# Protection of the kidney by thiazolidinediones: An assessment from bench to bedside

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**The global epidemic of diabetes mellitus has led to a continuous increase in the prevalence of diabetic nephropathy over the past years. Thus, diabetic nephropathy is currently the number one cause of end-stage renal disease in the Western world. It represents a major public health problem for which more effective prevention and treatment strategies are needed. Thiazolidinediones (TZDs) are a class of agents that lower blood glucose through reduction of insulin resistance in patients with type 2 diabetes. Growing evidence support the concept that TZDs have several beneficial effects on the cardiovascular system beyond their effects on glycemic control. These benefits include: blood pressure lowering, triglyceride reduction, high-density lipoprotein–cholesterol elevation, and reduction in subclinical vascular inflammation. Moreover, data from several animal and human studies support the notion that TZDs reduce urine albumin excretion and may prevent development of renal injury. The relative lack of evidence, however, demonstrating the effects of TZDs on hard renal outcomes mandates the need for well-designed trials with this particular objective. This paper summarizes all the data from clinical and experimental studies relevant to a possible renoprotective effect of TZDs and discusses actions of these compounds that may contribute toward this effect.**

*Kidney International* (2006) **70,** 1223–1233. doi:10.1038/sj.ki.5001620; published online 2 August 2006

KEYWORDS: thiazolidinediones; PPARγ agonists; renal protection; diabetic nephropathy

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**Received 24 February 2006; revised 21 April 2006; accepted 2 May 2006; published online 2 August 2006**

The incidence and prevalence of type 2 diabetes mellitus (DM) has been increasing worldwide since the decade of the 1980s, a rise that is estimated to continue in the future.[1,2] Conversely, the incidence of type 1 DM has been stable for many years.[2] Diabetic nephropathy is a common complication of DM, and represents one of the biggest challenges for modern nephrology, as it is the most common cause of end-stage renal disease, accounting for about 40% of all new cases.[3,4]

The earliest clinical sign of nephropathy in diabetic patients is the appearance of macroalbuminuria, that is, urine albumin excretion (UAE) $>300$ mg/day, as microalbuminuria previously thought to be as an early sign of nephropathy in type 1 diabetes has been recently shown not to correlate with the degree of morphologic change in the kidney.[4–6] The presence of microalbuminuria, that is, UAE between 30 and 300 mg/day is considered a marker of abnormal endothelial function and increased vascular permeability[7] as well as inflammation,[8–10] whereas progression to macroalbuminuria is a manifestation of overt nephropathy and associated with faster deterioration of kidney function.[11]

The so-called 'metabolic' or 'insulin resistance' (IR) syndrome, represents a cluster of disturbances that are risk factors for cardiovascular disease, including type 2 DM, abdominal obesity, hypertension, and dyslipidemia,[12,13] and its prevalence is today also particularly high in developed societies.[14,15] In the initial descriptions of the syndrome IR was proposed to be the underlying disorder of it, causally related to the other components through various mechanisms.[16,17] Several previous studies supported an association between IR or compensatory hyperinsulinemia and elevated urine albumin or kidney disease,[18] and thus microalbuminuria was proposed to be another main component of the metabolic syndrome.[12]

Thiazolidinediones (TZDs) represent a class of compounds currently used for the treatment of type 2 DM that exert their hypoglycemic properties through reduction of IR.[19,20] These agents act by stimulating a certain type of nuclear receptor, called peroxisome proliferator-activated receptor gamma (PPARγ). Such receptors are abundant in adipose tissue cells, but they are also present in various other cell types, such as vascular smooth muscle cells, macrophages, vascular endothelial cells, colon epithelial cells, as well as renal glomerular and tubular cells. Through

transcriptional regulation of various genes, PPARγ receptors play an important role in adipocyte differentiation and lipid and carbohydrate metabolism.[19,21,22] Apart from improving glycemic control in patients with type 2 DM, several lines of evidence support the notion that TZDs have beneficial effects on other components of the metabolic syndrome, such as blood pressure (BP) lowering, triglyceride reduction, high-density lipoprotein–cholesterol elevation, redistribution of body fat away from the central compartment, decrease of C-reactive protein and plasminogen activator inhibitor -1 (PAI-1) levels, and others.[19,20]

Additionally, several animal studies demonstrate that TZDs also reduce urine albumin or protein excretion and protect against injury to the kidney.[23–26] Moreover, experimental studies exposed numerous actions of TZDs in the kidney that could explain a possible renoprotective effect.[27–32] Human studies also report significant reductions in UAE among patients with type 2 diabetes by all TZDs examined (troglitazone, pioglitazone, and rosiglitazone).[33–35] This review summarizes data from clinical and experimental studies regarding the effect of TZDs on UAE levels, as well as systemic and local kidney actions of these compounds that can partly explain a possible renoprotective effect.

## EFFECTS OF TZDS ON UAE AND RENAL INJURY

The first indications for a possible renoprotective action of TZDs came from an animal study with troglitazone more than 10 years ago, in which this compound was associated with an important decrease in urine protein excretion, along with a reduction in BP in obese Zucker rats.[23] In the following years, a beneficial effect on urine albumin or protein excretion in animal models of IR, diabetes, and hypertension has been consistently reported for troglitazone,[26,36–39] pioglitazone,[24,26,40] and rosiglitazone,[25,41,42] whereas troglitazone showed a renal protective effect also in models of non-diabetic chronic kidney disease[43,44] (Table 1). In many of these studies, this reduction of UAE with TZDs was accompanied by other beneficial actions on the kidney, such as reduction of glomerular hyperfiltration, prevention of intrarenal arteriolosclerosis, and prevention of glomerulosclerosis and tubulointerstitial fibrosis.[24–26,38,40,41,43,44] It is noteworthy, that in studies where TZDs were compared to angiotensin-converting enzyme (ACE) inhibitors, the TZD exerted similar[43] or superior[41] protection against injury to the kidney.

For almost a decade, several human studies have examined the renoprotective properties of all TZDs, as shown in Table 2. In the first among these, Sironi *et al.*[45] found no significant effect of 200 mg of troglitazone daily on UAE in patients with type 2 DM, but the baseline levels of UAE in the group they studied were normal and the study period was only 8 weeks. In a subsequent study, 400 mg of troglitazone were compared to 500 mg of metformin in 30 patients with type 2 DM and microalbuminuria for 12 weeks.[33]

**Table 1 | Animal studies reporting renal effects of TZDs**

| Study | TZD compound | Animal model | Duration | Effect on urine albumin or protein excretion | Effect on BP | Hemodynamic and morphological renal effects |
|---|---|---|---|---|---|---|
| Yoshioka *et al.*[23] | Troglitazone | Obese Zucker rats | 4 and 8 weeks | ↓ | ↓ | NA |
| Fujii *et al.*[36] | Troglitazone | Streptozotocin-induced diabetic rats | 12 weeks | ↓ | ■ | NA |
| Fujiwara *et al.*[37] | Troglitazone | Heminephrectomized Wistar fatty rats | 24 weeks | ↓ | ↓ | NA |
| Isshiki *et al.*[38] | Troglitazone | Streptozotocin-induced diabetic rats | 12 weeks | ↓ | NA | Prevention of glomerular hyperfiltration |
| Nicholas *et al.*[39] | Troglitazone | Streptozotocin-induced diabetic rats | 3 months | ↓ | ■ | NA |
| Yoshida *et al.*[43] | Troglitazone | 5/6 nephrectomized SHR | 12 weeks | ■ | ↓ | Prevention of glomerulosclerosis |
| Ma *et al.*[44] | Troglitazone | 5/6 nephrectomized Sprague–Dawley rats | 12 weeks | ↓ | ■ | Prevention of glomerulosclerosis and glomerular cell proliferation |
| Yamashita *et al.*[26] | Troglitazone, pioglitazone | Streptozotocin-induced diabetic SHR | 12 weeks | ↓ | ■ | Prevention of the loss of anionic sites of glomerular basement membranes |
| Yoshimoto *et al.*[24] | Pioglitazone | Diabetic Wistar fatty rats | 13 weeks | ↓ | ↓ | Prevention of glomerulosclerosis and intrarenal arteriolosclerosis |
| Tanimoto *et al.*[40] | Pioglitazone | Diabetic KK/Ta mice | 4 and 8 weeks | ↓ | ■ | Prevention of glomerular enlargement |
| Buckingham *et al.*[25] | Rosiglitazone | Obese Zucker rats | 4 and 9 months | ↓ | ↓ | Prevention of glomerulosclerosis and tubulointerstitial fibrosis |
| Baylis *et al.*[41] | Rosiglitazone | Obese Zucker rats | 6 months | ↓ | ■ | Prevention of glomerulosclerosis and tubulointerstitial fibrosis |
| Khan *et al.*[42] | Rosiglitazone | Obese Zucker rats | 12 weeks | ↓ | ↓ | NA |

BP, blood pressure; NA, not applicable; SHR, spontaneously hypertensive rats; TZD, thiazolidinedione.
↓, significant reduction of urine albumin/protein excretion or blood pressure levels, or protection against elevation of urine albumin/protein excretion or blood pressure levels observed in untreated groups; ■, no significant effect.

**Table 2 | Human studies reporting renal effects of TZDs**

| Study | Type of subjects | N | Daily doses of regimens compared | Duration | Mean effect on UAE versus baseline in TZD groups (%) | Mean effect on SBP/DBP versus baseline in TZD groups (mmHg) |
|---|---|---|---|---|---|---|
| Sironi *et al.*[45] | DM2, (hyp)[a] | 40 | 200 mg Tro versus plb | 8 weeks | +11% | −4/−3[b] |
| Imano *et al.*[33] | DM2, mA, (hyp)[a] | 30 | 400 mg Tro versus 500 mg Met | 12 weeks | −39%[c] | −3/0 |
| Nakamura *et al.*[46] | DM2, mA or MA | 32 | 400 mg Tro versus 5 mg Gli | 12 months | −67%[c] in mA 0% in MA | −6[d] |
| Nakamura *et al.*[34] | DM2, mA | 45 | 30 mg Pio versus 5 mg Gli versus 0.6 mg Vog | 3 months | −66%[c] | −6/−4 |
| Nakamura *et al.*[49] | DM2, mA | 28 | 30 mg Pio versus plb | 6 months | −59%[c] | −4[d] |
| Aljabri *et al.*[50] | DM2, (mA)[e], (hyp)[a] | 62 | 30–45 mg Pio versus isophane insulin | 16 weeks | −44% | −8/−5 |
| Yanagawa *et al.*[51] | DM2, mA, (hyp)[a] | 40 | Pio versus Met or Gli | 12 weeks | −45%[c] | NA |
| Hanefeld *et al.*[52] | DM2, (mA)[e], (hyp)[a] | 639 | 15–45 mg Pio versus 850–2550 mg Met | 12 months | −15%[c] | NA[f] |
| Schernthaner *et al.*[53] | DM2, (hyp)[a] | 1199 | 15–45 mg Pio versus 850–2550 mg Met | 12 months | −19%[c] | NA[f] |
| Matthews *et al.*[54] | DM2, (hyp)[a] | 630 | 15–45 mg Pio versus 80–320 mg Gli | 12 months | −10%[c] | NA[f] |
| Agarwal R, *et al.*[55] | DM2, MA (hyp)[a] | 44 | Pio versus Glip | 4 months | −7% | +3.7/+2.2[b] |
| Lebovitz *et al.*[56] | DM2, (mA)[e], (hyp)[a] | 493 | 4 or 8 mg Rosi versus plb | 26 weeks | 4 mg group: −14% 8 mg group: −22%[c] | NA |
| Bakris *et al.*[35] | DM2, (mA)[e], (hyp)[a] | 129 | 8 mg Rosi versus Gli | 12 months | −30%[c] | −0.1[c]/−2.3[b,c] |
| Sarafidis *et al.*[57] | DM2, hyp, (mA)[e] | 20 | 4 mg Rosi | 6 months | −35%[c] | −5.4[c]/−4.1[b,c] |
| Pistrosch *et al.*[58] | DM2, (mA)[e], (hyp)[a] | 19 | non-mA patients: Rosi versus Nat, mA patients: Rosi versus plb | 12 weeks | non-mA patients: +18%[g], mA patients: −66%[c,g] | NA |
| Bakris *et al.*[59] | DM2, mA, (hyp)[a] | 389 | Rosi versus Gli | 32 weeks | −23%[c] | −1.3[c]/−2.3[b,c] |

DBP, diastolic blood pressure; DM2, subjects with type 2 diabetes mellitus; Gli, glibenclamide; Glip, glipizide; hyp, subjects with hypertension; mA, subjects with microalbuminuria; MA, subjects with macroalbuminuria, Met, metformin; NA, changes in blood pressure levels not applicable; Nat, nateglinide; plb, placebo; Pio, pioglitazone; SBP, systolic blood pressure; Tro, troglitazone; TZd, thiazolidinediones UAE, urine albumin excretion, Vog, Voglibose.
[a]A percentage of the total population was also hypertensive.
[b]Studies using ambulatory BP recordings.
[c]Significant change versus baseline levels or the other group compared.
[d]Mean change for systolic BP versus baseline in patients treated with the TZD.
[e]A percentage of the total population had microalbuminuria.
[f]Studies reporting nonsignificant decreases in BP versus baseline in both the groups compared, without giving detailed changes.
[g]Change versus the group compared.

Troglitazone treatment was associated with a significant reduction in albumin to creatinine ratio (ACR) of about 40%, whereas metformin treatment had practically no effect. In another study in 32 type 2 diabetic patients with micro- or macroalbuminuria but without hypertension, troglitazone produced a significant reduction in UAE in the microalbuminuric group after 12 months of treatment in contrast to glibenclamide. None of the drugs, however, affected UAE in the group with macroalbuminuria.[46] These findings were not corroborated as troglitazone was withdrawn from the market owing to severe hepatotoxicity and rare cases of liver failure and death.[19,47,48]

In the first human study of the field with pioglitazone, Nakamura *et al.*[34] compared the effect of this compound to glibenclamide and voglibose on 45 normotensive type 2 diabetic patients with microalbuminuria. After 3 months of treatment, the three drugs produced similar reductions in hemoglobin $A_{1c}$ ($HbA_{1c}$) levels, but only pioglitazone significantly reduced UAE, for about 70%. In a subsequent study from the same group, pioglitazone was again found to produce a similar reduction in UAE of type 2 diabetic normotensive subjects with microalbuminuria.[49] Aljabri *et al.*[50] compared the addition of pioglitazone with that of bedtime insulin in 62 type 2 diabetic patients already on metformin and a sulfonylurea. The two treatments produced a similar reduction in $HbA_{1c}$ levels after 16 weeks, as well as a similar decrease in the levels of urine ACR. However, although the authors report no significant differences in baseline characteristics between groups, patients treated with pioglitazone had three times lower baseline ACR levels than those treated with insulin (mean value 58 versus 178 mg/g). Similarly, in another study including 40 type 2 diabetic microalbuminuric patients, pioglitazone significantly reduced ACR after 12 weeks, but this decrement was comparable to that of gliclazide treatment. Again, baseline ACR levels in the pioglitazone group were about half of those in the gliclazide group (mean value 55 versus 95 mg/g).[51]

This beneficial effect of pioglitazone on UAE was also apparent in larger, multi-center intervention studies, which compared the general efficacy and safety of this TZD in comparison with other oral antidiabetic agents in patients with type 2 DM, the majority of whom had normoalbumi-

nuria. In one of these studies, Hanefeld *et al.*[52] randomized more than 600 patients already receiving a sulfonylurea to pioglitazone or metformin for 52 weeks. Although the two regimens had comparable effects on glycemic control, urinary ACR was reduced by 15% in the group receiving pioglitazone and increased by 2% in metformin group. Another study from the same group in drug-naive patients with type 2 DM showed that pioglitazone significantly reduced ACR, whereas metformin had no effect after 52 weeks of treatment.[53] In a similar follow-up study, the addition of pioglitazone to those who had previously received metformin therapy was associated with a decrease in ACR of 10%, whereas the addition of gliclazide with an increase of 6%. Both drugs, however, were associated with similar improvements in glycemic control.[54]

A recent pilot study evaluated the effect of pioglitazone and glipizide on urine protein excretion in 44 patients with overt diabetic nephropathy (median 24-h urine protein 2.6 and 2.8 g/g of creatinine in the two groups, respectively).[55] After 4 months of treatment, the pioglitazone group had a mean adjusted reduction of 7.2% in urine protein excretion, whereas the glipizide group had an adjusted mean increase of 6.1%. However, this adjusted mean reduction of 13.2% with pioglitazone was not statistically significant when compared to glipizide. These findings are consistent with the aforementioned data with troglitazone in macroalbuminuric patients.[46]

The effect of rosiglitazone on UAE was originally evaluated in two multi-center intervention studies in patients with type 2 DM. The first aimed to evaluate the efficacy of rosiglitazone monotherapy and randomized almost 500 subjects to 2 or 4 mg rosiglitazone b.i.d. or placebo for 6 months.[56] Only the higher dose group showed a significant reduction of 21.6% versus baseline in ACR. In the subgroup of patients with microalbuminuria, rosiglitazone reduced ACR by approximately 40% versus baseline and 30% versus placebo. The second study was part of a cardiac safety study where rosiglitazone 4 mg b.i.d. was compared to glyburide in 121 patients for 52 weeks.[35] After 28 weeks of treatment, both groups had significant reductions in ACR of about 30%, but after 52 weeks only rosiglitazone group continued to demonstrate a significant ACR reduction. This finding suggests that duration of follow-up is an important variable in assessing the effect of TZDs. Additionally, 24-h ambulatory BP was assessed in this trial and a difference of $-3.5/-2.7$ mmHg compared to glyburide group was noted at 52 weeks. This change in ambulatory BP correlated with changes in ACR and less so with $HbA_{1c}$.

We previously examined the effects of rosiglitazone in a cohort of patients with both type 2 DM and hypertension.[57] After 26 weeks of treatment, the drug reduced UAE, measured either in 24-h urine collections or with the use of ACR in spot specimens, by about 35%. In this study, changes in UAE were positively correlated with changes in ambulatory systolic BP, diastolic BP, and fasting insulin levels, and inversely correlated with changes in insulin sensitivity measured with the hyperinsulinemic euglycemic clamp. No associations were found between changes in UAE and changes in fasting glucose or $HbA_{1c}$. In a separate cross-over study, rosiglitazone was again shown to reduce UAE in patients with type 2 DM and microalbuminuria after 12 weeks of treatment, by about 60%.[58] Of note in this study was that rosiglitazone improved renal endothelial nitric oxide (NO) synthesis and produced also significant reductions of about 10% in glomerular filtration rate and filtration fraction in these subjects. This decrease in glomerular filtration rate was strongly correlated with the respective decrease in UAE produced by this compound. However, no significant correlations were found between changes of UAE and changes of BP, insulin sensitivity, fasting plasma glucose, or $HbA_{1c}$ with rosiglitazone.

A recent multi-center study examined specifically the effects on ACR of a TZD or a sulfonylurea compound added to metformin.[59] Three and eighty-nine patients with type 2 DM and microalbuminuria receiving metformin were randomized to rosiglitazone or glibenclamide. After 32 weeks of treatment, rosiglitazone was associated with a decrease in ACR of 23% versus baseline, whereas glibenclamide did not have any significant effect. As a result, a difference between treatment groups in ACR of 15% was evident, along with a difference of about 2.5 mmHg in both 24-h systolic BP and diastolic BP.

Overall, the above data clearly show that TZD treatment can reduce UAE. However, it should be emphasized that currently there are no trials that examine the effects of TZDs on kidney disease progression, that is, doubling of serum creatinine or time to onset of end-stage renal disease, in patients with diabetic nephropathy. Such studies are needed in order to provide the best evidence for a renoprotective effect of TZDs.

## ACTIONS OF TZDS POSSIBLY CONTRIBUTING TOWARD A RENOPROTECTIVE EFFECT

The mechanisms through which TZDs reduce UAE and renal injury are not totally clear at present. Several systemic actions of TZDs, such as reductions in blood glucose and insulin, as well as BP levels, could be involved into a renoprotective property of these compounds. Moreover, functional PPAR$\gamma$ receptors have been identified in renal glomerular and tubular segments. Expression of PPAR$\gamma$ protein is abundant in inner medulla, that is, the inner medullary collecting duct and inner medullary interstitial cells.[60,61] Various groups have also identified functional PPAR$\gamma$ in cultured animal glomerular mesangial cells.[28,39,62,63] These findings suggest that TZDs can also protect against renal injury through direct actions on these receptors. The systemic and local actions of TZDs possibly involved in a renoprotective effect are shown in Figure 1.

### Reduction in blood glucose and insulin levels

Strict glycemic control is associated with reduction of microalbuminuria and protection against injury to the

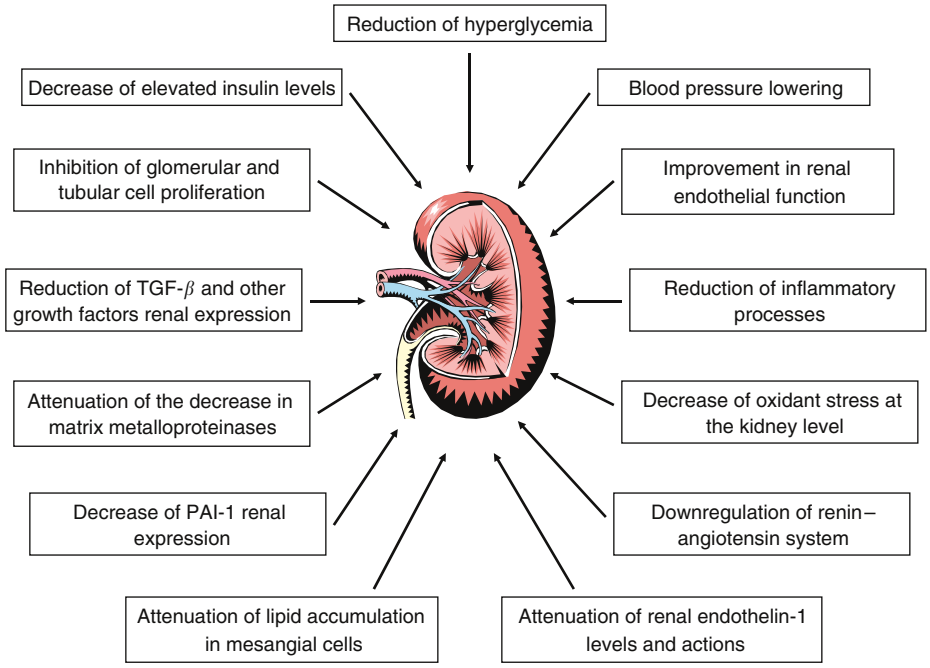


**Figure 1 | Actions of TZDs possibly contributing to the reduction of UAE and attenuation of renal injury.**

kidney in patients with DM.[64–66] Chronic hyperglycemia is connected to glomerular dysfunction through mechanisms that include enhancement of the polyol pathway, non-enzymatic glycation, accumulation of advance-glycation end products, increase in *de novo* diacylglycerol synthesis leading to protein kinase C (PKC) activation and others.[67] Inhibition of PKC has been shown to reduce albuminuria.[27,68,69] TZDs inhibit PKC in various cell types, including glomerular mesangial cells,[27,38,70,71] thus it has been postulated that troglitazone could reduce UAE through inhibition of the *de novo* diacylglycerol-PKC pathway.[27,36,38]

However, in most of the above studies comparing active treatments,[33–35,46,52–54] although glycemic control was similar between the different drug groups, UAE was reduced only in patients receiving a TZD. Moreover, all studies that examined correlations between changes in UAE and fasting glucose or $HbA_{1c}$ demonstrated weak or no associations.[35,57,58] Taken together, these data suggest that improvement in glycemic control cannot be eliminated as a mechanism by which TZDs improve UAE, but does not seem to be the only one.

Another possible mechanism for the renoprotective effect of TZDs is the reduction in elevated insulin levels. In experimental studies, insulin has been shown to promote renal injury through various actions, that is, direct induction of mesangial cell proliferation and extracellular matrix protein synthesis,[72] promotion of insulin-like growth factor I production,[73] which also induces mesangial cell proliferation,[72] increase in renal TGF-$\beta$ production,[74,75] upregulation of angiotensin II angiotensin 1 receptor mRNA expression,[74,76,77] and stimulation of endothelin-1 production in mesangial cells.[76] Conversely, insulin infusion *in vivo* selectively increases renal albumin excretion and clearance rate in type 2 diabetic patients by about 50%, but not in healthy subjects.[78] In most of the above studies that report changes in insulin levels, TZD treatment has resulted in significant reductions in fasting insulin levels in contrast to other oral antidiabetic agents.[33,53,54,57] This decrease in insulin levels has also been found to correlate with the reduction of UAE.[57] As a result, amelioration of hyperinsulinemia with TZDs could be another mechanism contributing to their renoprotective effects.

### BP lowering

Tight BP control is considered even more important than glycemic control in reducing UAE and protecting against renal disease progression in patients with type 2 DM and even small variations in BP can play a role in renoprotection.[79,80] A considerable number of previous animal and human studies have shown that all TZDs can produce small but significant changes in BP levels and various TZD actions, such as improvement in endothelial function, attenuation of sympathetic overactivity, or reduction of intracellular $Ca^{2+}$ content in vascular smooth muscle cells, could be responsible for this effect.[81] In many of the animal studies mentioned above, TZD treatment produced also significant reductions in BP[23–25,37,42,43] (Table 1). In some of the relevant human studies, changes in BP are not reported,[56] whereas in others BP did not present significant changes versus baseline in any of the groups compared,[33,34,46,49,52,53,55,58] as shown in Table 2. However, in many of the latter studies,[33,34,46,49,58] BP changes were toward different directions (decrease in the groups of TZDs, increase in the other groups) and no statistical comparison between the different groups took place. Therefore, the

possibility that differences in BP between groups (which varied up to 10/6 mmHg) have contributed in reductions of UAE levels with TZDs in these studies cannot be excluded. Moreover, in most relevant studies that recorded ambulatory BP so far, BP was significantly reduced with the TZD treatment[35,57,59] and there were strong and persistent correlations between the reductions in UAE and BP.

It has to be noted that in both animal models[82] and humans,[83,84] reduction of BP with TZD treatment has been documented even in the presence of fluid retention. Fluid retention in patients receiving TZDs is a well-documented observation and has been considered a major contributor toward weight gain, pedal edema, and heart failure development.[84,85] Several mechanisms have been proposed for this TZD action, including drop in systemic and intraglomerular BP.[82,85] However, recent studies shed more light on the field, suggesting a PPAR$\gamma$-dependent upregulation of the amiloride-sensitive epithelial $Na^+$ channel in the collecting duct as the most plausible mechanism.[86,87] It has been observed that treating cultured collecting ducts with TZDs increased epithelial $Na^+$ channel gene expression and amiloride-sensitive sodium absorption through a PPAR$\gamma$-dependent pathway.[86] Further, TZDs could not stimulate sodium transport in collecting duct epithelial cells with disruption of the PPAR$\gamma$ gene.[87] In this study, TZDs could not induce increases in plasma volume and weight gain in mice with this specific disruption, in contrast to control animals.[86,87] It should be noted, however, that in clinical studies that examined BP changes, this fluid retention did not mitigate against BP reduction for up to 1 year.[35] Overall, based on existing evidence an even small BP lowering action of TZDs should be considered as a basic mechanism of UAE amelioration.

#### Improvement in endothelial function

Endothelial dysfunction is central in the development of vascular complications of diabetes and the presence of microalbuminuria in the urine is considered as the earliest sign of impaired vascular function at the kidney level.[88,89] Numerous experimental and clinical studies have also established the close relationship between IR and endothelial dysfunction.[90,91] Although the role of renal endothelial function and NO availability in the progression of diabetic nephropathy is complex and not totally clear yet, different abnormalities of NO production contribute to renal injury in the various stages of the disease.[92,93] Amelioration of IR with TZDs has been shown to improve impaired endothelial function in patients with type 2 diabetes.[94,95] Previous animal studies support that TZDs can improve impaired renal NO availability.[30,96] Another experimental study suggested that troglitazone has an endothelium-independent vasodilating effect on the afferent renal arterioles and a vasodilating effect on efferent arterioles, leading to reduced capillary pressure and UAE, which seems to follow an increase in NO bioavailability.[97] Most importantly, the above-mentioned study from Pistrosch *et al.*[58] has elegantly shown that the addition of rosiglitazone in microalbuminuric type 2 diabetic patients simultaneously increased renal NO bioavailability and reduced hyperfiltration and UAE, suggesting that improvement in renal endothelial function plays a key role in TZD-associated renoprotection.

#### Antiproliferative actions

Growing evidence support a critical role in the pathogenesis of diabetic nephropathy of several growth factors, the most important of which seems to be transforming growth factor-$\beta$ (TGF-$\beta$).[98] As mentioned above, insulin was found to increase TGF-$\beta$1 production from mesangial cells[74] as well as proximal epithelial renal cells,[75] and thus reversion of hyperinsulinemia with TZDs can also protect against the increase in renal TGF-$\beta$. Troglitazone was previously associated with inhibition of mesangial cell proliferation and de-differentiation, inhibition of extracellular matrix protein increase, as well as promotion of mesangial cell apoptosis.[28,38,62,99] Moreover, TZDs were reported to also inhibit proliferation and increase apoptosis of renal proximal tubular cells.[100,101] Other experimental studies have shown that PPAR$\gamma$ agonists directly decrease glomerular TGF-$\beta$ expression independently of their effects on glucose or insulin[38,44] as well as suppress the glucose-induced production of TGF-$\beta$ from tubular cells.[101] TZDs can also inhibit the TGF-$\beta$-induced collagen type 1 and fibronectin synthesis from mesangial cells.[63,102,103] Apart from interference with TGF-$\beta$ actions, TZDs have been shown to inhibit proliferation of mesangial cells induced by other growth factors, such as platelet-derived growth factor[39,104] and vascular endothelial growth factor.[105]

Other actions of TZDs related to inhibition of growth procedures at the kidney level are their effects on matrix metalloproteinases and plasminogen activator inhibitor type 1 (PAI-1) levels. Matrix metalloproteinases are enzymes normally responsible for glomerular extracellular matrix degradation and their inhibition leads to extracellular matrix accumulation and renal fibrosis.[106] Pioglitazone was previously shown to attenuate the decrease of matrix metalloproteinases-2 and therefore to protect against collagen IV accumulation in the glomeruli of diabetic rats.[32] On the other hand, PAI-1, a major regulator of fibrinolysis has been also suggested to influence the progression of diabetic nephropathy. Although PAI-1 is not expressed in the normal kidney, renal PAI-1 expression is strongly induced in various forms of kidney diseases leading to renal failure, as well as in animals with diabetes even without nephropathy.[107,108] In epidemiological studies, plasma PAI-1 levels were significantly correlated with the UAE rate in patients with type 2 DM.[109] In terms of pathophysiology, PAI-1, apart from plasminogen, also inhibits plasmin-mediated matrix metalloproteinases activation.[107] Inhibition of PAI-1 activity or of PAI-1 synthesis by specific antibodies, peptidic antagonists, etc. *in vitro* was shown to prevent renal fibrosis.[107] TZDs have been repeatedly shown to reduce plasma PAI-1 levels in humans,[110] and to downregulate glomerular PAI-1

expression, along with a reduction in UAE, in animal models,[39,44] findings indicating another mechanism for their antifibrotic effects.

### Anti-inflammatory actions

During the last years, the association of inflammation markers with renal function deterioration has been repeatedly reported in epidemiological studies and an important role in the progression of diabetic nephropathy has also been attributed to inflammatory processes.[111,112] TZDs have been previously shown to suppress the production of proatherogenic inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-$\alpha$ from macrophages *in vitro*[113] and to reduce plasma levels of C-reactive protein, the most common marker of subclinical vascular inflammation, in type 2 diabetic patients.[84,114,115] In renal mesangial cells, TZDs attenuated the IL-1-mediated increase in the expression of IL-6 and tumor necrosis factor-$\alpha$,[29] and the stretch-induced increase in monocyte chemoattractant activity, by decreasing nuclear factor-$\kappa$B activation and monocyte chemoattractant protein-1 production.[116] In renal tubular cells, pioglitazone was shown to reverse the increase in monocyte chemoattractant protein-1, evoked from low-density lipoprotein[117] and to further suppress monocyte chemoattractant protein-1 levels after exposure to high glucose,[101] whereas rosiglitazone was reported to reduce the levels of IL-1, tumor necrosis factor-$\alpha$, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, as well as the infiltration of renal tissue with macrophages.[118] A reduction in C-reactive protein and IL-6 levels was also shown in patients with overt diabetic nephropathy treated with pioglitazone.[119]

Another possible mechanism of the renoprotective effect of TZDs is the attenuation of oxidant injury at the kidney level. Closely linked to inflammatory processes, oxidant stress is also considered a potent contributor in the progression of diabetic nephropathy,[120] as experimental data indicate an acceleration of renal injury when normal balance of the kidney shifts toward a pro-oxidant state and human studies have shown increased prevalence of oxidant stress both in patients with early and with overt nephropathy.[121,122] Hyperglycemia is a well-known cause of oxidant stress and IR is also associated with production of free radicals, which in turn is responsible for a deterioration of insulin action, leading to a vicious circle.[123] Thus, reversion of hyperglycemia and IR could lead in amelioration of oxidant stress. Studies in various animal models suggest that TZDs can act as a potent general antioxidant[124] and reduce oxidative stress at the kidney level.[31,96,125] Although further studies are necessary in the field, the above data suggest that the anti-inflammatory and antioxidant properties of TZDs could be also helpful in renal function protection.

### Interference with the renin–angiotensin system

*In vitro* studies have shown that TZDs can interfere with the renin–angiotensin system in several tissues. In particular, Harte *et al.*[126] showed that rosiglitazone lowers the production of angiotensins I and II from human subcutaneous adipocytes, whereas in other studies TZDs have been found to downregulate the expression of angiotensin 1 receptor mRNA and angiotensin 1 receptor protein in vascular smooth muscle cells.[127,128] These actions of TZDs could explain other findings regarding their ability to inhibit angiotensin II-induced vascular smooth muscle cells proliferation.[129] These TZDs' properties should also be examined in renal glomerular cells, having also in mind the above-mentioned upregulation of intrarenal renin–angiotensin system from insulin.[74] However, even if this inhibiting effect of TZDs on renin–angiotensin system actions is restricted on renal vasculature, it could also contribute to TZD's renoprotective properties.

### Attenuation of intracellular lipid accumulation

Dyslipidemia has long been proposed as an important contributor toward the progression of kidney disease and subsequent evidence from both experimental studies in animals and clinical studies in humans supported this notion.[130–132] As in the case of atherosclerotic damage, lipid accumulation and foam cell formation are basic features of the lipid-mediated glomerular and tubulointerstitial injury,[132,133] and previous studies have shed light to the mechanisms of lipid uptake and accumulation in renal cells.[134] Emerging evidence suggests that PPAR$\gamma$ agonism may attenuate lipid accumulation and its related injury in mesangial cells.[135,136] Inflammatory cytokines such as IL-1$\beta$ are known to promote mesangial lipid accumulation through dysregulation of low-density lipoprotein receptor expression, which allows increased lipid uptake,[137] as well as inhibition of cholesterol efflux.[135] A reduction of mesangial PPAR$\gamma$ expression was reported to be involved in the last action of IL-1$\beta$, whereas activation of PPAR$\gamma$ with the agonist prostaglandin J2 enhanced lipid efflux and protected against intracellular lipid accumulation.[135] In addition, liver-X-receptor-$\alpha$ (LXR$\alpha$), an important regulator of cholesterol efflux in macrophages, has been recently identified in mesangial cells.[136] Activation of LXR$\alpha$ increased cholesterol efflux, whereas both troglitazone and rosiglitazone were shown to markedly increase glomerular LXR$\alpha$ expression.[136] Taken together, these findings suggest that attenuation of mesangial lipid accumulation is another possible mechanism of TZD-associated renoprotection.

### Decrease in endothelin-1 levels

Previous studies reported an increase in endothelin-1 levels in patients with type 2 DM or hypertension and microalbuminuria,[138,139] suggesting an involvement of endothelin-1 in the development of nephropathy in these patients. Insulin has been shown to raise plasma endothelin-1 levels *in vivo* in type 2 diabetic subjects[140] and to stimulate endothelin-1 production from endothelial cells,[141] as well as renal mesangial cells[76] *in vitro*. Apart from being a potent vasoconstrictor of the renal vasculature that reduces renal

plasma flow and glomerular filtration rate, endothelin-1 has been shown in experimental studies to have mitogenic effects on mesangial cells.[142,143] Among others, endothelin-1 stimulates a PKC-regulated phospholipase D, which hydrolyzes phospholipid substrates and induces generation of phosphatidic acids that stimulate proliferation in mesangial cells[142] and potentiates the mitogenic effects of angiotensin II.[76] TZDs have been found to significantly reduce urine endothelin-1 secretion in humans,[34] as well as to inhibit PKC *in vitro*,[27,38,70,71] as mentioned above, indicating that a depressing action on endothelin-1 levels and the mechanisms through which endothelin-1 evokes renal injury could also take part in their renoprotective action.

Other actions of TZDs that can contribute to their renoprotective properties have also been reported. For example, PPAR$\gamma$ agonists could improve the mechanic properties of glomerular cells, as they have been found to reverse the contractile dysfunction of mesangial cells evoked from hyperglycemia.[144] Another mechanism involved in the reduction in UAE with TZDs could be an increase in tubular cell albumin uptake, as evident in a recent study.[117] Overall, future research should further elucidate actions like the above or investigate other potential renoprotective properties of TZDs.

## CONCLUSION

The contribution of PPAR$\gamma$ agonists to the armamentarium of treatment of the growing epidemic of patients with type 2 DM provides complementary mechanisms that affect both glycemic control and inflammatory reactions in this disease. TZDs act by multiple mechanisms to control glucose and reduce inflammation and will probably have a role more in prevention as well as treatment. For now we should use these agents to reduce inflammatory processes as manifested by microalbuminuria, as well as improve glycemic control in patients with type 2 DM.

New strategies for prevention of diabetic nephropathy as well as the delay of its progression are urgently needed. Several studies in various animal models have consistently shown that all TZDs are able to reduce albuminuria as well as prevent renal injury. Data from human studies in patients with type 2 DM and normal UAE levels or microalbuminuria are also promising, as in most of them TZD treatment significantly reduced UAE. These findings are strongly supported from both clinical and experimental evidence. Clearly, TZDs can act beneficially on most of the major players involved in the progression of diabetic nephropathy, namely hyperglycemia, hyperinsulinemia, elevated BP, dyslipidemia, endothelial dysfunction, inflammatory cytokines, renin–angiotensin system activation, and others. Hence, TZDs provide a promising approach for the prevention of nephropathy in diabetic patients. However, the total renoprotective effect of TZDs should be explored by future clinical trials that examine the effects of TZDs in patients with proteinuria and on harder renal outcomes, like progressive decline in renal function, as well as by experimental studies investigating the details of the above protecting mechanisms.

## ACKNOWLEDGMENTS

This paper was not supported by any source and represents an original effort on our part .

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