on sparing the pancreatic islet in patients with T2DM in a sort of antiapoptotic phenomenon [11]. Trials of various TZDs (troglitazone, rosiglitazone and pioglitazone) have been performed in patients with glucose intolerance (GI) in order to determine their ability to delay progression to overt T2DM. For troglitazone, the TRIPOD study reported a 52% decrease in the incidence of T2DM in high-risk patients [17]. A 62% decrease in the progression from GI to T2DM was described in the DREAM study for rosiglitazone [18], while the corresponding percentage for pioglitazone in the ACT NOW study was 72% [19].

In terms of HbA1c reduction, decreases in the range of 0.78 [7] to -1.7% [10] have been reported with pioglitazone monotherapy, and in combination with other antidiabetic agents (including insulin), decreases have ranged from -0.67 [8] to -1.3% [20–22]. Decreases in FPG concentrations with pioglitazone monotherapy range from -10.8 mg/dl [7] to -77 mg/dl [10], and in combination with other antidiabetic agents (including insulin), they range from -20.5 mg/dl [8] to -82.8 mg/dl [23].

Several publications have reported favorable effects of pioglitazone treatment on the lipid profile of T2DM patients. These phenomena are partly, and jointly, responsible, for the lower incidence of cardiovascular (CV) events observed in a long-term outcomes study in patients receiving this drug [24]. A noteworthy trial described decreases of around 30% in triglyceride (TG) concentrations in very low density lipoprotein, with concomitant increases of 14% in HDL-cholesterol. Triglyceride decrease was attributed

to an increase in the clearance of TG contained in very low density lipoprotein due to a greater activity of lipoprotein lipase induced by pioglitazone [25]. In a 6-month follow-up study, Aronoff *et al.* reported a 9.6% decrease from baseline in TG concentrations with pioglitazone as monotherapy at 30 mg daily, along with a 12.2% increase from baseline in HDL concentrations [26]. In part of the PROactive trial, with a sample of 2605 subjects receiving pioglitazone titrated from 15 mg to 45 mg daily, the average decrease in TGs observed after a follow-up of 34.5 months was 11.4%, together with an increase of 7.2% in HDL [24]. In an additional study with a follow-up of 16 weeks, 99 patients receiving pioglitazone at 45 mg daily, showed decreases of 16% in TG levels, with increases of 20% in HDL [27]. Finally, findings described by Goldberg *et al.* when comparing the effects on lipid profiles of pioglitazone and rosiglitazone are especially noteworthy. As regards TG, these showed a decrease of 51.9 ± 7.8 mg/dl with pioglitazone in contrast with an increase in 13.1 ± 7.8 mg/dl with rosiglitazone. The corresponding changes in HDL were +5.2 ± 0.5 mg/dl and +2.4 ± 0.5 mg/dl, respectively [28]. These elevations in high density lipoprotein-cholesterol seemed to be directly related to the beneficial effects described in the PROactive trial [29].

• Clinical efficacy: effects on the liver

As a key pathophysiological element in T2DM, insulin resistance has been closely associated with FFA infiltration in the hepatic parenchyma, with the resulting development of hepatic steatosis, which may progress to an inflammatory state with hepatocellular damage, known as nonalcoholic steatohepatitis, or NASH [4].

Much favorable data exist regarding the effects of pioglitazone on NASH and hepatic function [30–32]. In a study by Gastaldelli *et al.* 20 subjects with T2DM previously treated with sulfonylurea (SU) were randomized to receive pioglitazone 45 mg daily or placebo for 16 weeks in order to determine the endogen glucose production rate, gluconeogenesis (GNG) and insulin sensitivity. A significant decrease in hepatic GNG rate and in insulin resistance in the liver (up to 35%) was observed in the pioglitazone group. The changes observed in GNG directly correlate with changes in FPG and FFA concentrations. It is now known that this effect occurs due to the inhibition of key enzymes of GNG (PEPCK, pyruvate carboxylase and glucose-6-phosphatase) after PPARγ activation by pioglitazone [30]. It is important to clarify that the effect of pioglitazone on GNG is one of the primary determinants of its antihyperglycemic effect.

The study by Belfort *et al.* assessed the effects of pioglitazone 45 mg daily compared with placebo on 55 subjects with histological evidence of steatohepatitis and GI or T2DM by performing hepatic biopsies and magnetic resonance spectroscopy to estimate fat content in the liver. A 40 and 58% decrease in aspartate aminotransferase and alanine aminotransferase levels respectively was found, and a 54% decrease in hepatic fat content. From a histological point of view, pioglitazone treatment resulted in a marked improvement (85%) in necroinflammation rates. However, no significant changes in fibrosis were seen when compared with placebo, suggesting its benefit occurs earlier in the development of this complication [31]. Sanyal *et al.* described similar findings; after the planned treatment period, a greater proportion of patients treated with pioglitazone compared with placebo (42 vs 27%) showed histological evidence of steatohepatitis resolution [32].

Several meta-analyses and systematic reviews detail favorable outcomes from TZD use in patients with evidence of hepatic damage due to insulin resistance. These benefits have been more obvious in patients with insulin resistance before developing overt T2DM [33].

• Clinical efficacy: effects on endothelium & cardiovascular disease risk

Some phenomena linking pioglitazone use to improved CV risk markers include the decrease in TG plasma concentrations, the increase in HDL cholesterol levels as well as a lesser synthesis and release of proinflammatory cytokines to the bloodstream, impacting favorably on endothelial function [24–28,34–35]. Additional evidence of the effects on biological markers of inflammation and atherosclerosis were described by Schernthaner, including the decrease in MCP-1 levels, MMP-9, CRP and IL-6 [36,37]. In a similar study, with a follow-up period of 12 months, the effects of pioglitazone and rosiglitazone combined with metformin on glycemic control and blood pressure in patients with T2DM and metabolic syndrome were evaluated. Small (3–5 mmHg) but significant ($p < 0.05$) decreases in systolic and diastolic blood pressure were described [38].

After binding to PPARγ, TZDs not only increase the IRS-1 signaling pathway improving

insulin sensitivity, but also inhibit the MAPK pathway, responsible for the mitogenic effects of the hormone [4]. Today, this represents a potential explanation for the previously described decrease in carotids' and coronaries' intima and media thickness [39–41]. Nissen *et al.* in the PERISCOPE study compared the effects of pioglitazone with those of glimepiride on the progression of coronary atherosclerosis in patients with T2DM and known coronary disease (n = 543). By means of an intracoronary ultrasound after a follow-up of 18 months, patients in the pioglitazone group (15–45 mg/day) showed a 0.16% decrease in atheroma plaque size, compared with a 0.73% increase seen in the glimepiride group (p = 0.002), concluding that pioglitazone treatment in patients with T2DM and coronary disease decreases the progression of coronary atherosclerosis [39].

On the other hand, pioglitazone's effects (15–45 mg/day) were also compared with those of glimepiride (1–4 mg/day) in terms of the changes in the intima-media thickness of the common carotid in patients with T2DM after a follow-up of 72 weeks (n = 462). By means of ultrasonographic assessment, at the end of the study the mean difference in the carotid intimamedia thickness was smaller with pioglitazone than glimepiride (-0.013 mm; 95% CI: 0.024–0.0002; p = 0.02), also showing a favorable effect of pioglitazone on the progression of the carotid atherosclerotic disease, considered a marker of coronary atherosclerosis [39].

More recently, the change in plaque's inflammation at common carotids and the ascending aorta was assessed with pioglitazone (15–30 mg/day) compared with glimepiride (0.5–4 mg/day), through the use of ^{18}F-fluorodeoxyglucose in PET-CT In this case, 56 subjects with GI or T2DM were included for a follow-up period of 16 weeks. After study completion, the corrected uptake ratio of the radiolabeled drug (indicator of local inflammatory activity) significantly decreased with pioglitazone compared with glimepiride. Also, the change from baseline in this ratio was much greater for pioglitazone. The study concluded that pioglitazone use in patients with T2DM or GI decreases inflammation in atheromatous plaque regardless of its glycemic effect [41].

The inflammatory phenomena described thus far and their favorable effects on endothelial function have also yielded favorable results regarding the risk of CV events, especially in terms of secondary prevention [24]. However, results of the PROactive study are attributed to a combination of effects: an improvement in dyslipidemia, less endothelial inflammation, antihyperglycemic effect and a decrease in blood pressure [11].

The PROactive trial was designed to assess secondary prevention of cardiovascular disease (CVD) in patients with T2DM. All patients (n = 5238) had evidence of CVD (myocardial infarction, stroke or peripheral vascular disease) or multiple risk factors for developing CVD. The subjects were randomized to receive pioglitazone (45 mg daily) or placebo for an approximate follow-up period of 2.85 years. The primary endpoint, the composite of mortality for any cause, nonfatal myocardial infarction, acute coronary syndrome, stroke, amputation and coronary artery or extremity bypass, did not show a significant difference favoring pioglitazone (10% decrease in risk). However, significant decreases (18%) in favor of pioglitazone in the composite outcome of CV death, nonfatal myocardial infarction and nonfatal stroke were described [24].

Two *post hoc* PROactive analyses showed promising results in terms of secondary prevention of CVD. In one, some patients had a myocardial infarction before randomization (46.7%). Patients in the pioglitazone group (1230 subjects vs 1215 in the placebo group) showed a 28% decrease in the risk of suffering another infarction (fatal or nonfatal), and a 37% decrease in the risk of suffering acute coronary syndrome (p = 0.045 and 0.035, respectively) [42].

The second *post hoc* analysis for PROactive assessed the risk of stroke in previous stroke sufferers (n = 984) and in those without a history of cerebrovascular events (n = 4254). In the group that received pioglitazone and had a history of stroke (n = 486), the drug showed a 47% decrease (p = 0.0085) in the risk of another event (fatal or nonfatal) occurring. This effect was not seen in the group of subjects without previous vascular events [43].

In a more recent study (PROFIT-J), pioglitazone was compared with placebo in 522 Japanese subjects with T2DM, dyslipidemia and/or hypertension with one or more clinically silent strokes, advanced carotid atherosclerosis or microalbuminuria. Subjects were followed for an average of 672 days. After almost 2 years of treatment, pioglitazone did not show a significant decrease in the rate of CV events,

although it achieved significant decreases in HbA1c, diastolic blood pressure and low-density lipoprotein cholesterol and increases in high-density lipoprotein-cholesterol compared with placebo [44].

In a meta-analysis assessing the results of 19 randomized, controlled studies of pioglitazone with a sample of 16,390 subjects, a significant decrease in the risk of death, stroke or myocardial infarction was described in those patients receiving pioglitazone (hazard ratio [HR]: 0.82; 95% CI: 0.72 – 0.94; p = 0.005). The difference between pioglitazone and comparator therapies was observed after 1 year of therapy [45].

• Clinical efficacy: effects on the kidney

Several types of damage progressively occurring on the glomerular level have been described as a consequence of T2DM, including glomeruloesclerosis; mediated at least in part by TGF-β. This mediator, increased in patients with diabetes, causes various degrees of damage on epithelial glomerular cells and podocytes, taking part in the progressive proteinuria described by patients. It could also be responsible for some of the epithelial transformation at the glomerular level, causing interstitial fibrosis. Mesangial cells and podocytes express PPARγ, and its stimulation by TZDs has been associated with a decrease in proteinuria due to a cytoprotective effect [46].

A 2001 study determined the urinary excretion of albumin and the urinary elimination of podocytes (both markers of glomerular damage). Twenty-eight patients with T2DM and microalbuminuria were randomized to receive pioglitazone (30 mg/day; n = 14) or placebo (n = 14) for 26 weeks. Podocytes in urine were detected in 17 of the 28 patients (60.7%) at study initiation. In these patients, pioglitazone treatment was associated with a decrease in the number of urinary podocytes of 0.9 ± 1.0 cell/ml to 0.1 ± 0.2 cell/ml (p < 0.001). In addition, the urinary excretion of albumin was decreased in the pioglitazone group from 96.7 ± 50.5 μg/min to 39.7 ± 22.9 μg/min (p < 0.01). Both parameters remained unchanged in the placebo group [47]. However, in a study of patients with more advanced nephropathy (n = 44), pioglitazone treatment for 4 months did not result in a decrease in the urinary excretion of albumin compared with an SU [48].

More recently, the APRIME study showed that the combined use of pioglitazone and inhibitors of the renin–angiotensin system in patients with T2DM, hypertension and microalbuminuria significantly decreases the urinary excretion of albumin, which not only translates into a renoprotective effect but also decreases CV risk [49].

• Clinical efficacy: effects on ovarian function

Polycystic ovarian syndrome (PCOS) has been associated with insulin resistance and infertility. Thecha cells express insulin receptors and, in parallel, hyperinsulinemia resulting from insulin resistance is responsible for the excessive synthesis and release of androgens by the ovary with its varied clinical expressions [50,51].

Pioglitazone at 45 mg daily has been shown to improve the hyperandrogenic profile of women with PCOS and several other metabolic parameters associated with insulin resistance after 6 months of therapy [51]. In a study performed in Mexico by Ortega *et al.*, the effects of metformin 2550 mg daily were compared with those of pioglitazone 30 mg daily for 6 months in 52 obese women with PCOS, observing similar decreases in hirsutism, as well as in free testosterone and androstenedione concentrations [52]. A systematic review and meta-analysis of randomized, controlled studies that included 278 women with PCOS comparing the efficacy of pioglitazone and metformin concluded that pioglitazone is superior to metformin in improving insulin sensitivity (as measured by fasting insulin concentrations and the homeostasis model assessment of insulin resistance), metformin is superior to pioglitazone in weight loss (as measured by difference in BMI), and there are no significant differences between both drugs as regards testosterone concentrations and score in the Ferriman–Gallwey scale **(Figure 2)** [53]. It is worth mentioning that treatment of PCOS is not an approved indication for pioglitazone in the absence of T2DM.

• Safety: weight gain & fluid retention

Weight gain and fluid retention may represent the most frequent adverse events associated with TZDs, including pioglitazone. Weight increases between 1 and 6 kg have been reported in the first year of therapy, which may be attributed to the composite of adipogenesis (primarily in the subcutaneous compartment), redistribution of adipose tissue from the abdominal to the subcutaneous compartment and sodium and

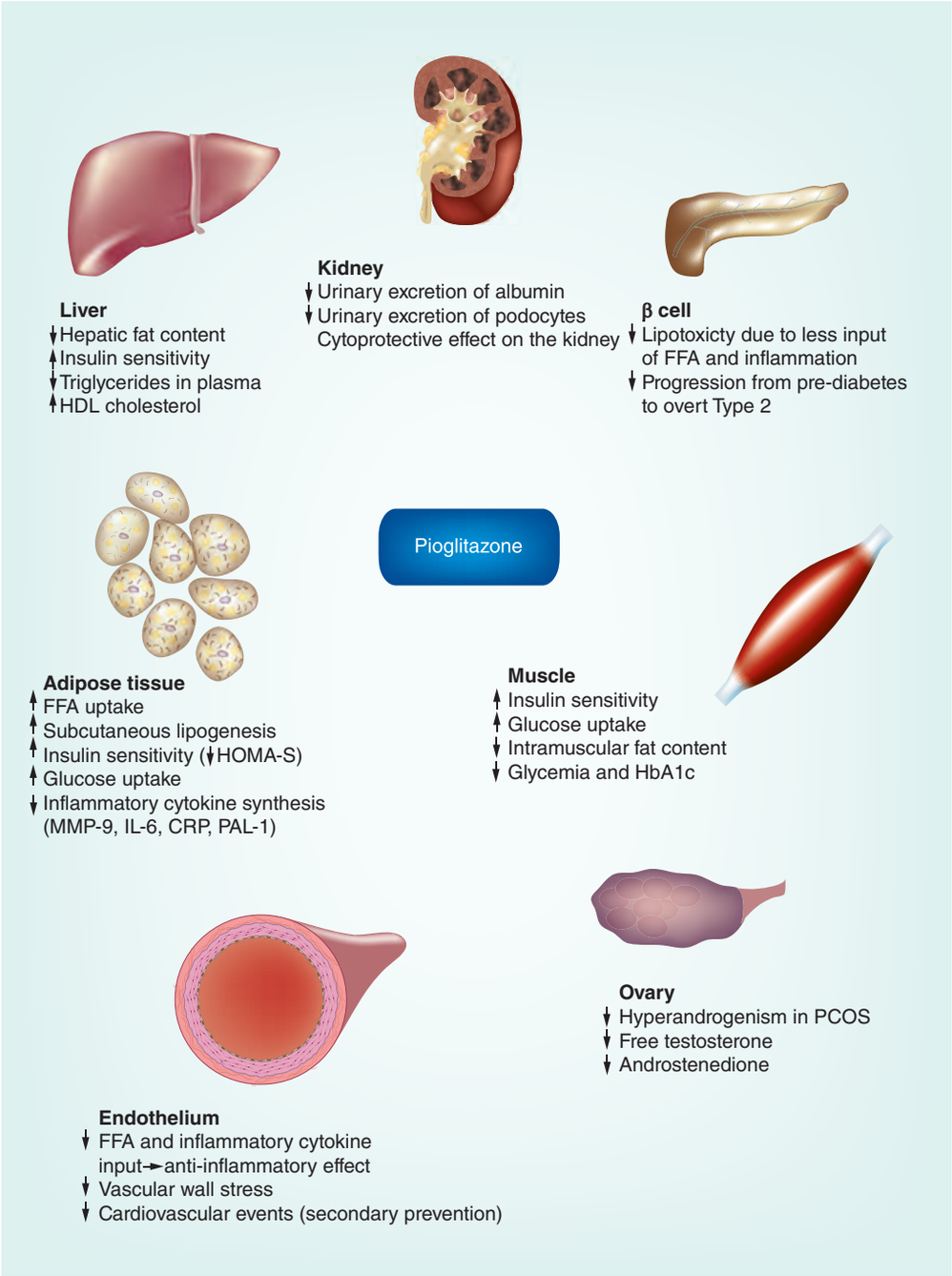

Figure 2. Direct and indirect effects of pioglitazone on different tissues.
FFA: Free fatty acids; HbA1c: Glycosylated hemoglobin AIc; HDL: High-density lipoprotein; HOMA-S: Homeostasis model assessment-sensitivity; PCOS: Polycystic ovarian syndrome.

water retention [11,54–55]. This adiposity may be reduced when therapy is combined with metformin or augmented when combined with insulin, thus close monitoring of patient compliance with diet and physical activity is essential [54]. In the PROactive trial, the weight gain described for pioglitazone was 3.6 kg [24]. An observational study in 2092 patients with T2DM, the IRIS III trial, described that pioglitazone treatment for 20 weeks, under routine conditions and medical advice regarding diet, does not cause significant changes in bodyweight and BMI [56].

The use of TZDs has been associated with the development of peripheral edema. The mean

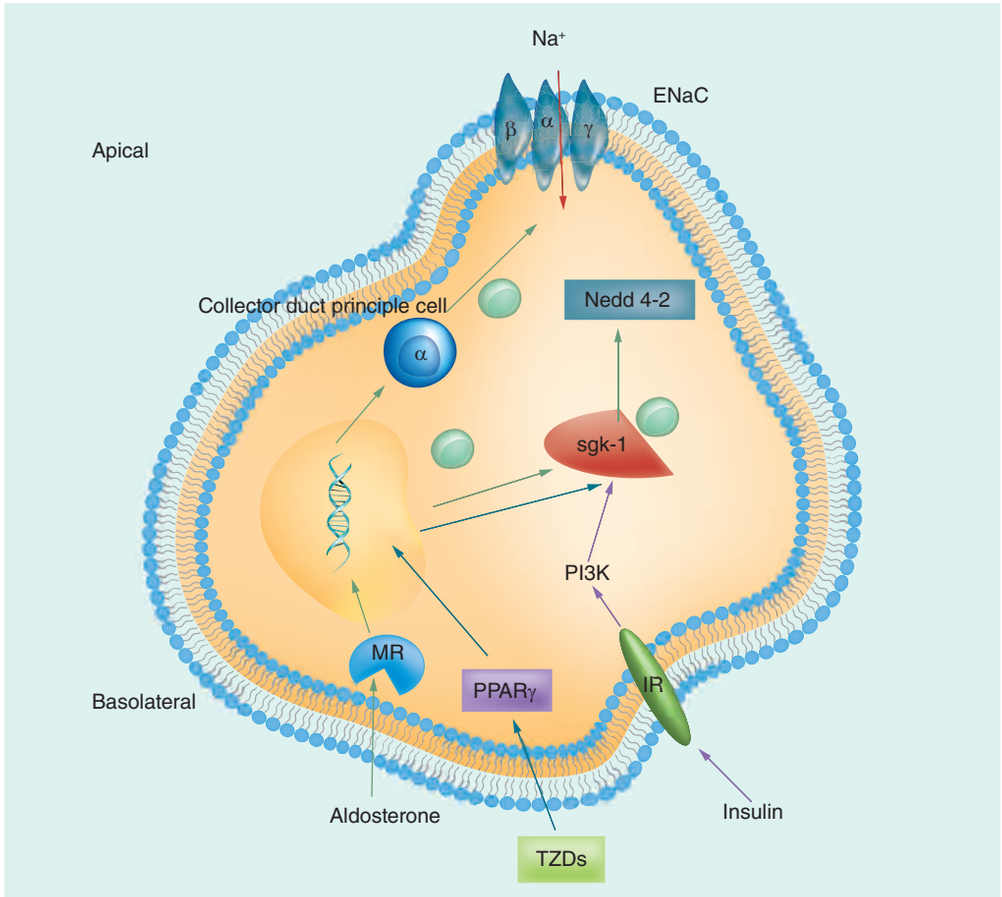

Figure 3. Sgk1-Nedd 4-2-ENaC system. The figure illustrates the effects on the persistence of degradation of the ENaC (epithelial sodium channel) in the main cell of the collecting tubule of aldosterone, insulin and TZDs (through the stimulation of the PPARγ receptor).
α, β and γ refer to the subunits in the ENaC.
ENaC: Epithelial sodium channel; IR: Insulin receptor; MR: Mineralocorticoid receptor; Nedd 4-2: Ubiquitin essential for ENaC degradation; TZD: Thiazolidinedione.

incidence of edema secondary to the use of these medications is 3–5% as monotherapy and up to 10–15% when combined with other antidiabetic agents, especially insulin [55,57–59]. Incidences are similar in studies using pioglitazone alone [11,60]. When added to an SU, pioglitazone reported a 7.5% incidence of edema compared with a 1.2% incidence with SU alone [61]. In an observational, prospective trial conducted in Canada with a sample of 1527 patients with T2DM receiving pioglitazone at 30 mg daily, the latter allowed for an odds ratio (OR) of 1.92 compared with patients who were not receiving this agent [62].

The pathophysiology of TZD-induced edema may involve genetic factors as well as the consequences of these drugs' interaction with PPARγ receptors found in renal tubules. Several single-nucleotide polymorphisms associated with a higher risk of developing edema after the use of glitazones have been described. The presence of those polymorphisms can dramatically increase the risk of developing edema (OR: 16.45; 95% CI: 3.05–88.76) [63,64].

It is important to understand the possible consequences of activating PPARγ receptors in the nephron, especially in its most distal portions (collecting tubules). At this level, there is an important structure responsible for sodium reabsorption from the tubule into the bloodstream; the epithelial sodium channel (ENaC). This is a protein composed of three subunits (α, β and γ), each of which is codified by an individual gene whose activity is regulated through several pathways. While the ENaC is anchored to the apical membrane of the collecting tubule cell, it remains active in reabsorbing sodium.

Stopping this activity requires that the channel be degraded by endocytosis. For this to happen, an ubiquitin (Nedd 4-2) must interact with the PY region of the α or γ subunit of the ENaC gene in the absence of protein sgk-1. The presence of this protein prevents the interaction of Nedd 4-2 with the gene's PY region and, thus, promotes ENaC's persistence in the membrane. This is known as the sgk-1-Nedd 4-2-ENaC pathway **(Figure 3)** [55].

Decreases in blood pressure and/or sodium concentrations through the macula densa activate the rennin–angiotensin–aldosterone axis. After interacting with its receptor (MR) in the collecting tubule's cell, aldosterone stimulates the transcription of the α subunit gene of the ENaC (upregulating its expression and, consequently, the tubular absorption of sodium and water) and the sgk-1 gene's transcription. This means that Nedd 4-2 is inactivated and ENaC will persist in the apical membrane of the tubular cell in order to continue reabsorbing sodium. The sgk-1-Nedd 4.2 pathway is also stimulated by insulin through the PI3K pathway, and after the activation of the PPARγ receptors [55]. This explains how drugs such as pioglitazone stimulate the tubular reabsorption of sodium, which can be notorious in patients with a genetic predisposition, as well as in those with therapies combining TZD with insulin. A trial was performed with 260 patients with T2DM who had developed edema, determined by a decrease of ≥0.5% in hematocrit. After 12 weeks of treatment with rosiglitazone, furosemide, hydrochlorothiazide and spironolactone were administered for 7 days (average daily dose 53, 34 and 69 mg, respectively). Those patients who discontinued TZD showed a discrete increase in hematocrit (0.77%; p = 0.073), which suggests that the discontinuation of the medication might not be enough to correct the edema. The most significant increase involving the hematocrit occurred in the group receiving spironolactone (1.14%; p = 0.004), followed by hydrochlorothiazide and furosemide, which is not surprising since spironolactone is an antagonist of aldosterone's action. The long-term safety and efficacy of these diuretics were not determined [58]. In the PROactive study, there was an increase in edema and heart failure among patients receiving pioglitazone, though mortality due to this condition did not differ between groups [24].

Finally, it should be noted that the 2003 American Diabetes Association/American Heart Association consensus recommends that TZDs should not be used in patients with persistent heart failure (especially New York Heart Association stages III and IV). If used, TZDs should be initiated at a low dose and slowly titrated according to patients' needs, alongside close clinical monitoring in order to detect any sign of cardiac failure, taking into account that labeling can vary across different geographies [59].

• Safety: effects on bone

A solid body of evidence indicates increases in surface bone mineral density (BMD) in patients with T2DM. Paradoxically, these increases are accompanied by a greater risk of fragility fractures [65]. More specialized studies have described significant increases in the total and specific volumetric BMD of the trabecular bone by means of a peripheral quantitative computed tomography [66]. This same technique has enabled us to discover that patients with T2DM have altered bone microarchitecture, primarily consisting of an important cortical porosity and a thickening of the peripheral portions of the trabecular bone (not the case in medullar portions). These changes result in reduced bone strength secondary to altered compressive properties and, therefore, an increase in fracture risk [67].

Several studies have associated the use of TZDs with a decrease in BMD, as well as an increase in fracture risk. Hypotheses explaining this have focused on a preferential differentiation from the pluripotent mesenchymal cells toward adipocytes over that of osteoblasts [68]. Also, the suppression of insulin-like growth factor 1 in the bone has been described, derived from *in vitro* and *in vivo* studies, after the activation of PPARγ receptors, which results in defective bone formation [68]. TZD use has also been associated with lower aromatase activity in adipose tissue and less biosynthesis of both testosterone and estradiol in the ovary [68]. The secondary decrease in sexual steroid concentrations also represents a factor in altered bone mass.

In a subanalysis of the population that participated in the ACT NOW study by Ralph DeFronzo's group, authors determined the surface BMD in patients with carbohydrate intolerance who received pioglitazone, compared with subjects with the same metabolic condition who received placebo [19]. After a follow-up of less than 3 years, pioglitazone use was associated with decreases in BMD in men and women in

various parts of the body [69]. Increases in the incidence of fragility fractures up to 39% have been described both in men and women, with no significant difference between pioglitazone and rosiglitazone [70]. Other studies associate TZD use with bone losses (only in women) in the whole body, the lumbar spine and the trochanter, per year of use of these medications of -0.61, -1.23 and -0.65% respectively [71]. Increases in fracture risk (OR 2.59; 95% CI: 0.96–7.01) have been reported for pioglitazone [72]. A similar phenomenon describing a negative effect of pioglitazone on bone only in women was observed in the PROactive study [24].

A meta-analysis including 10 randomized, controlled trials and a total of 13,715 patients with T2DM or carbohydrate intolerance treated with pioglitazone or rosiglitazone, concluded that the increase in fracture risk with these medications is significant in women but not in men (OR: 2.23; 95% CI: 1.65–3.01 vs OR: 1.00; 95% CI: 0.73–1.39, respectively). In this large study, fractures predominated in distal portions of upper limbs, with few cases of hip fracture. The risk increased after at least a year of continued use [73]. These results invite reflection on the use of TZDs in patients with a high risk of fragility fractures.

• Safety: pioglitazone & cancer

Several documents have described the increased incidence of various malignancies in patients with T2DM or obesity, due to such factors as the increase in circulating insulin concentrations (hormone with mitogenic activity), of insulin-like growth factors and the presence of inflammatory cytokines: a cascade of phenomena favoring tumor growth [74]. Influences of antidiabetic therapies in the development of some cancer types have also been observed [74]. In the case of TZD, the relationship between diabetes and the incidence of malignancy can be altered [75].

In a systematic review and meta-analysis of controlled trials, cohort studies and case reports including 2.5 million subjects, TZD use was associated with a decrease in the risk of colorectal, lung and breast cancers, compared with patients who had never received therapy with these drugs. Randomized studies did not show any significant trend in favor or against TZD use in terms of cancer risk. Part of this large study was a separate analysis of pioglitazone trials which found an association between pioglitazone and a reduced risk of cancer in general, including colorectal, lung, breast, prostate and renal cancers (risk ratio: 0.95; 95% CI: 0.91–0.99; p = 0.009) [75]. The activation of neoplastic suppressors upon PPARγ stimulation (e.g., *LKB-1* and *mTOR*), as well as other possible independent PPARγ pathways, were mentioned as possible mechanisms responsible for the above [75]. That same year, a study including more than 600,000 patients with T2DM in Taiwan reported that pioglitazone was associated with a significant decrease in the risk of developing liver cancer (OR: 0.83; 95% CI: 0.72–0.95) [76].

In another cohort examining the relationship between pioglitazone use and various malignancies in more than 250,000 patients, a trend toward an increased risk of melanoma and lymphoma was observed (HR: 1.3; 95% CI: 0.9–2.0, and HR: 1.3; 95% CI: 1.0–1.8, respectively), as well as a tendency toward a decreased risk of renal cancer (HR: 0.7; 95% CI: 0.4–1.1). The models used to obtain these risk ratios were adjusted for variables such as age, sex, ethnicity, economic status, smoking status and glycemic control. These patients were followed for less than 6 years; therefore a true association between pioglitazone use and the risk of developing these neoplasms cannot be ruled out [77].

In 2011, the results of a French cohort alerted the international medical community to a possible increase in the risk of bladder cancer with pioglitazone. After a 4-year follow-up in a population of 155,535 insured patients using pioglitazone, the adjusted hazard ratio was 1.22 (95% CI: 1.05 – 1.43) [78]. HRs increased as the cumulative dose rose to more than 28,000 mg, and the continuous use-time to more than 24 months (HR: 1.75; 95% CI: 1.22–2.5 and HR: 1.36; 95% CI: 1.04–1.79) [79]. In view of previous observations regarding this association, as in the PROactive study [24], a randomized, controlled study was requested with the purpose of examining the existing risk between pioglitazone use and the development of bladder cancer. In order to conduct this study, the Kaiser Permanente of Northern California records were examined for a follow-up of 10 years. The first interim report, published in 2011 by J Lewis, described a discrete increase in the risk of bladder cancer after the use of pioglitazone for more than 24 months (HR: 1.4; 95% CI: 1.03–2.0), which was not seen

for shorter periods (HR: 1.2; 95% CI: 0.9–1.5). It should be noted that 95% of the detected malignancies were *in situ*, in very early clinical stages [79]. Subsequent reports (8-year analysis) and the final study report by pharmaceutical company Takeda®, show a clear decrease in the risk of bladder cancer after pioglitazone use over time [80]. The final report (ahead of publishing) announcing the submission of the information for publication in 2014, asserts that there is no statistically significant change in the aforementioned risk [81]. Finally, a meta-analysis including six studies with an average follow-up of 44 months described a discrete but significant increase in the risk of developing bladder carcinoma with pioglitazone. This therapy, therefore, should be considered after discussing the benefits and risks, and including other risk factors such as smoking, family history and exposure to certain risk chemotherapies. This study does not recommend pioglitazone use in patients who have or have had bladder cancer [82].

• Safety: macular edema

Diabetic macular edema (DME) is a manifestation of non-proliferative diabetic retinopathy, and contributes to vision loss in patients with T2DM. The thickening of the retina in the macular region secondary to the presence of microaneurysms and exudates is partly responsible for this phenomenon [83].

A prospective cohort study with approximately 170,000 patients with diabetes using TZDs, determined that treatment with these drugs is associated with a discrete increase in the development of DME, which may increase further in concomitant therapy with insulin. It has been suggested that sodium and water retention might be a key factor in this phenomenon's pathophysiology [84].

The risk of developing DME in TZD users versus patients with T2DM who did not use these drugs was examined in a more recent cohort in the short term (1 year) and in the long term (10 years). In both cases, HR increased for TZD users (HR: 2.3; 95% CI: 1.5–3.6 for 1 year, and HR: 2.3; 95% CI: 1.7–3.0 for 10 years). No differences were observed between pioglitazone and rosiglitazone, and a trend toward an increased risk of DME was noted in combination therapies with insulin (HR: 3.0; 95% CI: 1.5–5.9), which was decreased with concomitant treatment with aspirin or angiotensin converting enzyme inhibitors [85].

With existing evidence, the cause–effect relationship between pioglitazone use and the development of DME is weak. Therefore, routine ophthalmologic assessments should be continued in patients with T2DM.

Conclusion

As an agonist of the PPARγ, pioglitazone has been shown to increase insulin sensitivity, particularly in peripheral tissues: skeletal muscle and adipose tissue, and in the liver, resulting in a better uptake of glucose with the subsequent decrease in the production of oxygen reactive species, a decrease in FFA plasma concentrations, and in proinflammatory cytokines and a lower hepatic glucose production. From a clinical standpoint, the average reduction in HbA1c with pioglitazone is between 1.0 and 1.5% (with basal values reported from 7.6 to 10.2%). However, the effects of improving insulin sensitivity as well as reducing inflammatory status have allowed for the description of pleiotropic effects such as the decrease in TG concentrations, the increase in high-density lipoprotein cholesterol, an important reduction in the rate of CV events in terms of secondary prevention and in the ovarian function of patients with PCOS.

Bodyweight gain, in large measure due to an increase of sodium and water retention in renal tubules, are the most frequent secondary effects of pioglitazone and caution is therefore advised when using this drug in patients with higher risk of heart failure. Regarding the possible relationship between pioglitazone use and cancer risk, most evidence indicates that pioglitazone may, in fact, be associated with a lower risk of developing certain types of malignancies, especially colorectal, liver and lung cancers. The discrete increase in the risk of bladder cancer with pioglitazone has weakened according to recent publications with longer follow-up periods. Macular edema and the risk of fragility fractures must be considered when selecting patients to be included in treatment protocols with pioglitazone. In summary, pioglitazone continues to be a valid and attractive alternative for the management of patients with T2DM and evidence of insulin resistance. With an awareness of its strengths and weaknesses, an adequate balance should be established in order to obtain maximum benefit with minimum risk.

TZDs, such as pioglitazone, are drugs that exert their mechanism of action through a complex interaction with other molecules like coactivators and corepressors, leading to various influences on gene expression. Further and deeper understanding on the functions of the PPARγ, interaction between isoforms and manipulation of gene expression is needed so a clearer balance between gains and side effects can be achieved as new drugs are being developed. Insulin resistance is still a cornerstone of T2DM pathophysiology and must be targeted for appropriate management at all times. As long as the number needed to harm for the most frequent side effects of pioglitazone remains high, appropriate use should continue, with the additional benefit of increasing knowledge and experience regarding its functioning.

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