Peroxisome proliferator-activated receptor-γ agonists in atherosclerosis: current evidence and future directions

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Purpose of review

The prevalence of type 2 diabetes globally is reaching epidemic proportions. Type 2 diabetes is strongly associated with increased risk of cardiovascular disease. Atherosclerosis is thought to arise as a result of a chronic inflammatory process within the arterial wall. Insulin resistance is central to the pathogenesis of type 2 diabetes and may contribute to atherogenesis, either directly or through associated risk factors. The peroxisome proliferator-activated receptor-γ agonists, the thiazolidinediones, pioglitazone and rosiglitazone, are insulin sensitizing agents, that are licensed for the management of hyperglycaemia. Growing evidence supports an array of additional effects of thiazolidinedione therapy, both immunomodulatory and antiinflammatory, which may attenuate atherogenesis in type 2 diabetes.

Recent findings

Studies have shown that thiazolidinedione therapy may lead to risk factor modulation in type 2 diabetes. Thiazolidinediones treatment has been shown to reduce blood pressure, modify the atherogenic lipid profile associated with type 2 diabetes, reduce microalbuminuria and ameliorate the prothrombotic diathesis. Further evidence suggests that thiazolidinediones therapy inhibits the inflammatory processes which may be involved in atherosclerotic plaque initiation, propagation and destabilization.

Summary

Modification of insulin resistance by thiazolidinedione therapy in type 2 diabetes and the range of pleiotropic effects may not only impact on incident type 2 diabetes, but also on associated cardiovascular disease. Numerous large clinical endpoint studies are under way to investigate these issues.

Keywords

atherosclerosis, PPARγ, thazolidinediones, rosiglitazone, pioglitazone, troglitazone

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Abbreviations

BARI-2D	Bypass Angioplasty Revascularisation Investigation 2 Diabetes
CRP	C-reactive protein
MMP	metalloproteinase
PAI-1	plasminogen activator inhibitor type 1
PPAR	peroxisome proliferator-activated receptor
T2D	type 2 diabetes
VCAM-1	vascular cell adhesion molecule-1
VSMC	vascular smooth muscle cells

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Introduction

Type 2 diabetes (T2D) confers an increased risk of cardiovascular disease of two to four times that of the general population [1,2]. The UK Prospective Diabetes Study confirmed a significant prevalence of cardiovascular disease at diagnosis of T2D [3]. Insulin resistance and β-cell dysfunction are central to the pathogenesis and progression of T2D. Insulin resistance is an independent predictor of the disease [4], and also independently associates with cardiovascular disease in T2D [5••]. Insulin resistance is a cardinal feature of the metabolic syndrome, and T2D patients with insulin resistance have a pro-atherogenic cardiovascular risk profile [6].

Treatment of insulin resistance thus represents an important target with respect to reducing the incidence of T2D, together with cardiovascular disease risk reduction. Metformin exerts modest insulin sensitizing effects, metformin monotherapy in the UK Prospective Diabetes Study demonstrating significant benefits on cardiovascular outcome [7].

Furthermore, metformin therapy has beneficial effects on markers of cardiovascular risk, but the mechanisms by which metformin may influence atherogenesis are not fully elucidated [8•].

Peroxisome proliferator-activated receptor (PPAR)γ is a nuclear receptor with a pivotal role in cell metabolism, in particular in relation to adipogenesis and lipid metabolism [9]. The thiazolidinediones, pioglitazone (Takeda) and rosiglitazone (GlaxoSmithKline), are PPARγ agonists currently licensed for the management of hyperglycaemia in T2D. Troglitazone, the first thiazolidinedione made available to world markets, has been withdrawn due to reports of hepatic toxicity. The thiazolidinediones improve insulin sensitivity by complex mechanisms, and

facilitate glucose disposal primarily by enhanced glucose uptake in subcutaneous adipose tissue [10••].

Insulin resistance can be decreased by 78% by thiazolidinedione treatment [11]. Consequently, thiazolidinediones may improve cardiovascular risk through beneficial effects on insulin resistance, and modification of associated risk factors. In particular, atherosclerosis is increasingly recognised as an inflammatory diathesis [12], and considerable interest has been generated by the observations that PPARγ activation may modulate the inflammatory process.

Atherogenesis is believed to be initiated by passage of lipid through the vascular endothelium, and deposition in the arterial intima, where it stimulates an inflammatory response. The subsequent immune response, monocyte chemotaxis, T-cell activation and vascular smooth muscle cell migration, forms the fundamental lesion of atherosclerosis: the atherosclerotic plaque. Destabilization of this lesion relates to an ongoing inflammatory diathesis, causing acute thrombosis, and resulting in clinical cardiovascular events.

The antiinflammatory properties of thiazolidinediones, in addition to their metabolic effects, may confer further cardiovascular benefits. At present there is a lack of definitive outcome evidence to support this hypothesis, although large, endpoint studies are under way. There is, however, considerable experimental data, and small clinical studies, to support the cardiovascular benefits of thiazolidinedione therapy.

Peroxisome proliferator-activated receptor-γ vascular effects

In the context of insulin resistance and T2D, dyslipidaemia is characterized by modification of plasma lipids, mainly through the processes of oxidation and glycation. Thus, the predominant lipoprotein moiety involved in the initiation of atherogenesis is likely to be modified. The inflammatory response initiated by these modified lipids, considered to be a consequence of nuclear factor $\kappa\beta$ activation, leads to the increased expression of vascular cell adhesion molecule-1 (VCAM-1) on the overlying endothelial cells [13]. Together with increased expression of intercellular adhesion molecule-1, this leads to monocyte and T-lymphocyte recruitment to the vascular wall. PPARγ agonists have been shown to reduce endothelial expression of VCAM-1 [14] and intercellular adhesion molecule-1 [15], on activated endothelium. Thus, PPARγ agonists, through modulating inflammation, may influence the early stages of atherosclerosis.

Once the monocyte is adherent to the endothelial wall a sequence of events causes its diapedeses through the endothelium. This process occurs along a gradient of chemoattractant cytokines, which facilitate recruitment of leukocytes into the intima. One of these chemokines, monocyte chemoattractant protein-1, is overexpressed in developing atheromatous plaques, and plays a role in leukocyte recruitment [16]. Other candidates for the role of chemoattractants in atherogenesis are interleukin-8, and the CXC chemokines induced by interferon-γ. It was recently demonstrated that pioglitazone has direct antiinflammatory effects in a rat model of atherosclerosis via interference with monocyte chemoattractant protein-1, and its monocyte receptor, CCR2 [17•]. A rat model of long-term inibition of nitric oxide synthesis was used in this study, and with a pioglitazone dose equivalent to that in humans the investigators demonstrated significantly decreased levels of CCR2 in lesional and circulating monocytes. The reduced signalling between monocyte chemoattractant protein-1 and CCR2 may explain the reduced entry of monocyte/macrophages into the intima, and the observed reduction in coronary atherosclerosis in this study. These data are in accordance with those from a similar study in a mouse model of atherosclerosis, using rosiglitazone [18].

T lymphocytes also bind to VCAM-1, and atherosclerotic plaques are associated with large numbers of activated T-cells [19–21]. Major histocompatibility complex class II molecules are involved in antigen presentation to T lymphocytes thus provoking an immune response. The histocompatibility complex molecules are expressed in endothelial cells, macrophages and vascular smooth muscle cells (VSMCs) in proximity to activated T lymphocytes in the atherosclerotic plaque [22,23]. PPARγ ligands have been demonstrated to downregulate major histocompatibility complex class II expression, induced by interferon-γ, in atheroma-associated cells [24•]. This may result in suppression of CD4+ T lymphocyte activation and proliferation in the atherosclerotic plaque, thus attenuating the immune response to modified lipids in the arterial intima.

As monocytes enter the intima they undergo transformation into macrophages. Macrophages take up lipid deposited in the intima via a number of receptors, including scavenger receptor-A, and CD 36. Scavenger receptors are not subject to negative control by lipids, and hence the macrophage may become lipid saturated, forming the 'foam cell'. Foam cells may play a pivotal role in atherogenesis, secreting reactive oxygen species, inflammatory cytokines, matrix metalloproteinases (MMPs) and tissue factor into the local matrix.

PPARγ is upregulated in activated macrophages [25,26], while PPARγ agonists have been shown to attenuate the inflammatory response in activated monocytes and macrophages. Thus activation of PPARγ receptors in

macrophages within the arterial intima may reduce cytokine production, limiting the local inflammatory response, hence arresting atherogenesis.

However, subsequent work has cast a different light on the role of PPARγ in the developing atherosclerotic plaque. Oxidized LDL, and macrophage colony stimulating factor have been shown to induce expression of PPARγ [27]. Moreover, PPARγ activation stimulates binding and uptake of oxidized LDL, and facilitates its entry into the cell by upregulating transcription at the gene encoding for the CD36 receptor [28]. Thus PPARγ activation by ligands such as oxidized LDL could be driving the inflammatory process. Furthermore, given that PPARγ agonism by oxidized LDL stimulates CD36, a means by which oxidized LDL enters the cell, then theoretically a feedforward loop may be created.

However, Chawla *et al.* [29] showed that PPARγ agonism may upregulate the cholesterol efflux pathway involving the ATP-binding cassette protein A1 [29]. Thus PPARγ activation may stimulate the means of influx, and efflux, of lipid from the macrophage, and not favour foam cell formation. Theoretically, therefore, there may be a synergistic role for thiazolidinediones and novel ATP-binding cassette transporter protein A1 lipid modifying drugs under development.

Plaque progression

Plaque progression occurs with the recruitment of VSMCs from the media to the intima. VSMCs contribute to the formation of a fibrous cap, rich in collagen fibrils overlying the conglomeration of foam cells. Some foam cells may undergo apoptosis, leading to the formation of a lipid rich core within the lesion. Small vessels may be stimulated to grow into the lesion providing it with its own blood supply. It is thought that further plaque progression is dynamic, occurring by one of three processes: (1) endothelial cell injury and exposure of collagen and von Willebrand factor to the systemic circulation; (2) disruption of the vascular supply within the plaque leading to haemorrhage and release of potent growth factors and other blood-borne contents; and (3) rupture of the fibrous cap overlying the lesion and exposure of the thrombogenic contents of the lipid-rich core to the systemic circulation.

PPARγ agonists have been shown to inhibit tube formation induced by vascular endothelial growth factor *in vitro* and *in vivo* [30]. They thereby reduce the possibility of intra-lesional micro-haemorrhage from these friable vessels, which may be potent stimulators of plaque extension, through the release of such growth factors as thrombin, platelet derived growth factor and transforming growth factor-β, a potent stimulus of extracellular matrix production by VSMCs. Thus PPARγ activation may inhibit plaque progression.

PPARγ receptors have been identified in VSMCs at the site of atherosclerotic plaques, and current evidence suggests that PPARγ agonists may inhibit VSMC migration and proliferation [31]. This group used troglitazone and rosiglitazone, in human VSMCs, and demonstrated inhibition of platelet derived growth factor-induced VSMC migration. These data are consistent with previous studies with troglitazone in rat VSMCs [32].

The activity of MMPs has engendered much interest in recent years, especially in relation to atherosclerotic plaque stability. The MMPs are secreted from vascular cells and play an important part in the degradation of extracellular matrix. The action of MMPs is antagonized by the tissue inhibitors of metalloproteinases 1 and 2. It is known that MMPs co-localize in the shoulder regions of atherosclerotic plaques (the area where the fibrous cap meets vascular endothelium), as well as in the plaque core and microvasculature [33]. It is the uncontrolled activity of MMPs, particularly at the shoulder regions of the plaque, which may weaken the structure of the fibrous cap, creating a lesion prone to rupture. MMP-9, also known as gelatinase B, appears particularly important in migration of VSMCs after arterial injury. Clinical studies of rosiglitazone treatment in T2D patients have demonstrated a reduction in serum concentrations of MMP-9 [34,35]. Troglitazone has been shown to suppress MMP-9 expression, and gelatinase activity in human VSMCs [36], and also in human macrophages [37]. PPARγ agonists, in the first study at least, did not have any effect on tissue inhibitor of metalloproteinase expression, thus favouring the preservation of the extracellular matrix, maintaining arterial structure, inhibiting migration of VSMCs, and possible stabilization of the atherosclerotic lesion.

Clinical evidence

The potential clinical benefits of these effects on VSMC proliferation were recently illustrated by Choi *et al.* [38••]. Coronary artery stent restenosis rates are significantly higher in T2D patients than in non-T2D subjects. This study assessed the degree of restenosis after coronary artery stent insertion in 100 T2D patients, 45 of whom had rosiglitazone in addition to their pre-study medication. A significant reduction in stent restenosis was seen in the rosiglitazone treated patients, along with significantly reduced high-sensitivity C-reactive protein (CRP) levels, independent of glycaemic control. The effect of rosiglitazone treatment in this study may thus be a manifestation of its antiinflammatory effects in the vessel wall. These data may provide support for the potential benefits of thiazolidinedione

therapy in the ongoing Bypass Angioplasty Revascularisation Investigation 2 Diabetes (BARI-2D) study [39].

Thiazolidinedione therapy with rosiglitazone for 26 weeks in T2D has been shown to reduce serum levels of MMP-9 and CRP [34], which may reflect a direct antiinflammatory effect on the vascular endothelium, or metabolic effects. Since raised CRP levels are now recognized in association with increased cardiovascular risk [40•], the effects of thiazolidinedione therapy on CRP suggest potential cardiovascular benefits of the treatment. However the effects of CRP lowering on cardiovascular outcome have yet to be elucidated.

Carotid intimal thickness is a surrogate marker of atherosclerotic risk, and has been linked to incidence and prevalence of coronary heart disease [41–45] and stroke [42,44,45]. Pioglitazone therapy of 6 months' duration has been shown to significantly reduce carotid intima thickness in the treatment of T2D [46], further suggesting the inhibitory potential of T2D on atherosclerosis.

Endothelial dysfunction is an early, potentially reversible event in the development of cardiovascular disease [47]. Endothelial dysfunction may facilitate the passage of lipid through the vascular wall into the intimal space, which is thought to be one of the earliest stages in the development of atherosclerosis [48]. Troglitazone in a number of small studies has been shown to reverse endothelial dysfunction in T2D [49,50]; and rosiglitazone, in a small study of 93 patients randomized to placebo, metformin or rosiglitazone, was shown to improve endothelial dysfunction [51]. Furthermore, treatment with troglitazone has reversed the endothelial dysfunction associated with the polycystic ovarian syndrome [52], an insulin resistant state.

Microalbuminuria is an indicator of incipient diabetic nephropathy in T2D [53], but increasingly is being seen as a potent, early indicator of cardiovascular disease [54,55]. It has been shown that rosiglitazone produces a significant, sustained reduction in microalbuminuria, independent of glycaemic control, when compared with the sulphonylurea glyburide [56••]. Improvements in microalbuminuria occurred in patients without overt microalbuminuria, and in those with established microalbuminuria. These effects may relate to both the blood pressure lowering properties of thiazolidinediones as well as potential effects on vascular function. Small numbers of patients ($n = 203$) were involved in this open-labelled study, designed as a cardiac safety study, but the results suggest that PPARγ agonists have sustained, beneficial effects on the vasculature and endothelial cell function.

Blood pressure lowering has been demonstrated in mammalian models of diabetes treated with PPARγ agonists [57]. Both pioglitazone and rosiglitazone were found to lower angiotensin II induced hypertension, reduce markers of inflammation, correct structural abnormalities in the vessel wall, and improve endothelial function in a rat model of hypertension. Modest effects on blood pressure with thiazolidinedione treatment in humans have been described, as evidenced by a superior reduction in diastolic blood pressure with rosiglitazone as compared with glyburide [58]. The blood pressure lowering effects of thiazolidinediones in humans require further studies to elaborate on these data.

Endothelin-1 is a potent vasoconstrictor, as well as a mediator of smooth muscle mitogenesis, and thus a determinant of systemic blood pressure [59]. Thiazolidinedione treatment has been shown to attenuate the release of endothelin-1 from bovine vascular cells, which may contribute to the observed blood pressure lowering effect with these drugs [60]. Coupled with this is the modest blood pressure lowering effect demonstrated in a rat model of hypertension [61]. Rosiglitazone in this study suppressed endothelin-1 production, reduced markers of inflammation, and improved endothelial function.

Acute thrombosis, for instance during plaque rupture in a coronary artery leading to myocardial infarction, might, in part, be due to an imbalance of pro and antithrombotic factors in plasma. Plasminogen activator inhibitor type 1 (PAI-1) inactivates tissue plasminogen activator in plasma, thus creating a pro-thrombotic milieu. PAI-1 levels are increased in insulin resistance and T2D [62], and elevated PAI-1 levels have been linked to an increased risk of myocardial infarction [63]. In human vein endothelial cells, cultured with tumour necrosis factor-α to induce elevation of PAI-1, pioglitazone reduces PAI-1 levels [64], as does rosiglitazone [65]. Thiazolidinedione therapy may redress the balance of the coagulation system in favour of thrombolysis, normalizing the pro-thrombotic milieu observed in the metabolic syndrome.

The typical lipid profile of T2D is characterized by low HDL, hypertriglyceridaemia, and the generation of atherogenic, small, dense LDL-cholesterol particles, which are prone to oxidation and glycation [66]. Data on file from Takeda show that a 30 mg dose of pioglitazone for 26 weeks results in triglyceride lowering of 9.6%, an HDL increase of 12%, and a neutral effect on LDL and total cholesterol (pioglitazone data sheet). Data for rosiglitazone, in combination with metformin at a dose of 8 mg for 26 weeks, showed an HDL increase of 13.3%, LDL increase of 18.6% and a neutral effect on total cholesterol and the total cholesterol to HDL ratio [67].

Low HDL cholesterol is increasingly recognized as a cardiovascular risk factor at any given level of total cholesterol or LDL-C [68]. The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) demonstrated an 11% risk reduction in coronary heart disease events with each 0.13 mmol/l increase in HDL [69]. Thus the HDL elevating properties of thiazolidinediones therapy may further add to their putative cardiovascular benefits.

In a study comparing the thiazolidinediones in patients taken off troglitazone, treatment with rosiglitazone and pioglitazone increased HDL levels by approximately 2% [70]. This study also suggested that rosiglitazone and pioglitazone altered lipoprotein metabolism, with triglyceride depletion of lipoprotein resulting in the generation of larger less dense particles. This was confirmed in recent data [71].

Since triglyceride-rich lipoprotein particles, in particular small, dense LDL cholesterol, are associated with increased cardiovascular risk, endothelial dysfunction, enhanced inflammation and oxidative stress, the lipoprotein effects of the thiazolidinediones may account for potential cardiovascular benefits, as well as contributing to the suggested anti-inflammatory effects.

However, two important features of the lipid effects of the thiazolidinediones must be considered. Firstly, there are no clear data relating to the differential effects of rosiglitazone versus pioglitazone on lipid metabolism. It is possible that differences in PPARγ binding domain activity, or differential PPARγ activation, may result in differing effects of the thiazolidinediones on plasma lipoproteins. Direct comparative studies are however required to evaluate these issues. Secondly, no long-term clinical study data are available to assess the lipid effects of the thiazolidinediones, and thus the effects on long-term cardiovascular risk are unclear.

Conclusion

Thiazolidinedione therapy appears to result in a variety of effects independent of blood glucose lowering which may have the potential to revolutionize cardiovascular risk management in T2D. Although there are considerable experimental data supporting the cardiovascular benefits of thiazolidinediones, there is at present a shortage of clinical outcome data. A number of large randomized studies are under way to evaluate this theme including PROACTIVE (Prospective Pioglitazone Clinical Trial in Macrovascular Events), RECORD (Rosiglitazone evaluated for cardiac outcomes and regulation of glycaemia in diabetes), ADOPT (A diabetes outcomes progression trial), ACCORD (Action to control cardiovascular risk in diabetes), PPAR (PPARγ agonists for the prevention of late adverse events following percutaneous revascularisation), and BARI-2D.

The thiazolidinediones, by improving insulin sensitivity, along with the additional pleiotropic effects, in particular immunomodulatory and antiinflammatory, may have a powerful impact on the cardiovascular disease burden associated with T2D.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Kannnel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham Study. Diabetes Care 1979; 2:120–126.

2 Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12 year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; 16:434–444.

3 UKPDS Group. UK Prospective Diabetes Study 6: Complications in newly diagnosed type 2 diabetic patients and their association with different clinical and biochemical risk factors. Diabetes Res 1990; 13:1–11.

4 Lillioja S, Mott DM, Spraul M, *et al.* Insulin Resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. N Engl J Med 1993; 329:1988–1992.

5 •• Bonora E, Formentini G, Calcaterra F, *et al.* HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects. Diabetes Care 2002; 25:1135–1141.
This study related insulin resistance to increased risk of cardiovascular disease in a large cohort of patients studied prospectively. The paper also discusses some possible mechanisms by which hyperinsulinaemia may result in atherosclerosis.

6 Haffner S, D'Agostini R, Mykkanen L, *et al.* Insulin sensitivity in subjects with type 2 diabetes. Diabetes Care 1999; 22:562–568.

7 UK Prospective Study Group. Effects of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998; 352:854–865.

8 • Kirpichnikov D, McFarlane S, Sowers J. Metformin: an update. Ann Intern Med 2002; 137:25–31.
This is an excellent review of metformin, in particular in relation to possible beneficial effects on cardiovascular risk.

9 Debril M-B, Renaud J-P, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor γ. J Mol Med 2001; 79:30–47.

10 •• Berger J, Moller D. The mechanism of action of PPARs. Annu Rev Med 2002; 53:409–435.
This is a comprehensive review of PPAR mechanisms of action including discussion of some of the pleiotropic effects of PPARγ activation.

11 Carey D, Cowin G, Galloway G, *et al.* Rosiglitazone increases insulin sensitivity and reduces factors associated with insulin resistance in type 2 diabetics. Diabetes Res Clin Pract 2000; 50 (Suppl 2):S64.

12 Libby P. Inflammation in atherosclerosis. Nature 2002; 420:868–874.

13 Iiyama K, Hajra L, Iiyama M, *et al.* Patterns of VCAM-1 and ICAM-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. Circ Res 1999; 85:199–207.

14 Pasceri V, Wu H, Willersen J, Yeh E. Modular of vascular inflammation in vitro and in vivo by peroxisome proliferator activated receptor-γ activators. Circulation 2001; 101:235-238.

15 Wang N, Verna L, Chen N-G, Chen J, *et al.* Constitutive activation of peroxisome proliferator-activated receptor-γ suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. J Biol Chem 2002; 277:34176–34181.

16 Gu L, Okada Y, Clinton SK, Gerard C, *et al.* Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998; 2:275–281.

17 Ishibashi M, Egashira K, Hiasa K, Inoue S. Antiinflammatory and antiarterio-
• sclerotic effects of pioglitazone. Hypertension 2002; 40:687–693.
Much research is focussed at this early stage of atherogenesis; PPARγ activation appears to be effective in preventing monocyte adhesion to the vascular endothelium which is expressing adhesion molecules, thus inhibiting atherogenesis at an early stage.

18 Han K-H, Chang MK, Boullier A, *et al.* Oxidized LDL reduces monocyte CCR2 expression through pathways involving peroxisome proliferator-activated receptorγ. J Clin Invest 2000; 106:793–802.

19 Hansson G, Holm J, Jonasson L. Detection of activated T lymphocytes in the human atherosclerotic plaque. Am J Pathol 1989; 135:169–175.

20 Van der Wal AC, Das PK, Bentz, van de Berg D, *et al.* Atherosclerotic lesions in humans: in situ immunophenotypic analysis suggesting an immune mediated response. Lab Invest 1989; 61:166–170.

21 Zhou X, Stemme S, Hansson GK. Evidence for a local immune response in atherosclerosis: CD4+ T cells infiltrate lesions of apolipoprotein-E-deficient mice. Am J Pathol 1996; 149:359–366.

22 Jonasson L, Holm J, Skalli O, *et al.* Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. J Clin Invest 1985; 76:125–131.

23 Whitman SC, Ravisankar P, Elam H, Daugherty A. Exogenous interferon-γ enhances atherosclerosis in apolipoprotein $E^{-/-}$ mice. Am J Pathol 2000; 157:1819–1824.

24 Kwak B, Myit S, Mulhaupt F, *et al.* PPARγ but not PPARα ligands are potent
• repressors of major histocompatibility complex class II induction in atheroma-associated cells. Circ Res 2002; 90:356–362.
This paper associates PPARγ activation with suppressed MHC-II, and a postulated reduction in T-cell activity at the site of atherogenesis. Inhibition of T-cell activation may have synergistic effects with the observed antiinflammatory effects of PPARγ activation on monocytes.

25 Jiang C, Ting A, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391:82–86.

26 Ricote M, Li C, Wilson T, *et al.* The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature 1998; 391:79–82.

27 Ricote M, Huang J, Fajas L, *et al.* Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1998; 95:7614–7619.

28 Tontonoz P, Nagy L, Alvarez JGA, *et al.* PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998; 93:241–252.

29 Chawla A, Boisvert WA, Lee C-H, *et al.* A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001; 7:161–171.

30 Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor-γ ligands are potent inhibitors of angiogenesis *in vitro* and *in vivo*. J Biol Chem 1999; 274:9116–9121.

31 Law R, Goetze S, Xi X-P, *et al.* Expression and function of PPARγ in rat and human vascular smooth muscle cells. Circulation 2000; 101:1311–1318.

32 Law R, Meehan W, Xi X-P. Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia. J Clin Invest 1996; 98:1897–1905.

33 Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994; 94:2493–2503.

34 Haffner SM, Greenberg A, Weston WM, *et al.* Effect of rosiglitazone treatment on non-traditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. Circulation 2002; 106:679–684.

35 Marx N, Froehlich J, Siam L. Antidiabetic PPARγ-activator rosiglitazone reduces MMP-9 serum levels in type 2 diabetic patients with coronary artery disease. arteriosclerosis, thrombosis and vascular biology 2003; 23:283–288.

36 Marx N, Schönbeck U, Lazar MA, *et al.* Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998; 83:1097–1103.

37 Marx N, Sukhova G, Murphy C, *et al.* Macrophages in human atheroma contain PPARγ: differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes in vitro. Am J Path 1998; 153:17–23.

38 Choi SH, Choi DH, Ko YK, *et al.* Preventive effects of rosiglitazone on
•• restenosis after coronary stenting in patients with type 2 diabetes. Diabetes 2003; 52 (Suppl 1):A19 [oral presentation].
Basic science theory was tested in a clinical study, which related the possible anti-inflammatory effects of rosiglitazone in the endothelium to real clinical benefits observed as reductions in coronary stent restenosis rates.

39 Sobel BE, Frye R, Detre KM, *et al.* BARI-2D Burgeoning dilemmas in the management of diabetes and cardiovascular disease: rationale for the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) Trial. Circulation 2003; 107:636–642.

40 Ridker PM, Rifai N, Rose L, *et al.* Comparison of C-reactive protein and low-
• density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002; 347:1557–1565.
This provides further evidence in support of the role of inflammation in atherosclerosis.

41 Burke GL, Evans GW, Riley WA, *et al.* Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Stroke 1995; 26:386–391.

42 Bots ML, Hoes AW, Koudstaal PJ, *et al.* Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96:1432–1437.

43 Wofford JL, Kahl FR, Howard GR, *et al.* Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. Arterioscler Thromb 1991; 11:1786–1794.

44 O'Leary DH, Polak JF, Kronmal RA, *et al.* Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. N Engl J Med 1999; 340:14–22.

45 Chambless LE, Folsom AR, Clegg LX, *et al.* Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol 2000; 151:478–487.

46 Koshiyama H, Shimono D, Kuwamuru N, *et al.* Inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes. J Clin Endocrinol Metab 2001; 86:3452–3456.

47 Ross R. The pathogenesis of atherosclerosis:a perspective for the 1990s. Nature 1993; 362:801–809.

48 Ross R. Mechanisms of disease: Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340:115–126.

49 Watanabe Y, Sunayama S, Shimada K, *et al.* Troglitazone improves endothelial dysfunction in patients with insulin resistance. J Atheroscler Thromb 2000; 7:159–163.

50 Caballero AE, Saouaf R, Lim SC, *et al.* The effects of troglitazone, an insulin-sensitising agent, on the endothelial function in early and late type 2 diabetes: a placebo-controlled randomised clinical trial. Metabolism 2003; 52:173–180.

51 Natali A, Baldeweg S, Toschi E, *et al.* Rosiglitazone directly improves endothelial function in type 2 diabetic patients. Diabetes 2002 51(Suppl 2); P-573:A142.

52 Paradisi G, Steinberg HO, Shepard MK, *et al.* Troglitazone therpy improves endothelial function to near normal levels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003; 88:576–580.

53 Bertani T, Gambara V, Remuzzi G. Structural basis of diabetic nephropathy in microalbuminuric NIDDM patients: a light microscopy study. Diabetologia1996; 39:1625–1628.

54 Savage S, Estacio RO, Jeffers B, Schrier RW. Urinary albumin excretion as a predictor of diabetic retinopathy, neuropathy, and cardiovascular disease in NIDDM. Diabetes Care 1996; 19:1243–1248.

55 Mietinnen H, Haffner SM, Lehto S, *et al.* Proteinuria predicts stroke and other atherosclerotic vascular disease events in nondiabetic and non-insulin-dependent diabetic subjects. Stroke 1996; 27:2033–2039.

56 Bakris G, Viberti G, Weston WM, *et al.* Rosiglitazone reduces urinary
•• albumin excretion in type 2 diabetes. J Hum Hypertens 2003; 17:7–12.
Microalbuminuria is increasingly recognized as a marker of cardiovascular risk in type 2 diabetes. Reduction in microalbuminuria, as demonstrated here with rosiglitazone, is discussed as a result of the effects of the thiazolidinediones on blood pressure, vascular function and other mechanisms.

57 Diep QN, Mabrouk M El, Cohn JS. Structure, endothelial function, cell growth, and inflammation in blood vessels of angiotensin II-infused rats: role of peroxisome proliferator–activated receptor-γ. Circulation 2002; 105:2296–2302.

58 Sutton M St J, Rendell M, Dandona P. A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycemic control in patients with type 2 diabetes. Diabetes Care 2002; 25:2058–2064.

59 Miller RC, Pelton JT, Huggins JP. Endothelins: from receptors to medicine. Trends Pharmacol Sci 1993; 2:54–60.

60 Satoh H, Tsukamoto K, Hashimoto Y. Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: a new possible role of PPARγ on vascular endothelial function. Biochem Biophys Res Commun 1999; 254:757–763.

61 Iglarz M, Touyz RM, Amiri F. Effect of peroxisome proliferator-activated receptor-α and -γ activators on vascular remodeling in endothelin-dependent hypertension. Arterioscler Thromb Vasc Biol 2003; 23:45–51.

62 Juhan-Vague I, Roul C, Alessi MC, *et al.* Increased Plasminogen Activator Inhibitor Activity in Non Insulin Dependent Diabetic Patients-Relationship with Plasma Insulin. Thrombosis and Haemostasis 1989; 61(3):370–373.

63 Thögersen AM, JanssonJ-H, Boman K, *et al.* High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998; 98:2241–2247.

64 Kato K, Satoh H, Endo Y, *et al.* Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: a possible role for PPARγ in endothelial function. Biochem Biophys Res Commun 1999; 258:431–435.

65 Chu J, Abbasi F, Lamendola C, *et al.* Improvements in vascular and inflammatory markers in rosiglitazone treated insulin-resistant subjects are independent of changes in insulin sensitivity or glycaemic control. Diabetes 2003; 52 (Suppl 1):A76–A325.

66 Hoeg JM. Lipoproteins and atherogenesis. Endocrinol Metab Clin North Am 1998; 27:569–584.

67 Fonseca V, Rosenstock J, Patwardhan R, Salzman A. Effect of metformin and rosiglitazone combination therapy in patients with type 2 diabetes mellitus: a randomized controlled trial. JAMA 2000; 283:1695–1702.

68 Assman G, Schulte H. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAN experience): prospective cardiovascular Munster study. Am J Cardiol 1992; 85:37–45.

69 Robins SJ, Collins D, Wittes JT, *et al.* Relation of Gemfibrozil treatment and lipid levels with major coronary events. VA-HIT: a randomised controlled trial. JAMA 2001; 285:1585–1591.

70 Khan MA, St Peter JV, Xue JL. A prospective, randomized comparison of the metabolic effects of pioglitazone or rosiglitazone in patients with type 2 diabetes who were previously treated with troglitazone. Diabetes Care 2002; 25:708–711.

71 Lee C, Edwards G, Maher L, *et al.* Favourable effects of Pioglitazone mono or combination therapy on the atherogenic index of plasma: a surrogate marker of LDL particle size. Diabetes 2003; 52 (Suppl 1):A160–A688.