



Review

Vascular effects of TZDs: New implicationsFlorian Blaschke^a, Robert Spanheimer^b, Mehmood Khan^b, Ronald E. Law^{b,*}^a *Division of Endocrinology, Diabetes and Hypertension, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA*^b *Department of Medical and Scientific Affairs, Takeda Pharmaceuticals North America, Lincolnshire, IL 60069, USA*

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Abstract

The incidence of diabetes, now affecting more than 170 million individuals is growing rapidly. Type 2 diabetes, which accounts for 90% of all diabetes cases, is associated with increased cardiovascular morbidity and mortality. Thiazolidinediones (TZDs), used for the treatment of patients with type 2 diabetes improve insulin sensitivity and endothelial dysfunction and exert beneficial effects on the lipid profile by activating the peroxisome proliferator-activated receptor gamma (PPAR- γ). Moreover, a large body of evidence indicates that TZDs exhibit antiatherogenic effects independent of their antidiabetic and lipid-lowering properties by modulating inflammatory processes.

This review will focus on the role of PPAR- γ agonists in the vessel wall and summarize their effects on C-reactive protein (CRP), plasminogen activator inhibitor type-1 (PAI-1), matrix metalloproteinase-9 (MMP-9), adiponectin and ATP-binding cassette transporter A1 (ABCA1) and their implications for treatment of advanced stages of atherosclerosis, particularly in a setting of type 2 diabetes.

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1. Introduction

Current evidence suggests a pivotal role for inflammation in all phases of atherosclerosis from the formation of fatty streaks to subsequent rupture of the lesion, and acute coronary syndromes (Ross, 1999; Libby, 2002). This concept is supported by epidemiological and clinical studies, where systemic inflammatory markers such as high-sensitive C-

reactive protein (hs-CRP), interleukin-6 (IL-6) or serum amyloid A have been shown to be strong predictors of cardiovascular complications in various settings (Ridker et al., 2000). Besides the potential use of inflammatory biomarkers as risk predictor for cardiovascular events, these markers might also serve as targets for pharmacological therapy.

The prevalence of type 2 diabetes, a leading cause of morbidity and mortality in Western societies, is increasing exponentially around the globe (Zimmet et al., 2001). Cardiovascular disease has been shown to account for up to 80% of deaths in type 2 diabetic patients (Kirpichnikov and

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Sowers, 2001). Besides an elevated incidence of atherosclerosis, diabetes mellitus is also associated with poor outcomes after vascular occlusion compared with the nondiabetic population (Kannel and McGee, 1979; Wei et al., 1998; Kornowski et al., 1997). Plaque composition is known to determine the risk of plaque disruption and thrombosis, which is the main cause of the acute coronary syndrome (Falk et al., 1995; Libby, 1995). Plaques prone to rupture are characterized by rich inflammatory infiltrates and decreased collagen and vascular smooth muscle cells (VSMCs) in their caps and shoulders (Lendon et al., 1991; Moreno et al., 1994; Van der Wal et al., 1994). Atherosclerotic lesions in patients with type 2 diabetes exhibit increased macrophage infiltration, decreased VSMC content and a larger lipid core than lesions in subjects without diabetes (Moreno et al., 2000). Thus, type 2 diabetes is not only associated with accelerated and premature coronary atherosclerosis, but also with an increased vulnerability for plaque rupture and thrombosis (Haffner et al., 1998). However, the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that intensive blood glucose control with insulin or sulfonylurea in type 2 diabetic patients had only a limited effect on the incidence of cardiovascular events (UKPDS Group, 1998), indicating the necessity of new treatment strategies to reduce cardiovascular morbidity and mortality associated with this syndrome. Thiazolidinediones (TZDs), agonists for the peroxisome proliferator-activated receptor- γ (PPAR- γ) are strong candidates for realizing this aim.

PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPARs regulate transcription of target genes by heterodimerizing with the retinoid X receptor (RXR) and binding to PPAR response elements (PPRE) in the promoter region of their target genes (Gearing et al., 1993). In the absence of ligands, PPAR/RXR heterodimers can actively repress gene transcription through recruitment of corepressor complexes (Krogsdam et al., 2002). Ligand binding induces a conformational change in the heterodimeric unit, resulting in activation of gene transcription through dissociation of corepressors and recruitment of coactivators (Nolte et al., 1998). In addition, PPARs can repress gene expression in a DNA-binding-independent manner by antagonizing the activity of other transcription factors (Ricote et al., 1998). Three isoforms, PPAR- γ , PPAR- α and PPAR- β/δ have been identified so far. Synthetic ligands for two forms of the receptor, PPAR- γ and PPAR- α , are clinically available; ligands for PPAR- β/δ are currently under development, as well as dual and pan PPAR ligands. Troglitazone was the first PPAR- γ agonist developed for clinical use, but was withdrawn because of rare, but serious hepatotoxicity (Tolman and Chandramouli, 2003). Two other TZDs, pioglitazone and rosiglitazone, are currently available and are not associated with hepatotoxicity (Isley, 2003). TZDs reduce peripheral insulin resistance by increasing insulin-dependent glucose disposal and reducing hepatic glucose output and are widely used for the treatment of type 2 diabetic patients (Saltiel and Olefsky, 1996).

In numerous studies, PPAR- γ ligands have been found to attenuate atherosclerotic lesion formation in genetically prone mouse models and reduce inflammatory gene expression in

vascular cells in vitro (Ricote et al., 1998; Li et al., 2000; Collins et al., 2001; Jiang et al., 1998). Therefore, besides the beneficial effect of TZDs on glucose haemostasis and plasma lipids in humans (Gerber et al., 2003; Khan et al., 2002), direct effects on vascular cells may also contribute to the potential antiatherogenic properties of TZDs. Clinical studies have shown that TZD treatment reduced inflammatory surrogate markers of vascular risk, such as CRP, tumor necrosis factor- α (TNF- α) or serum amyloid A (Haffner et al., 2002; Mohanty et al., 2004). In small clinical trials with type 2 diabetics, troglitazone and pioglitazone have been found to attenuate progression of common carotid arterial intima-media thickness (Koshiyama et al., 2001; Minamikawa et al., 1998). Recently, results from the first TZD cardiovascular outcomes trial, PROactive, were published (Dormandy et al., 2005). Outcomes trials with other TZDs and non-TZD PPAR- γ ligand are necessary to further elucidate the role of TZDs as potential candidates for the treatment of atherosclerosis.

This review focuses on the effects of PPAR- γ agonists on CRP, plasminogen activator inhibitor type 1 (PAI-1), matrix metalloproteinase 9 (MMP-9), adiponectin and ATP-binding cassette transporter A1 (ABCA1), which are known to play important roles especially in the late stages of atherosclerosis.

2. C-reactive protein (CRP)

Many epidemiological studies have shown that CRP, a classic acute-phase protein, correlates with increased risk of cardiovascular disease, myocardial infarction, and stroke among apparently healthy individuals (Ridker et al., 1998). Furthermore, CRP levels have been found to predict the risk of recurrent coronary events among patients with stable and unstable angina (Haverkate et al., 1997; Liuzzo et al., 1994), in the acute phase after myocardial infarction (Takahashi et al., 2003), and after revascularization procedures (Buffon et al., 1999). CRP is a stronger predictor of future cardiovascular events than LDL cholesterol levels and adds prognostic information to the conventional Framingham risk assessment (Ridker et al., 2002).

The principal source of circulating CRP is the hepatocyte; however, previous studies demonstrated the presence of CRP mRNA and protein in human atherosclerotic lesions (Yasojima et al., 2001). Both in vitro and in vivo studies suggest that CRP, in addition to being a risk marker, promotes atherosclerotic processes by direct proinflammatory effects on vascular cells (Jialal et al., 2004).

In human endothelial cells (ECs), CRP increased expression of adhesion molecules like ICAM (intracellular adhesion molecule), VCAM (vascular cell adhesion molecule) and E-selectin (Pasceri et al., 2000), which play crucial roles in the development of atherosclerosis by mediating macrophage adherence to ECs (Patel et al., 1998). CRP also upregulates ET-1 (endothelin-1) and tissue factor expression and decreases endothelial nitric oxide (NO) release (Verma et al., 2002a,b; Cermak et al., 1993). In addition, CRP induced expression of MCP-1 (monocyte chemoattractant protein-1), which was significantly inhibited by pretreatment with the

PPAR- γ ligand ciglitazone (Pasceri et al., 2001). Accordingly, Verma et al. found that rosiglitazone attenuated the CRP-mediated effect on cell adhesion molecules and MCP-1 expression both under basal and hyperglycemic conditions (Verma et al., 2003). The findings that hyperglycemia potentiated the proatherogenic effects of CRP on ECs in vitro might have important implications for patients with diabetes (Verma et al., 2003). Hyperglycemia may exaggerate the proatherogenic effects of CRP, thus contributing to the observed accelerated atherosclerosis in type 2 diabetics compared with nondiabetics.

Moreover, previous studies have shown that CRP causes a decrease in eNOS expression and bioactivity in human ECs (Venugopal et al., 2002; Verma et al., 2002a,b). Nitric oxide derived from endothelial NO synthase (eNOS) reduces vessel tone and inhibits VSMC proliferation and migration, LDL oxidation, and platelet/monocyte adhesion to endothelium, all known to be key events in the development of atherosclerosis (Wever et al., 1998; Loscalzo, 1995; Boger et al., 1996). In addition, CRP has been shown to promote endothelial cell apoptosis in a NO-dependent fashion and upregulate nuclear factor kappa B (NF- κ B), a key transcription factor that induces the transcription of numerous proatherosclerotic genes (Verma et al., 2002a,b; Thurberg and Collins, 1998). More recently, CRP was found to inhibit endothelial progenitor cell (EPC) differentiation and promote EPC apoptosis. Bone marrow-derived EPCs contribute to angiogenesis and the subsequent development of collateral circulation, and might therefore play an important role in maintaining and/or revitalizing cardiac tissue after infarction (Asahara et al., 1999). Pretreatment with the PPAR- γ agonist rosiglitazone attenuated the detrimental effects of CRP on EPCs, further supporting the growing body of evidence which indicate beneficial effects of PPAR- γ agonists that extend beyond improving insulin sensitivity (Verma et al., 2004). In patients with type 2 diabetes, EPC number and function are impaired, which might contribute to the poor outcome in diabetics after vascular occlusion (Tepper et al., 2002). Thus, these findings indicate that improving EPCs number and function might be beneficial in terms of atherosclerotic lesion development, especially in high-risk populations like type 2 diabetics.

CRP has been found to activate the complement system and be chemotactic for human blood monocytes in vitro (Wolbink et al., 1996; Torzewski et al., 2000). Han et al. demonstrated that CRP promotes MCP-1 mediated chemotaxis by increasing CC chemokine receptor 2 (CCR2) expression, the most dominant chemotaxis receptor in monocytes (Han et al., 2004). CRP was also found to promote the uptake of oxidized LDL, thus contributing to foam cell formation (Chang et al., 2002). In addition, Williams et al. demonstrated that CRP-stimulated matrix metalloproteinase-1 (MMP-1) mRNA and protein expression in human monocytes/macrophages, indicating that CRP may contribute to plaque vulnerability by promoting matrix degradation (Williams et al., 2004).

The angiotensin type 1 receptor (AT₁R) plays a central role in the development and clinical course of atherosclerosis (Nickenig and Harrison, 2002). CRP markedly upregulates

AT₁R mRNA and protein expression in cultured human VSMCs, which leads to increased VSMC migration and proliferation and enhanced reactive oxygen species production (Wang et al., 2003). In a carotid artery balloon angioplasty model in rats, exposure to CRP increased AT₁R expression in the vessel wall, resulting in increased VSMC proliferation and migration, as well as an increase in neointima formation (Wang et al., 2003). Moreover, Hattori et al. demonstrated that CRP causes NF- κ B activation and subsequent MCP-1, IL-6 and iNOS gene expression, which was inhibited by rosiglitazone and pioglitazone (Hattori et al., 2003). In atherosclerotic plaques obtained from directional coronary atherectomy, enhanced immunoreactivity of CRP was associated with histological and clinical features of coronary plaque instability (Kobayashi et al., 2003). In addition, CRP has been shown to induce apoptosis in human coronary VSMCs, which may contribute to a weakening of the intimal plaque texture and to reduced deposition of extracellular matrix proteins, both increasing plaque vulnerability (Blaschke et al., 2004).

In apoE^{-/-} mice, human CRP transgene expression resulted in accelerated aortic atherosclerosis, although the effect was only modest (Paul et al., 2004). These data further support and extend the in vitro observation of a direct proatherogenic role for CRP.

Numerous clinical studies clearly established CRP as a risk marker for coronary artery disease (CAD) in a variety of settings. In type 2 diabetic patients with CAD higher levels of CRP were observed compared with type 2 diabetics without CAD, suggesting the inclusion of CRP in the risk assessment of diabetic patients (Mojiminiyi et al., 2002). In addition, elevated levels of CRP were found to be a strong independent predictor for the future development of type 2 diabetes (Pradhan et al., 2001; Hu et al., 2004). The findings that CRP levels are increased in patients with the metabolic syndrome and diabetes suggest that low-grade inflammation might be an important player in the pathogenesis of insulin resistance and type 2 diabetes, and subsequent increased CAD risk.

Previous clinical studies in type 2 diabetic patients have shown that pioglitazone and rosiglitazone treatment significantly reduced plasma CRP levels (Haffner et al., 2002; Satoh et al., 2003; Chu et al., 2002). Within a week, TZDs have been shown to reduce plasma CRP levels by 30%, while the corresponding effect of statins was only 14% (Sjoholm and Nystrom, 2005). In addition, Satoh et al. demonstrated that pioglitazone treatment of type 2 diabetic patients resulted in a significant reduction of CRP levels in both responders and nonresponders, classified according to the reduction in HbA1c levels (Satoh et al., 2003). These findings provided initial evidence that the antiatherogenic effects of TZDs are independent of their antidiabetic actions. Accordingly, rosiglitazone treatment reduced CRP levels in nondiabetic patients with CAD and nondiabetic hypertensive patients and in nondiabetic and diabetic obese patients (Mohanty et al., 2004; Sidhu et al., 2003, 2004; Raji et al., 2003). Since adipocytes have been proposed to be the missing link between insulin resistance and cardiovascular disease, the TZD-mediated decrease in CRP levels in

obese subjects may have important implications for treatment strategies in this high-risk population.

In summary, CRP has been shown to be a powerful predictor of future cardiovascular events and may also play a direct role in the development of atherosclerosis and its thrombotic complications. Numerous studies demonstrated that TZDs attenuate the proatherogenic effects of CRP *in vitro*, in concentrations known to be associated with increased risk of future cardiovascular events. In addition, clinical trials have shown that TZD treatment reduces plasma CRP levels both in diabetic and nondiabetic patients. However, whether these results translate into a therapeutic benefit with respect to atherosclerotic coronary disease awaits the completion of ongoing clinical trials.

3. Plasminogen activator inhibitor type-1 (PAI-1)

Plasminogen activator inhibitor type-1 (PAI-1), a member of the serpin superfamily of proteinase inhibitors, plays an important role in the cardiovascular and renal system to promote both thrombosis and fibrosis (Eddy, 2002). PAI-1 inhibits plasminogen activators, including tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), thereby preventing the conversion of plasminogen into plasmin. In plasma, PAI-1 promotes clot formation, which plays an important role in the pathogenesis of cardiovascular events (Thogersen et al., 1998; Johansson et al., 2000). In tissue, PAI-1 promotes accumulation of extracellular matrix and regulates vascular remodelling, cardiac fibrosis and glomerulosclerosis (Oda et al., 2001; Eitzman et al., 2000).

A variety of clinical studies have demonstrated an association between circulating PAI-1 levels and cardiovascular events (Thogersen et al., 1998; Kohler et al., 2000; Zunker et al., 1999). In addition, previous studies indicate a correlation between PAI-1 levels and outcomes after acute coronary syndromes, indicating that PAI-1 might also be a useful prognostic marker (Collet et al., 2003). It has been shown that throughout the spectrum of insulin resistance, from the metabolic syndrome to type 2 diabetes, PAI-1 levels are increased (Festa et al., 1999; Pannaciuoli et al., 2002). Plasma PAI-1 levels have been found to correlate with each component of the insulin resistance syndrome and, in particular with fasting insulin levels, triglycerides and body mass index (BMI) (Vague et al., 1986; Rosito et al., 2004; Eliasson et al., 1994). Thus, PAI-1 may provide a link between insulin resistance, obesity, type 2 diabetes and increased incidence of CAD.

PAI-1 is produced by several cell types, including hepatocytes, endothelial cells, fibroblasts, mesangial cells and adipocytes (Peraldi et al., 1992; Lucore et al., 1988; Alessi et al., 1997). In clinical trials, treatment with TZDs has been shown to reduce plasma levels of PAI-1 in insulin-resistant patients in proportion to the insulin-sensitizing activity of the drug. In a double-blind, randomized study comparing the effect of glibenclamide alone or in combination with rosiglitazone, significant reductions in plasma levels of PAI-1 antigen and PAI-1 activity were observed in the rosiglitazone-treated group compared with placebo (Freed et al., 2004). Previous studies

with troglitazone also demonstrated a decrease in circulating PAI-1 levels (Kato et al., 2000; Kruszynska et al., 2000). Both direct and indirect mechanisms are likely to mediate the effect of TZDs on plasma PAI-1 levels. Treatment of insulin-resistant subjects with TZDs reduced plasma insulin levels, which are generally correlated with PAI-1 plasma concentrations (Suter et al., 1992). Both insulin and pro-insulin has been shown to increase PAI-1 expression *in vivo* and *in vitro* (Nordt et al., 1998; Schneider and Sobel, 1991). TZDs also have been shown to decrease free fatty acids (FFAs), which are reported to stimulate PAI-1 production (Suter et al., 1992; Chen and Schneider, 2002). Depending on the cell type, different direct effects of TZDs on PAI-1 expression have been observed *in vitro*. Overexpression of PPAR- γ in human fibroblasts resulted in increased PAI-1 secretion in proportion to the amount of transfected PPAR- γ DNA (Marx et al., 1999). In addition, treatment of human ECs with natural PPAR- γ ligands (15d-PGJ₂, 9(s)-HODE, 13(s)-HODE) augmented PAI-1 mRNA and protein expression, thus implying that activation of the PPAR- γ signalling pathway may promote PAI-1-related atherogenicity (Marx et al., 1999). In contrast, Hong et al. reported that either PPAR- γ overexpression or troglitazone treatment modestly reduced both PAI-1 mRNA and protein expression in ECs, while a significant reduction was observed with a combination of PPAR- γ overexpression and troglitazone (Hong et al., 2003). In addition, troglitazone and pioglitazone were also found to inhibit both basal and TNF- α -stimulated PAI-1 secretion (Kato et al., 1999; Nordt et al., 2000; Hamaguchi et al., 2003). Interestingly, studies with ECs employing endogenous PPAR- γ ligands showed an increase in PAI-1 expression, while those using synthetic ligands demonstrated a decrease in PAI-1 expression.

Clinical studies have shown that weight loss significantly reduces plasma PAI-1 levels in obese humans (Kockx et al., 1999). Studies in human adipose tissue revealed significantly higher expression of PAI-1 per fat cell in adipocytes from obese than from lean subjects (Eriksson et al., 1998). These data indicate that adipose tissue is an important source of circulating PAI-1 and that secretion of PAI-1 by adipocytes contributes to elevated plasma PAI-1 seen in obese subjects. In human adipocytes Zirlik et al. demonstrated that the TZDs troglitazone, rosiglitazone, pioglitazone and ciglitazone significantly decreased PAI-1 expression under basal conditions and in response to TGF- β stimulation (Zirlik et al., 2004). In addition, rosiglitazone was shown to reduce both basal and insulin-induced PAI-1 production in isolated subcutaneous adipocytes. These results suggest that the effect of TZDs on adipose tissue may contribute to the observed reduction of serum PAI-1 levels in patients treated with TZDs. In contrast to the decrease in PAI-1 expression seen with TZDs in human adipocytes, treatment of 3T3-L1 preadipocytes with a combination of insulin and a TZD dramatically increased PAI-1 expression and accelerated the rate of adipocyte differentiation (Ihara et al., 2001). Whether the increase in PAI-1 is required for cell differentiation or a consequence of adipogenesis is unclear.

In type 2 diabetic subjects, PAI-1 levels were increased both in the plasma and the atheroma compared with those in non-

diabetic patients with comparable atherosclerotic lesions (Vague et al., 1986; Sobel et al., 1998). In addition, Shindo et al. demonstrated that PAI-1 expression in coronary atherectomy specimens is increased in acute coronary syndromes compared to stable angina pectoris, and correlates with plaque macrophage density (Shindo et al., 2001).

Recently, Schneider et al. demonstrated that overexpression of PAI-1 in VSMCs in apoE^{-/-} mice reduced VSMC cellularity of vascular lesions. Accordingly, migration of VSMCs isolated from PAI-1 transgenic mice was attenuated (Schneider et al., 2004). Taken together, these data suggest that increased PAI-1 expression during the development of atherosclerotic lesions may, as a result of decreased VSMC migration, promote the formation of VSMC-poor plaques with thin fibrous caps that are more prone to rupture. In addition, increased PAI-1 may also contribute to chronic plaque growth and thrombogenicity because of its effects on the fibrinolytic system. Therefore, TZD administration, which is associated with a decrease in PAI-1 plasma levels in the circulation and in the atheroma could lead to decreases in the prothrombotic state and promote plaque stabilization.

VSMCs play not only a decisive role in the development of atherosclerosis but are also crucial for the development of restenosis after percutaneous transluminal coronary angioplasty (PTCA). Because proliferation of VSMCs rather than increased migration might be pivotal in restenosis, decreases in VSMC migration, implicated in the formation of vulnerable plaques, would not necessarily be expected to attenuate the restenotic process. Numerous studies investigated the role of PAI-1 in neointima formation using different animal species, strains, and diverse intervention techniques. However, these studies revealed contradictory results. Carmeliet et al. demonstrated that neointima formation after electrical or mechanical injury was significantly increased in PAI-1^{-/-} mice, while adenovirus-mediated overexpression of PAI-1 reduced neointima formation (Carmeliet et al., 1997). Accordingly, de Waard et al. showed an increase in neointima formation in PAI-1^{-/-} mice after carotid artery ligation (de Waard et al., 2002). In contrast, Ploplis et al. demonstrated that copper-cuff-induced neointima formation was decreased in PAI-1^{-/-} mice compared to wild type controls (Ploplis et al., 2001). In addition, deYoung et al. found that adenovirus-mediated increases in PAI-1 expression in the artery wall promotes neointima growth in a balloon injury model in rats (deYoung et al., 2001). The diversity of results in the animal models of neointima formation may reflect differences in species, strains, and different types or location of induced arterial injury.

PPAR- γ ligands are known to inhibit proliferation of VSMCs in vitro and prevent neointima formation in animal models, independent of the presence of diabetes (Law et al., 2000; Yoshimoto et al., 1999; Phillips et al., 2003). In clinical trials, troglitazone, rosiglitazone, and pioglitazone reduced neointimal tissue proliferation after coronary stent implantation in type 2 diabetic patients (Takagi et al., 2000, 2003; Choi et al., 2004), although one small study demonstrated no effect with rosiglitazone (Osman et al., 2004).

In conclusion, these results suggest that elevated PAI-1 levels, which are associated with all components of the metabolic syndrome, might be the link to increased cardiovascular risk in these patients. Therefore, TZD-mediated decreases in circulating PAI-1 levels might offer a new pharmacological approach to reduce the incidence of CAD and its complications in this population.

4. Matrix metalloproteinase-9 (MMP-9)

Remodelling of the extracellular matrix is known to play an important role in various vascular disorders including progression of atherosclerosis, rupture of atherosclerotic plaques, and development of restenotic lesions (Wills et al., 1996; Galis and Khatri, 2002).

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-containing endoproteinases that have the ability to degrade components of the extracellular matrix (ECM). The MMPs are classified according to the components of the extracellular matrix they degrade. Group 1 contains the collagenases (MMP-1, MMP-8, MMP-13), group 2 the gelatinases (MMP-2, MMP-9), group 3 the stromelysins (MMP-3, MMP-10, MMP-11) and group 4 the membrane-type MMPs, which degrade various extracellular matrix components (Wainwright, 2004). The activity of MMPs is regulated at the transcriptional level, by activation of the latent proenzymes by proteolytic cleavage, and by inhibition through specific tissue inhibitors of metalloproteinases (TIMPs) (Brew et al., 2000).

The activity of MMPs is generally low in normal adult tissue, but is upregulated during certain pathological processes, including inflammation and cardiac remodelling. A variety of extracellular stimuli such as cytokines or growth factors have been shown to induce MMP transcription (Chakraborti et al., 2003; Hanemaaijer et al., 1993). Many of these stimuli such as TNF- α , platelet-derived growth factor (PDGF) or interleukin-1 (IL-1) mediate atherosclerosis and restenosis. A variety of cells like macrophages, VSMCs, and leukocytes have been shown to produce MMPs (Galis et al., 1994a,b; Xu et al., 1999; Trocme et al., 1998; Di Girolamo et al., 1998).

In vitro, Marx et al. found that treatment of VSMCs with troglitazone and the natural occurring PPAR- γ ligand 15d-PGJ₂ decreased PMA-induced MMP-9 expression and activity (Marx et al., 1998a,b). In activated macrophages 15d-PGJ₂ inhibited the expression of MMP-9 by antagonizing the activities of the transcription factors AP-1, STAT and NF- κ B (Ricote et al., 1998). In agreement with these studies, MMP-9 release from human monocyte-derived macrophages was found to be suppressed by rosiglitazone and troglitazone (Patel et al., 2002; Marx et al., 1998a,b). In addition, Kintscher et al. demonstrated that rosiglitazone and troglitazone blocked MCP-1 directed monocyte migration, at least in part, through inhibition of MMP-9 expression (Kintscher et al., 2000).

Elevated MMP expression and activity was found in human atherosclerotic lesions and in experimental animal models of atherosclerosis and restenosis, suggesting that MMPs play an important role in initiation and progression of atherosclerosis

and in restenosis after PTCA. Migration and proliferation of VSMCs play not only a decisive role in the development of atherosclerosis but are also the primary pathophysiologic mechanism resulting in postangioplasty restenosis (Dzau et al., 2002). Migration of VSMCs into and within the intima requires the degradation of basal laminae and interstitial ECM, processes that involve MMPs (Galis et al., 1994a,b).

Numerous animal studies reported an induction of MMP-9 expression in VSMCs within the first days after balloon injury in rat carotid arteries (Bendeck et al., 1994; Zempo et al., 1994). Furthermore, administration of an MMP inhibitor after balloon injury or overexpression of TIMP-1 and TIMP-2, inhibitors of MMP-9 and MMP-2, respectively, reduced neointima formation in vivo (Bendeck et al., 1994; Forough et al., 1996; Cheng et al., 1998). These data further underline the crucial role of MMPs in the breakdown of ECM, allowing migration of VSMC from the outer layers. Accordingly, MMP inhibitors dramatically reduced VSMC migration in vitro (Pauly et al., 1994). Using an angioplasty model in rabbits, Coats et al. found that the collagen content was significantly decreased in restenotic versus nonrestenotic vessels, concurrent with an increase in MMP activity (Coats et al., 1997). In addition, Southgate et al. demonstrated that MMP inhibitors diminish VSMC proliferation in vitro (Southgate et al., 1992). Taken together, these results further support a role for MMPs in intimal thickening.

Clinical studies have shown that patients with previous acute myocardial infarction had significantly elevated serum MMP-9 levels compared with control subjects without history of CAD (Ferroni et al., 2003). Accordingly, Kalela et al. found that serum MMP-9 levels were higher in patients with 3-vessel CAD compared to patients with 1- or 2-vessel CAD or subjects with no evidence of CAD, indicating a potential role for MMP-9 in the noninvasive evaluation of the severity of CAD (Kalela et al., 2002). Moreover, clinical studies suggest that MMP-9 participates in the mechanism of the acute coronary syndrome. Analysis of human coronary atherectomy specimens from patients with unstable angina revealed elevated MMP-9 expression by VSMCs and macrophages compared to patients with stable angina (Brown et al., 1995; Jiang et al., 2004). Furthermore, significantly higher plasma levels of MMP-9 were found in patients with acute coronary syndrome compared with patients with stable angina or subjects with no evidence of CAD (Nomoto et al., 2003; Inokubo et al., 2001). Similarly, Kai et al. observed an immediate elevation in systemic MMP-9 plasma levels in patients with an acute myocardial infarction or unstable angina compared to control subjects (Kai et al., 1998). In addition, increased MMP-9 plasma levels were found in type 1 and type 2 diabetic patients (Maxwell et al., 2001; Tayebjee et al., 2004).

In a small clinical trial, Marx et al. showed that rosiglitazone treatment of type 2 diabetic patients with CAD decreased MMP-9 plasma levels (Marx et al., 2003). Interestingly, a significant reduction of MMP-9 plasma levels was already observed after 2 weeks of rosiglitazone treatment, an effect which persisted over the 12-week study period. Since previous studies have shown that rosiglitazone exhibits its maximal glucose-lowering

effect after 8 to 12 weeks (Nolan et al., 2000), these findings indicate that the effect of TZDs on MMP-9 levels are independent of their antidiabetic action. These results are in accordance with a previous study by Haffner et al. showing significantly reduced MMP-9 levels after 26 weeks of rosiglitazone treatment in type 2 diabetics.

Overexpression of MMP-9 in the vulnerable shoulder region of human plaques and elevated MMP-9 levels in the coronary circulation in patients with acute coronary syndrome suggest, that MMP-9 plays a crucial role in plaque destabilization (Galis et al., 1994a,b; Inokubo et al., 2001). Because previous studies have demonstrated that lesions from diabetic patients are more vulnerable than those from nondiabetic subjects (Moreno et al., 2000) reduction of MMP-9 levels by TZD treatment might be a promising tool to influence the incidence of acute coronary syndrome in these patients. TZD-mediated inhibition of MMP-9 expression might also contribute to the observed reduction of neointima formation after coronary stent implantation in type 2 diabetic subjects (Takagi et al., 2000, 2003).

Previous studies also suggest that MMPs, and in particular MMP-9, play a crucial role in both acute and chronic stages of acute myocardial infarction. The function and expression of MMPs during infarct healing and left ventricular remodeling after myocardial infarction have been investigated in several animal studies using MMP inhibitors and genetically modified mice. Numerous studies revealed that MMP-9 expression and activity is upregulated within minutes after experimental myocardial ischaemia (Etoh et al., 2001; Lu et al., 2000). Rohde et al. demonstrated that administration of a nonspecific MMP inhibitor attenuated early left ventricular dilatation 4 days after experimental myocardial infarction in mice (Rohde et al., 1999). Consistent with these observations, MMP-9^{-/-} mice had less left ventricular dilatation compared to controls until at least 15 days after experimental myocardial infarction (Ducharme et al., 2000). Moreover, ventricular rupture rate following myocardial infarction was also reduced in MMP-9^{-/-} mice compared to wild type controls (Heymans et al., 1999). Besides attenuated left ventricular dilatation, myocardial infarct size was reduced in MMP-9^{-/-} mice (Romanic et al., 2002).

A study of the time course of MMP-9 plasma expression in patients with myocardial infarction demonstrated that MMP-9 was elevated on admission and returned to baseline by 1 week after myocardial infarction, further supporting the hypothesis that MMP-9 plays a pathophysiological role in the early phase of acute myocardial infarction (Kaden et al., 2003).

In conclusion, these data suggest that inhibition of MMP-9 expression and activity may be a promising therapeutic approach to influence vascular disease, especially in the high-risk population of patients with diabetes mellitus. Because MMP-9 plasma levels are elevated in this population, the effect of TZDs on MMP-9 expression might contribute to a potential beneficial effect of TZDs on cardiovascular risk.

5. Adiponectin

Adiponectin, a hormone exclusively produced by the adipocyte (Maeda et al., 1996), is abundantly present in

circulating blood and has been shown to enhance insulin sensitivity (Berg et al., 2001; Yamauchi et al., 2002) and exhibit antiinflammatory (Yokota et al., 2000; Ouchi et al., 2000) and antiatherogenic properties (Okamoto et al., 2002; Yamauchi et al., 2003a,b). Adiponectin circulates as either the full-length protein or as a putative proteolytic cleavage fragment consisting of the globular C-terminal domain (Fruebis et al., 2001). Two adiponectin receptors, AdipoR1 and AdipoR2, which serve as receptors for globular and full-length adiponectin have been identified (Yamauchi et al., 2003a,b). AdipoR2 is predominantly expressed in the liver, whereas AdipoR1 is abundantly expressed in skeletal muscle (Yamauchi et al., 2003a,b). In contrast to the increase in plasma levels of most adipokines, adiponectin mRNA and serum levels are decreased in obese patients, and the level of adiponectin is negatively correlated with BMI (Hu et al., 1996; Arita et al., 1999). In addition to obesity, plasma levels of adiponectin are also decreased in insulin resistance, type 2 diabetes, and in patients with CAD (Jansson et al., 2003; Hotta et al., 2000). Weight loss has been shown to significantly increase plasma adiponectin levels in obese patients as well as diabetic and nondiabetic subjects (Hotta et al., 2000; Bruun et al., 2003; Yang et al., 2001). This is particularly interesting, as adiponectin increases occurred despite significant reductions in adipose tissue, the sole source of adiponectin production.

Numerous animal and in vitro studies indicate that adiponectin might play a pivotal role in protecting against atherosclerosis. The protective effect of adiponectin has been attributed to the ability of adiponectin to decrease adhesion molecule expression on ECs, to inhibit foam cell formation and VSMC proliferation/migration and to exert anti-inflammatory effects on macrophages. Full-length adiponectin has been shown to inhibit TNF- α —induced adhesion molecule expression on ECs, including VCAM-1, ICAM-1 and E-selectin and to suppress adhesion of THP-1 monocytes on ECs (Ouchi et al., 1999).

In addition, adiponectin directly stimulates NO production in ECs and protects ECs from apoptosis, thus contributing to the antiatherogenic activities of this adipocytokine (Chen et al., 2003; Kobayashi et al., 2004). Adiponectin inhibits macrophage to foam cell transformation by reducing the expression of class A scavenger receptor (SR-A), thus providing a mechanism for the link between obesity and insulin resistance and CAD (Ouchi et al., 2001; Furukawa et al., 2004). Moreover, adiponectin inhibits PDGF-BB—induced migration and proliferation of VSMCs through direct binding to PDGF-BB and suppression of growth factor-induced MAP kinase signaling (Arita et al., 2002). In addition, adiponectin may also be involved in plaque stability through increasing TIMP expression and secretion in human monocyte-derived macrophages (Kumada et al., 2004), which may affect development and progression of atherosclerosis.

Previous studies have shown that both globular and full-length adiponectin attenuates the development of atherosclerosis in genetically prone mouse models, despite similar plasma glucose and lipid levels. Adenovirus-mediated human full-length adiponectin administration to apoE^{-/-} mice significantly

attenuated atherosclerotic lesion formation compared to control mice (Okamoto et al., 2002). Adenovirus-synthesized adiponectin associated with foam cells in the fatty streak lesions and suppressed VCAM-1 and SR-A mRNA expression (Okamoto et al., 2002). Similarly, apoE^{-/-} mice overexpressing globular adiponectin showed an amelioration of atherosclerotic lesion formation, which was associated with decreased SR-A and TNF- α expression (Yamauchi et al., 2003a,b).

In humans, decreased plasma adiponectin levels are independently associated with the prevalence of CAD and predict development of atherosclerosis in both nondiabetic and type 1 diabetic patients, independently of other cardiovascular risk factors (Kumada et al., 2003; Maahs et al., 2005). In a large case-control study among men without any evidence of CAD at the time of the blood draw, Pischon et al. demonstrated that high plasma adiponectin concentrations were associated with decreased risk of myocardial infarction (Pischon et al., 2004).

In addition to the effect on vascular cells, adiponectin has been shown to increase fatty acid oxidation in skeletal muscle and to augment insulin-induced inhibition of glucose production in hepatocytes, thereby reducing plasma glucose levels (Berg et al., 2001; Yamauchi et al., 2002; Fruebis et al., 2001). Studies with adiponectin knockout mice showed impaired insulin sensitivity, further providing evidence for a role of adiponectin as an endogenous insulin sensitizer (Maeda et al., 2002; Kubota et al., 2002).

Numerous studies in humans demonstrated an association between low adiponectin levels and insulin resistance and type 2 diabetes, regardless of the obesity status of the individual subject (Kern et al., 2003; Abbasi et al., 2004). Moreover, in a study of Pima Indians, individuals with high adiponectin levels were shown to less likely develop type 2 diabetes than those with low adiponectin levels (Lindsay et al., 2002). In accordance with this observation, studies in different populations demonstrated that low plasma adiponectin levels are an independent predictor of future development of insulin resistance and type 2 diabetes, suggesting that adiponectin might play a central role in the development of type 2 diabetes (Snehalatha et al., 2003; Yamamoto et al., 2004; Spranger et al., 2003).

Previous studies demonstrated that TZD treatment increased adiponectin levels in rodents both in vivo and in vitro (Maeda et al., 2001; Combs et al., 2002). Administration of metformin or PPAR- α agonists had no effect on adiponectin levels, suggesting that the effect of PPAR- γ ligands on adiponectin levels are not just a consequence of an improved metabolic phenotype. In accordance, TZD treatment of cultured 3T3-L1 adipocytes increased expression and secretion of adiponectin (Maeda et al., 2001). In addition, Iwaki et al. recently identified a functional PPAR-response element in the human adiponectin promoter, thus demonstrating a direct effect of PPAR- γ on adiponectin transcription (Iwaki et al., 2003). Moreover, human studies have shown the beneficial effect of TZD treatment on adiponectin levels in a variety of clinical settings. One report in lean, obese, and type 2 diabetic subjects showed that troglitazone treatment increased plasma adiponectin levels in all three groups, even in normal subjects in whom no other TZD effects were observed

(Yu et al., 2002). An increase in adiponectin levels in type 2 diabetics was also demonstrated by the administration of rosiglitazone and pioglitazone over a period of 2 to 6 months (Boden et al., 2003; Wallace et al., 2004; Yang et al., 2002; Hirose et al., 2002; Miyazaki et al., 2004; Osei et al., 2004). In addition, short-term treatment with pioglitazone (3 weeks) increased adiponectin serum levels and mRNA expression in adipocytes and improved insulin sensitivity in insulin-resistant and in type 2 diabetic subjects, while no effect was seen on plasma free fatty acids or glucose levels (Hammarstedt et al., 2005; Tonelli et al., 2004). In pioglitazone-treated type 2 diabetics, Satoh et al. found an increase in plasma adiponectin concentrations in both responders and nonresponders, classified with respect to its antidiabetic effect (Satoh et al., 2003). These data indicate a beneficial effect of TZDs on adiponectin levels, independently of their antidiabetic properties. Recently, rosiglitazone was found to induce AdipoR2 expression in primary and THP-1 macrophages, providing a novel mechanism by which PPAR- γ agonists affect the adiponectin pathway (Chinetti et al., 2004).

In conclusion, these data indicate that pharmacological modulation of adiponectin levels might offer new treatment options for cardiovascular disease. TZD treatment might therefore reduce the increased risk of cardiovascular disease associated type 2 diabetes, at least in part through the effect of these agents on adiponectin levels.

6. ATP-binding cassette transporter A1 (ABCA1)

ABCA1 is a member of the ATP-binding cassette (ABC) transporter family, which is involved in the control of cholesterol efflux from macrophages and HDL assembly (Lee and Parks, 2005; Oram and Lawn, 2001). A key step in the formation of the HDL particle is the transport of cholesterol to the plasma membrane where it becomes available for desorption onto lipid-poor apoA-I particles, the precursors of HDL. Absence or defects in the ABCA1 gene result in decreased cellular cholesterol efflux and formation of cholesterol-depleted HDL particles that are rapidly catabolized (Marcil et al., 1999). Mutations in the ABCA1 gene cause a severe HDL deficiency syndrome in humans, known as Tangier disease. These patients are characterized by accumulation of cholesterol esters in peripheral tissues due to the cellular defect in HDL apolipoprotein-mediated cholesterol efflux, resulting in splenomegaly, hepatomegaly, neuropathies and CAD (Brooks-Wilson et al., 1999; Francis et al., 1995).

Selective inactivation of ABCA1 in macrophages of both LDLR^{-/-} and apoE^{-/-} mice significantly increased atherosclerosis in the absence of changes in plasma lipid profiles, demonstrating the antiatherogenic properties of ABCA1 (Aiello et al., 2002). However, complete ABCA1 knockout mice on a LDLR^{-/-} or apoE^{-/-} background had significantly lower plasma cholesterol levels, with no difference in atherosclerosis compared to control mice. These findings suggest that the atherogenic effect resulting from ABCA1 deficiency was compensated for by a less atherogenic lipid profile (Aiello et al., 2002).

ABCA1, known to be ubiquitously expressed, has been suggested to be regulated both transcriptionally and post-transcriptionally (Wellington et al., 2002). In macrophages, ABCA1 has been shown to be regulated at the transcriptional level by acetylated LDL, cAMP analogs and sterols (Langmann et al., 1999; Oram et al., 2000; Costet et al., 2000). In addition, Wang and Oram found that unsaturated fatty acids reduce macrophage ABCA1 content by enhancing its degradation (Wang and Oram, 2002). These results have important clinical implications, since one of the lipid abnormalities in type 2 diabetes is elevated FFAs (Reaven et al., 1988). Therefore, impaired ABCA1-mediated cholesterol efflux from macrophages might contribute to premature and accelerated atherosclerosis in type 2 diabetic patients.

Modulating lipid accumulation or efflux from the arterial wall has the potential to affect the progression of atherosclerotic lesions. Previous work has shown that PPAR- γ activators transcriptionally induce the expression of the macrophage scavenger receptor CD36, indicating an important role of PPAR- γ in cholesterol trafficking (Tontonoz et al., 1998). These data indicated that PPAR- γ might promote foam cell formation and the development of atherosclerosis, raising serious concerns about the potential long-term side-effects of TZDs in the treatment of type 2 diabetes. However, recent studies revealed a complex mechanism of lipid metabolism regulation by PPAR- γ , indicating that PPAR- γ modulates both uptake and efflux of cholesterol. Although PPAR- γ ligands increase CD36 expression, there is a compensatory decrease in macrophage-scavenger receptor class A expression, resulting in no substantial increase in cholesterol uptake (Moore et al., 2001).

In addition, previous *in vitro* studies demonstrated that PPAR- γ agonists induce cholesterol efflux from human macrophages and macrophage-derived foam cells through stimulation of the ABCA1 pathway (Chinetti et al., 2001). The effect of PPAR- γ agonists is possibly mediated through their inductive effect on liver X receptor alpha (LXR α) expression, as ABCA1 transcription has been shown to be induced by LXR ligands and LXR α is a direct target gene of PPAR- γ (Costet et al., 2000; Schwartz et al., 2000; Chawla et al., 2001). In contrast, Li et al. reported, that PPAR- γ agonists did not induce ABCA1 expression in peritoneal macrophages isolated from hypercholesterolemic LDLR^{-/-} mice or within the artery wall of animals with extensive atherosclerotic lesions (Li et al., 2004). The authors of this study suggested that one possible explanation for the discrepancy between *in vitro* and *in vivo* results might be that ABCA1 is already maximally expressed in the setting of extreme hyperlipidemia. Consistent with this hypothesis, ABCA1 expression was found to be upregulated in response to PPAR- γ ligand treatment at early time points after initiation of a high-cholesterol diet (Li et al., 2004). In addition, this study demonstrated that PPAR- γ ligands induced expression of the ABC transporter ABCG1 in macrophages and within the vessel wall. Previous studies demonstrated a direct involvement of ABCG1 in cholesterol and phospholipid transport in macrophages (Klucken et al., 2000).

Utilizing PPAR- γ -null macrophages revealed that LXR ligand induced ABCA1 and ABCG1 mRNA expression was reduced, and that the ability to stimulate cholesterol efflux was completely abolished in these macrophages. This model is supported by transplantation of PPAR- γ -null bone marrow into atherosclerosis-prone LDLR^{-/-} mice, resulting in a significant increase in atherosclerosis (Chawla et al., 2001). These results are consistent with the hypothesis that PPAR- γ signaling is protective in vivo through stimulation of reverse cholesterol transport. This concept is supported by a variety of animal studies, demonstrating an inhibitory effect of TZDs on lesion development—despite the ability of PPAR- γ ligands to induce CD36 expression in macrophages (Li et al., 2000; Minamikawa et al., 1998; Chen et al., 2001).

In conclusion, these data have important implications for therapeutic approaches to prevent and treat cardiovascular diseases in humans. Based on their ability to stimulate ABCA1 expression, TZDs might exert an antiatherogenic effect by modulating cholesterol efflux in macrophages independently of their antidiabetic properties.

7. The clinical significance of TZDs — results from the PROactive study

The vascular effects of TZDs and their beneficial activity against multiple proinflammatory and prothrombotic factors provide a compelling rationale for conducting cardiovascular outcomes trials with these oral antidiabetic agents.

Results from the first TZD cardiovascular outcomes trial, PROactive, were recently published (Dormandy et al., 2005). PROactive was a prospective, double-blind, randomized, placebo-controlled, secondary prevention study in 5238 type 2 diabetes subjects who were at high risk for cardiovascular events. Subjects received either oral pioglitazone, force titrated from 15 mg/daily to 45 mg/daily or placebo in addition to their existing glucose-lowering drugs, lipid modulators, anti-hypertensive agents, and other cardiovascular medications. Insulin usage as sole antidiabetic therapy or prior TZD usage were key exclusion criteria for the study. Subjects receiving pioglitazone had a 10% relative risk reduction, which did not achieve statistical significance, at study end after 3 years, in the primary composite endpoint of time to first occurrence of one of the following: all-cause mortality, non-fatal myocardial infarction (including silent myocardial infarction), stroke, acute coronary syndrome, leg or coronary artery revascularization, and amputation above the ankle. For the main secondary composite endpoint of time to first occurrence of all cause mortality, myocardial infarction (excluding silent myocardial infarction) or stroke, subjects treated with pioglitazone benefited from a statistically significant 16% relative risk reduction compared to placebo. This cardioprotective effect of pioglitazone was observed even though there was a 3% increase in the reported incidence of heart failure compared to placebo (no difference in fatal heart failure between arms).

Were any of the mechanisms discussed in this review responsible for the vascular protective effects of pioglitazone

manifested in PROactive? This is difficult to determine, as PROactive was not designed to elucidate pioglitazone's mechanism of action or that of TZD PPAR- γ ligands in general. In PROactive, pioglitazone decreased HbA1c by 0.5%, increased HDL by 9%, reduced triglycerides by 12%, and lowered systolic blood pressure by 3 mm Hg compared to placebo. The impact of pioglitazone on this set of well-recognized cardiovascular risk factors alone or in combination with its pleiotropic vascular effects could explain the findings in PROactive.

Are the results of PROactive translatable to all TZD PPAR- γ ligands? Although this question cannot be definitively answered, it is important to remember that PPAR- γ is a ligand-activated transcription factor in the nuclear hormone receptor superfamily. Ligand activation of PPAR- γ induces a conformational change in the nuclear receptor, resulting in the displacement of corepressors and recruitment of coactivators required for the transcription of PPAR- γ -regulated genes. Differential recruitment of coactivators and concomitant distinct patterns of gene regulation have been observed in comparing troglitazone to rosiglitazone (Camp et al., 2000). Each TZD PPAR- γ ligand, therefore, can be considered to be a unique selective PPAR- γ modulator or SPPARM with a distinct profile of target genes regulated compared to every other PPAR- γ ligand (Olefsky, 2000; Hsiao et al., 2004). Among the TZD class, both shared and distinct clinical effects would be predicted reflective of the pattern of genes regulated in common by multiple PPAR- γ ligands, as well as from genes uniquely targeted by a specific TZD. This hypothesis has been proven in a recent head-to-head clinical study comparing the glucose-lowering and lipid effects of pioglitazone and rosiglitazone (Goldberg et al., 2005). While both pioglitazone and rosiglitazone lowered HbA1c to a similar extent, each demonstrated a distinct profile in modulating lipids. Both raised HDL cholesterol, although pioglitazone's effect was greater (12% versus 7%). Effects on triglyceride levels were markedly different, with pioglitazone lowering them by 12%, while rosiglitazone treatment led to a 14% increase in levels. Differences in the average size and number of atherogenic small dense LDL cholesterol particles were also observed between the two TZDs, with pioglitazone eliciting a more favorable profile.

TZDs ameliorate insulin resistance, implement better glycemic control, and improve lipids in persons with type 2 diabetes who are at elevated risk for cardiovascular disease. The anti-inflammatory activity of TZDs and their pleiotropic vascular effects may also function to protect the diabetic vasculature. These mechanisms provided a strong scientific rationale for conducting TZD cardiovascular outcomes trials. Results from PROactive provide the first validation of the hypothesis that TZD PPAR γ ligands might reduce diabetes-associated cardiovascular events. Since each PPAR γ ligand has a unique molecular fingerprint that can potentially impact metabolic effects and other clinical parameters, it will be important to conduct outcomes trials with other TZD and non-TZD PPAR γ ligands, as well as dual PPAR α/γ agonists to see if they can replicate the findings of PROactive.

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