

# Insulin resistance and cardiovascular disease: the role of PPAR $\gamma$ activators beyond their anti-diabetic action

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## Abstract

Over the past few years it has been recognised that insulin resistance (IR) is an independent risk factor for major cardiovascular events. In addition, IR is associated with other factors such as hypertension, dyslipidaemia and endothelial dysfunction, and this cluster of metabolic disorders contributes to the cardiovascular risk of patients with IR. Given the increasing number of patients with IR, the modulation of their cardiovascular risk is a major task in diabetology and vascular medicine. This review will focus on the role of IR as a cardiovascular risk factor and on the potential of activators of the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) to modulate these risk factors associated with IR.

*Diabetes Vasc Dis Res* 2004;2:76–81

**Key words:** insulin resistance, PPAR $\gamma$  activators, thiazolidinediones, cardiovascular risk.

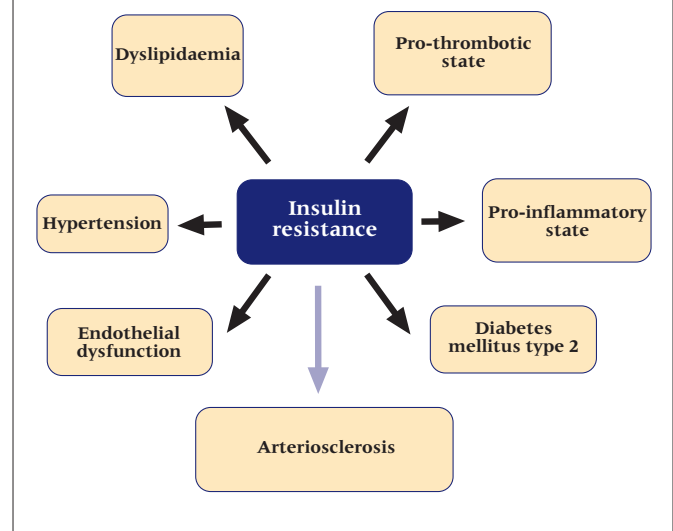
## Introduction

For many years insulin resistance (IR) was considered to be just an early stage in the development of type 2 diabetes mellitus. Moreover, increased cardiovascular mortality was attributed only to overt diabetes mellitus. The increasing number of patients with IR and type 2 diabetes mellitus, and the fact that these patients exhibit an increased propensity to develop vascular disease with its sequelae of acute myocardial infarction and stroke, has changed our view and now IR is recognised as an independent risk factor for cardiovascular disease. The following review will focus on the role of IR as a cardiovascular risk factor and on the potential of activators of the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) to modulate risk factors associated with IR.

## Insulin resistance syndrome and cardiovascular risk

IR is currently understood to be a state in which cells and

Figure 1. Insulin resistance is associated with other cardiovascular risk factors, and the complexity of this cluster contributes to the development of arteriosclerosis with its macrovascular complications



organs exhibit a limited sensitivity to insulin; hyperinsulinaemia results in order to keep blood glucose levels within the normal range. Recent data from various clinical and epidemiological trials have led to the recognition that IR is an independent risk factor for cardiovascular disease in non-diabetic subjects as well as in those with manifest type 2 diabetes mellitus.<sup>1-3</sup> In addition, IR is associated with other cardiovascular risk factors, such as hypertension, dyslipidaemia, obesity and hypercoagulability (figure 1),<sup>4-7</sup> all clustered in what Reaven termed Syndrome X in 1988<sup>8</sup> (and was later named metabolic syndrome or IR syndrome). Moreover, recent data suggest that IR is associated with a pro-inflammatory and pro-thrombotic state. The cluster of these factors contributes to the overall cardiovascular risk of IR patients.<sup>9</sup>

## Functions of PPAR $\gamma$ activators

PPARs belong to the group of nuclear hormone receptors, like the vitamin D or steroid receptors, and act as ligand-activated transcription factors regulating the expression of certain target genes. PPARs consist of a ligand- and a DNA-binding domain; upon activation, PPARs build heterodimers with another nuclear receptor, the retinoic X recep-

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tor (RXR), and these heterodimers bind to PPAR response elements (PPRE) in the promoter region of target genes, thus regulating their expression. These PPREs consist of a direct-repeat-1 (DR-1) sequence, meaning two half consensus motifs separated by one spacing base pair.<sup>10</sup> Three members of the PPAR family have been identified, PPAR $\alpha$ , PPAR $\gamma$  and PPAR $\beta/\delta$ , all sharing a 60–80% homology in their ligand- and DNA-binding domain, thus differing in the ligands they are activated by and in the target genes they regulate. PPAR $\alpha$  is involved in gene regulation in lipid metabolism and can be activated by certain polyunsaturated fatty acids as well as by lipid-lowering fibric acid derivatives like fenofibrate.<sup>11,12</sup> Less is known about the role of PPAR $\delta$ , its activators and target genes.

PPAR $\gamma$  is a key mediator in adipogenesis.<sup>13</sup> It regulates the expression of crucial genes in adipocytes, such as the genes encoding for lipoprotein lipase, fatty acid binding protein and acyl-CoA-synthetase, and overexpression of PPAR $\gamma$  in fibroblasts has been shown to induce adipocyte differentiation.<sup>14,15</sup> Activators of PPAR $\gamma$  include naturally occurring ligands, including the prostaglandin D<sub>2</sub> derivative, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>)<sup>16</sup> or oxidised linoleic acid [9(S)- and 13(S)-HODE] as components of oxidised LDL,<sup>17,18</sup> and a group of new, synthetic antidiabetic drugs, thiazolidinediones (TZDs, glitazones),<sup>19</sup> that are currently used to treat patients with type 2 diabetes mellitus.

Members of the insulin-sensitising TZD class include rosiglitazone and pioglitazone as well as the previously used troglitazone, which was withdrawn from the US market due to liver toxicity. The underlying mechanisms of TZD-mediated improvement of IR are not completely understood, but the induction of fat cell differentiation from large insulin-resistant adipocytes to smaller, more insulin-sensitive cells seems to be of particular importance. This differentiation leads to a reduced release of free fatty acids from these cells and diminishes circulating levels of IR-mediating adipocytokines such as TNF- $\alpha$ , leptin and resistin. These changes improve IR in liver and skeletal muscle.<sup>10</sup> In addition, TZDs seem to exhibit a direct effect in these organs, for example, by modulating the expression of glucose receptors. Furthermore, recent results suggest that TZDs may improve and conserve beta cell function.<sup>20</sup>

### The lipid profile

Patients with IR exhibit a characteristic dyslipidaemic pattern, with increased plasma triglycerides (TG), minimally elevated total cholesterol but decreased high-density lipoprotein cholesterol (HDL). In addition, these patients show increased levels of pro-atherogenic small, dense low-density lipoproteins (LDL),<sup>21,22</sup> known to be associated with increased cardiovascular mortality.<sup>23</sup> TZDs increase HDL levels and moderately enhance total cholesterol and LDL cholesterol. However, the increase in LDL is predominantly due to a shift of small dense LDL particles to large buoyant particles of LDL, which are considered to be less atherogenic.<sup>24</sup> Pioglitazone treatment leads to a decrease in triglycerides, while no such effect has been observed with rosiglitazone. This difference is most likely due to addition-

al PPAR $\alpha$ -activating properties of pioglitazone.<sup>25</sup> In addition, TZDs also lower free fatty acid (FFA) levels by stimulating FFA uptake into adipocytes, by reducing lipolysis and by enhancing fatty acid oxidation.<sup>26</sup> These effects of TZDs seem to be independent of their glucose-lowering action and can be observed in both diabetic and non-diabetic patients.<sup>24,27</sup>

### Blood pressure

Hypertension, an independent risk factor for coronary heart disease (CHD), is associated with IR and is an integral component of the metabolic syndrome. Often, patients are diagnosed with hypertension some years before developing overt type 2 diabetes. In addition, Hirose *et al.* reported newly diagnosed hypertension in 9.5%, 15.7% and 20.6% of initially normotensive Japanese men (379 subjects) in the lowest, intermediate and highest tertiles of IR, respectively, at seven years' follow-up. The two-fold increase in hypertension in the highest tertile of IR compared to the lowest tertile suggests that IR may be a predictor of hypertension.<sup>28</sup> Various studies have shown that TZDs reduce blood pressure in patients with type 2 diabetes mellitus as well as in obese subjects without diabetes.<sup>27</sup> Potential mechanisms for the hypotensive effects of TZDs include improved endothelium-dependent vasodilatation, a decrease in calcium influx and calcium sensitivity of the contractile apparatus,<sup>29,30</sup> and inhibition of endothelin-1 expression in endothelial cells through activation of PPAR $\gamma$ .<sup>31</sup> In clinical studies, TZDs have been shown to lower blood-pressure by 6–8 mmHg.<sup>32</sup>

### Haemostasis

Data from several groups have demonstrated convincingly that patients with IR show abnormalities in coagulation and fibrinolysis. Atherothrombotic complications in insulin-resistant patients are partly attributed to a pro-thrombotic state resulting from endothelial activation, hyperreactivity of platelets, hypercoagulability and hypofibrinolysis.<sup>21,33–36</sup> In particular, levels of PAI-1, the primary physiological inhibitor of fibrinolysis, are elevated in persons with IR, reflecting an elevated risk of vascular events, and increased levels of von Willebrand factor (vWF) have been reported in patients with diabetes mellitus. Beside their effects on lipid metabolism, hypertension and glucose metabolism, TZDs also exhibit a lowering effect on these pro-thrombotic mediators. As such, clinical studies have shown that TZDs can reduce serum levels of both PAI-1 and vWF.<sup>27,37</sup> In addition, recent data in non-diabetic patients with coronary artery disease suggest that rosiglitazone may reduce circulating platelet activity, as assessed by quantification of P-selectin-positive platelets by flow cytometry.<sup>38</sup>

### Arteriosclerosis

Patients with IR and type 2 diabetes mellitus have an increased risk of accelerated atherogenesis, and typically develop a diffuse and extensive pattern of arteriosclerotic lesions.<sup>39</sup> Lesion formation is preceded by endothelial dysfunction, which is characterised by an increase in endothelial permeability, permitting penetration of plasma

constituents into the vessel wall, and accumulation of monocytes. In the subendothelium, these cells then become macrophages, major effectors of inflammation during atherogenesis. Subsequently, over time, fatty streaks and complex atherosclerotic lesions develop.<sup>40</sup>

Recent data from our own group suggest that C-peptide may play a causal role in early atherogenesis in patients with IR and early type 2 diabetes mellitus. These patients exhibit increased levels of C-peptide and typically develop a diffuse and extensive pattern of arteriosclerosis at a very early stage. We have been able to show that C-peptide co-localises with monocytes and CD4-positive T cells in the intima of early arteriosclerotic lesions of diabetic subjects and that C-peptide induces monocyte and T cell migration *in vitro*. This process involves activation of pertussis toxin-sensitive G-proteins as well as PI 3-kinase gamma. Thus, C-peptide may be deposited in the vessel wall in early atherogenesis in diabetic subjects and may promote monocyte and T cell migration into developing lesions<sup>41,42</sup> However, additional studies, in particular in *in vivo* models, are necessary to prove this hypothesis.

Clinically, endothelial function can be assessed by measuring flow-dependent vasodilatation: disturbed flow-associated (endothelium-dependent) vasodilatation is regarded as an early marker in the development of vascular disease.<sup>43</sup> Balletshofer *et al.* showed a significant association between endothelial dysfunction and IR in young first-degree relatives of patients with type 2 diabetes,<sup>44</sup> as well as reduced flow-mediated vasodilatation in very young insulin-resistant subjects.<sup>45</sup> Still, further studies are needed to show that these findings indeed reflect an increased risk of future cardiovascular events in IR patients.

Experimental and clinical data suggest that PPAR $\gamma$  activators may exhibit direct protective effects in the vessel wall by modulating the expression of proatherogenic mediators in vascular cells. TZDs operate as ligands for the nuclear transcription factor PPAR $\gamma$ , which is highly expressed in endothelial cells, vascular smooth muscle cells, lymphocytes and macrophages *in vitro* and *in vivo*.<sup>46</sup> A number of *in vitro* studies support the supposition that PPAR $\gamma$  activators can modulate critical processes in all phases of atherogenesis.

In the early phase of atherogenesis, PPAR $\gamma$  activation by TZDs inhibits leukocyte-endothelial cell interaction, an inflammatory response that is critical at the beginning of plaque development.<sup>47</sup> Consistent with this finding, TZDs inhibit the expression of vascular cell adhesion molecules (VCAM-1) and intracellular adhesion molecules (ICAM-1) in activated endothelial cells.<sup>48</sup> This significantly reduces the recruitment of monocytes/macrophages and CD4-positive T cells to atherosclerotic plaques. In addition, PPAR $\gamma$ -activating TZDs limit the activation of monocytes and T-lymphocytes e.g. by inhibiting cytokine expression and cell migration.<sup>49-51</sup>

In advanced lesions such inflammatory mediators determine plaque stability by regulating monocyte/macrophage and vascular smooth muscle cell expression of matrix metalloproteinases (MMPs), a family of highly regulated enzymes that can degrade collagen and other matrix

proteins. PPAR $\gamma$  activators may stabilise atherosclerotic plaques by inhibiting the expression and activity of MMP-9 in human monocytes and human vascular smooth muscle cells.<sup>51,52</sup> These data, in conjunction with studies in various animal models, support a role of PPAR $\gamma$ -activating TZDs as modulators of the atherogenic process in the vessel wall. In animal models of early lesions, TZDs and non-TZD PPAR $\gamma$  activators reduced atherosclerosis by 20–40%;<sup>53,54</sup> in these studies, this effect was independent of changes in insulin, glucose or circulating lipids, suggesting direct vascular action.

Early clinical studies suggest that these vasoprotective effects of PPAR $\gamma$  activators may also be present in treated patients. As such, TZD treatment improves endothelial function in type 2 diabetics.<sup>55</sup> This effect has been demonstrated by enhanced endothelium-dependent vasodilatation and a decrease of sE-selectin levels.<sup>56,57</sup> In addition, urinary albumin excretion, a predictive marker for CHD in diabetic and non-diabetic subjects,<sup>58,59</sup> is reduced by TZD treatment in type 2 diabetic patients.<sup>60</sup> Beside these functional aspects, TZD treatment leads to morphological changes in the vessel wall, as shown by a reduction of carotid intima-media thickness.<sup>61,62</sup>

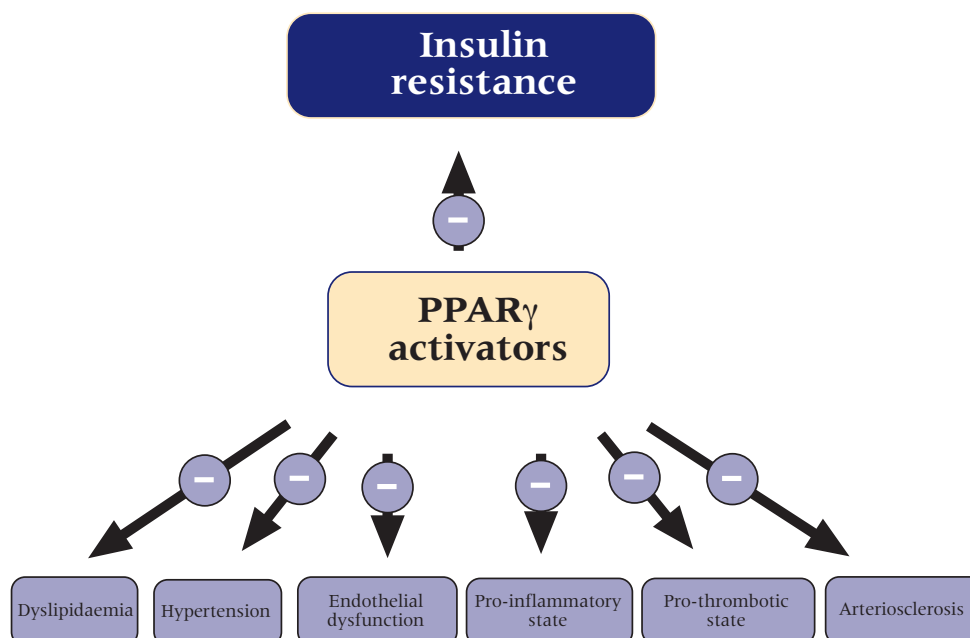
### Inflammation

According to our current understanding, inflammatory processes in the vessel wall contribute to atherogenesis and lesion development. In addition, several lines of evidence from clinical and epidemiological trials indicate a connection between atherosclerosis and inflammation.<sup>40</sup> Data from the last decade have led to the recognition that serum levels of inflammatory biomarkers of arteriosclerosis such as CRP, TNF- $\alpha$  and sCD40L, or of soluble adhesion molecules, like sICAM or sVCAM, predict cardiovascular risk in patients and healthy subjects.<sup>63-67</sup> In the multicentre Insulin Resistance Atherosclerosis Study (IRAS) Festa *et al.* showed a correlation between IR and plasma CRP levels,<sup>68</sup> and it has been suggested that inflammation may represent the 'common soil' for arteriosclerosis and diabetes mellitus.<sup>69</sup> Adipocytokines such as resistin and TNF, when released from visceral adipose tissue in patients with IR, most likely contribute to this inflammatory state, which may also promote lesion development. Moreover, levels of adiponectin, an adipocytokine with anti-inflammatory properties, are decreased in patients with IR, which could further promote atherogenesis in these patients.<sup>70</sup>

Independently, several groups have shown that PPAR $\gamma$  activators reduce serum levels of inflammatory biomarkers of arteriosclerosis, such as CRP, TNF- $\alpha$ , or sCD40L, in diabetic as well as in non-diabetic individuals.<sup>62,71-73</sup> In some of the studies this effect could be seen after only two weeks, whereas the glucose-lowering action was observed after 6–8 weeks, suggesting that these anti-inflammatory properties may be independent of PPAR $\gamma$  activators' metabolic action.<sup>72</sup> Moreover, rosiglitazone treatment has been shown to decrease serum levels of MMP-9, a marker considered to reflect plaque stability.<sup>73</sup>

Additional evidence for PPAR $\gamma$  activators' vasoprotective potential comes from restenosis studies in CAD

**Figure 2. PPAR $\gamma$  activators, like thiazolidinediones (TZDs), do not only influence insulin resistance itself, but also modulate associated risk factors and the development of arteriosclerosis**



patients: preliminary clinical data suggest that TZDs may limit restenosis after angioplasty in patients with type 2 diabetes.<sup>74,75</sup> Taken together, these data suggest that PPAR $\gamma$  activators, in addition to their metabolic action, may have direct protective effects in the vessel wall and modulate the inflammatory process in atherogenesis.

### Cardiac safety

After the introduction of TZDs as oral antidiabetic drugs, the observation that patients develop peripheral oedema and increased body weight raised major concerns about the cardiac safety of these agents. Although the underlying mechanisms for oedema development are not completely understood, enhanced sodium reabsorption due to increased insulin sensitivity in the proximal tubule as well as vasodilatory effects in pre-capillary vessels probably contribute to this clinical side effect. Data from clinical studies suggest that oedema while on TZD treatment can be classified as mild and moderate,<sup>76</sup> but it remains unclear whether fluid retention may aggravate pre-existing heart failure. In line with this concern, some authors have described patients who developed lung oedema during TZD treatment.<sup>77-79</sup> A recent study provided a more detailed overview of this issue: in 111 consecutive TZD-treated diabetic patients with chronic systolic heart failure 17.1% developed fluid retention, which presented predominantly as peripheral oedema and was reversible after drug withdrawal. The authors concluded that there is no direct association between the risk of fluid retention and the baseline degree of severity of heart failure.<sup>80</sup> Nonetheless, large clinical trials on cardiac safety are on the way and should answer the remaining questions on TZD treatment and cardiac safety.

### Conclusion

Work from various groups has shown that IR is an independent risk factor for macrovascular complications. In addition, IR is associated with other independent risk factors such as hypertension, endothelial dysfunction, dyslipidaemia, alterations in haemostasis and coagulation, and inflammatory activity. Recent data suggest that PPAR $\gamma$ -activating TZDs may not only address IR itself, but may also modulate these associated risk factors and exhibit direct protective effects in the vessel wall (figure 2). The combination of these favourable effects should reduce overall cardiovascular mortality and morbidity, but as yet this is still a hypothesis based on TZD-mediated risk factor modulation and effects on surrogate parameters. Therefore, we have to await the forthcoming results of large clinical trials with cardiovascular end points to judge whether this concept of vasculoprotection of PPAR $\gamma$ -activating TZDs translates into clinical practice.

### Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB451, project B9) and from the Else-Kröner-Fresenius Stiftung to Dr N Marx.

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