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COMMENTARY

Hepatic inflammation and insulin resistance in pre-diabetes – further evidence for the beneficial actions of PPAR-γ agonists and a role for SOCS-3 modulation

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Pre-diabetes is a condition affecting increasing numbers of the population who find themselves caught in the grey area between normal glucose regulation and diabetes mellitus and who experience impaired glucose tolerance or fasting glucose. The ability of thiozolidinediones (TZDs) to ameliorate the clinical signs of diabetes mellitus is well-known but there is also emerging evidence for the benefits of PPAR-γ agonists in pre-diabetes. In this issue of the *British Journal of Pharmacology*, Collino and colleagues report that pioglitazone can reduce hepatic inflammation and insulin resistance in rats administered a high cholesterol and fructose diet. Furthermore, pioglitazone reduced the expression of suppressor of cytokine signalling (SOCS)-3 – considered to be a key link between inflammation and insulin resistance. Although much work remains to be performed in fully understanding how TZDs modulate the cellular mechanisms which underlie pre-diabetes, these findings provide preliminary evidence that administration of TZDs to pre-diabetics could be beneficial.
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Pre-diabetes (also known as borderline diabetes *inter alia*) describes a condition affecting increasing numbers of the population who have abnormal glucose homeostasis but not diabetes mellitus and who suffer impaired glucose tolerance or fasting glucose (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Latest estimates suggest that up to 60 million people have some degree of pre-diabetes in the USA and recent figures released by Diabetes UK suggest that 7 million people in the UK may be affected. A steady increase in the amounts of sugar and saturated fat consumed in Western diets has contributed to the prevalence of pre-diabetes and a consequent increased risk of developing type 2 diabetes and cardiovascular disease. For example, high consumption of fructose, often in the form of high fructose corn syrup which is being used increasingly as a dietary sweetener, has been suggested to be a major contributor to the increasing incidence of pre-diabetes and the development of type 2 diabetes mellitus in the population (Johnson *et al.*, 2009; Miller and Adeli, 2008). Such a diet puts considerable stress on normal physiological function and under these conditions, chronic inflammation appears to be an important factor in the development of insulin resistance in metabolically active tissues such as the liver (Schenk *et al.*, 2008). The suppressor of cytokine signalling (SOCS) family of proteins appears to be implicated in the development of chronic inflammation and insulin resistance and the evidence for this is compelling: SOCS is highly expressed in the livers of obese animals, they can inhibit the activity of cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) and they can reduce insulin sensitivity by binding to the insulin receptor in the liver and preventing the coupling of insulin receptor substrate-2 (IRS-2) (Lebrun and Van Obberghen, 2008).

Thiozolidinediones (TZDs), which are agonists of the peroxisome proliferator-activated receptor-γ (PPAR-γ), are

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anti-diabetic drugs widely used for the management of insulin resistance associated with obesity and type 2 diabetes.

Synthetic TZDs such as rosiglitazone and pioglitazone increase glucose and lipid uptake, glucose oxidation and decrease free fatty acid concentration and insulin resistance in different tissues via activation of a range of genes. Furthermore, it is well-known that these PPAR-γ agonists also have several pleiotropic, non-hypoglycaemic actions beyond the control of glucose and lipid metabolism, including modulation of blood pressure, endothelial function, atherosclerosis, coagulation, albuminuria and lipid metabolism and inflammation (Rizos *et al*., 2008). These benefits have been well characterized in animal models of diabetes mellitus and good progress made in understanding the intracellular mechanisms involved. For example, pioglitazone has been shown to increase overall insulin sensitivity, in part via reduction of SOCS-3 expression which is associated with increased STAT-3 phosphorylation and adiponectin production in the fat tissues of *db/db* diabetic mice (Kanatani *et al*., 2007). Furthermore, pioglitazone promotes hepatic regeneration and improves survival after partial hepatectomy in obese and diabetic KK-A^y mice via similar modulation of SOCS-3 and STAT-3 phosphorylation (Aoyama *et al*., 2009).

Promising preliminary data have also emerged from studies investigating the potential benefits of TZDs in pre-diabetes (Dumasia *et al*., 2005), however, the intracellular mechanisms involved remain to be fully elucidated.

In this issue of the *British Journal of Pharmacology*, Collino and colleagues describe an investigation involving a non genetic insulin-resistant animal model of pre-diabetes established by giving rats a high cholesterol and fructose (HCF) diet (Collino *et al*., 2010). Wistar rats administered a HCF diet for 15 weeks developed several classic clinical signs of pre-diabetes including hyperlipidaemia, hyperinsulinaemia, impaired glucose tolerance and insulin resistance. However, daily administration of 3 $mg \cdot kg^{-1} \cdot day^{-1}$ pioglitazone to rats during the last 4 weeks of the HCF diet not only provided significant improvements in lipid metabolism and insulin responsiveness but also reduced markers of inflammation such as circulating levels of IL-6 and TNF-α, evidence of polymorphonuclear neutrophil infiltration and expression of intracellular adhesion molecule-1. Furthermore, pioglitazone also reduced the hepatic expression of SOCS-3 – a SOCS isoform which has been suggested to be a key link between hepatic inflammation and the development of insulin resistance (Lebrun and Van Obberghen, 2008). The authors conclude that chronic administration of pioglitazone reduces the hepatic inflammatory response induced in rats fed a HCF diet and that the changes in the hepatic expression of SOCS-3 produced by pioglitazone could be the link between reduction of hepatic inflammation and improved insulin signalling.

While this characterization of the effects of pioglitazone on the hepatic inflammation and SOCS-3 expression within the livers of pre-diabetic rats is novel, there are several issues of interest which may be raised from such a study, which could be addressed in future investigations. (i) High blood pressure is a recognized risk factor for pre-diabetes which itself is known to predispose patients to cardiac dysfunction. One previous study which used a comparable HCF diet to generate pre-diabetes in Sprague-Dawley rats described elevated blood pressure in their model (Deng *et al*., 2007) and in another investigation, pioglitazone, administered to genetically obese diabetic Wistar rats at the same dose and frequency as used by Collino and colleagues (3 $mg \cdot kg^{-1} \cdot day^{-1}$), lowered blood pressure (Yoshimoto *et al*., 1997). It would be interesting to see if there was also an increase in blood pressure observed by Collino and colleagues in their model and if so, could administration of pioglitazone have had a significant beneficial effect? (ii) Some of the protection afforded by TZDs appears to be mediated via non-genomic mechanisms independent of the PPAR-γ (Gardner *et al*., 2005). Collino and colleagues certainly provide good evidence that pioglitazone can induce genes on which PPAR response elements (PPRE) have been identified such as liver glucokinase and fatty acid synthase – an effect which appears to be mediated via activation of PPAR-γ which demonstrate increased expression in the livers of their pre-diabetic animals. However, in the future, the contribution of direct PPAR-γ activation by pioglitazone could be determined by examining the effects of selective PPAR-γ antagonists such as GW9662 or T0070907 or even via the use of PPAR-γ hepatic-specific knockout mice (global PPAR-γ knockout being lethal) similar to those developed for the study of role of the PPAR-γ in the brain and adipose tissues (Jones *et al*., 2005; Zhao *et al*., 2009). This aspect of pioglitazone action is of particular interest as the beneficial actions of TZDs can be observed even in the presence of reduced PPAR-γ expression, e.g. in the heart tissues of rats fed a high fat diet (Baranowski *et al*., 2008). (iii) There is good evidence from both *in vitro* and clinical studies for a link between hepatic inflammation and development of insulin resistance. For example, incubation of human hepatocytes with IL-6 can induce insulin resistance (Senn *et al*., 2002) and administration of rosiglitazone to non-diabetic patients with metabolic syndrome reduced circulating IL-6 levels which correlated with improvement of insulin resistance (Samaha *et al*., 2006). In this study, although pioglitazone lowered hepatic expression of both IL-6 and TNF-α and reduced insulin resistance, a causal relationship was not demonstrated. Thus the specific intracellular links between the hepatic inflammatory response in the pathogenesis of local insulin resistance in this *in vivo* model of pre-diabetes certainly warrants further investigation. (iv) Associated with this, the mechanistic association between pioglitazone, the PPAR-γ and the observed reduction of hepatic SOCS-3 expression also requires further study. In the liver, cytokines such as IL-6 and TNF-α can induce SOCS-3 expression which in turn attenuates further IL-6 and TNF-α release and cytokine-activated signal transduction pathways (e.g. those associated with IFN-γ) thereby providing a negative feedback effect on hepatic inflammation (Hong *et al*., 2001). *In vitro*, overexpression of SOCS-3 inhibits hepatic insulin autophosphorylation which contributes to insulin resistance (Senn *et al*., 2003). As the authors state in their Discussion, the consensus sequence of the PPAR-γ binding site has not yet been identified in the promoter region of the SOCS-3 gene, however, it would be interesting to investigate if TZDs such as pioglitazone can directly modulate the increased SOCS-3 expression observed under pre-diabetic conditions via transcriptional transrepression or indirectly via PPAR-γ-induced

reduction of the inflammatory response (or even a combination of both).

In this study, Collino and colleagues provide clear evidence that the TZD and PPAR-γ agonist pioglitazone can provide benefits against the liver inflammation and hepatic insulin resistance in an animal model of pre-diabetes and suggest that SOCS-3 may be a key link between these two events. Could these findings have clinical relevance to humans who find themselves in the grey area of borderline diabetes? If we allow ourselves to extrapolate these findings from the rat to the human, the dose of pioglitazone used in this study (0.75 mg daily to a 250 g rat – equivalent to 3 $mg \cdot kg^{-1} \cdot day^{-1}$) is higher but of a similar magnitude to that administered clinically (45 mg daily in the UK – equivalent to 0.64 $mg \cdot kg^{-1}$ to a 70 kg human) with pioglitazone reportedly providing greater benefit than rosiglitazone (Rizos *et al*., 2008). The findings of this study could therefore be very preliminary evidence for the future administration of TZDs to pre-diabetic patients. However, the primary (and best) clinical advice to give to pre-diabetic patients will undoubtedly always remain the same – make some significant lifestyle changes and eat a better diet!

References

Aoyama T, Ikejima K, Kon K, Okumura K, Arai K, Watanabe S (2009). Pioglitazone promotes survival and prevents hepatic regeneration failure after partial hepatectomy in obese and diabetic KK-A(y) mice. *Hepatology* **49**: 1636–1644.

Baranowski M, Blachnio-Zabielska A, Zabielski P, Gorski J (2008). Pioglitazone induces lipid accumulation in the rat heart despite concomitant reduction in plasma free fatty acid availability. *Arch Biochem Biophys* **477**: 86–91.

Collino M, Aragno M, Catiglia S, Miglio G, Tomasinelli C, Boccuzzi G *et al*. (2010). Beneficial effects of PPARγ agonism in overweight rats fed a Western-type diet involve SOCS-3 hepatic modulation. *Br J Pharmacol* **160**: 1892–1902.

Deng JY, Huang JP, Lu LS, Hung LM (2007). Impairment of cardiac insulin signaling and myocardial contractile performance in high-cholesterol/fructose-fed rats. *Am J Physiol Heart Circ Physiol* **293**: H978–H987.

Dumasia R, Eagle KA, Kline-Rogers E, May N, Cho L, Mukherjee D (2005). Role of PPAR-gamma agonist thiazolidinediones in treatment of pre-diabetic and diabetic individuals: a cardiovascular perspective. *Curr Drug Targets Cardiovasc Haematol Disord* **5**: 377–386.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* **26**: S5–S20.

Gardner OS, Dewar BJ, Graves LM (2005). Activation of mitogen-activated protein kinases by peroxisome proliferator-activated receptor ligands: an example of nongenomic signaling. *Mol Pharmacol* **68**: 933–941.

Hong F, Nguyen VA, Gao B (2001). Tumor necrosis factor alpha attenuates interferon alpha signaling in the liver: involvement of SOCS3 and SHP2 and implication in resistance to interferon therapy. *FASEB J* **15**: 1595–1597.

Johnson RJ, Perez-Pozo SE, Sautin YY, Manitius J, Sanchez-Lozada LG, Feig DI *et al*. (2009). Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? *Endocr Rev* **30**: 96–116.

Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y *et al*. (2005). Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. *Proc Natl Acad Sci USA* **102**: 6207–6212.

Kanatani Y, Usui I, Ishizuka K, Bukhari A, Fujisaka S, Urakaze M *et al*. (2007). Effects of pioglitazone on suppressor of cytokine signaling 3 expression: potential mechanisms for its effects on insulin sensitivity and adiponectin expression. *Diabetes* **56**: 795–803.

Lebrun P, Van Obberghen E (2008). SOCS proteins causing trouble in insulin action. *Acta Physiol (Oxf)* **192**: 29–36.

Miller A, Adeli K (2008). Dietary fructose and the metabolic syndrome. *Curr Opin Gastroenterol* **24**: 204–209.

Rizos CV, Liberopoulos EN, Mikhailidis DP, Elisaf MS (2008). Pleiotropic effects of thiazolidinediones. *Expert Opin Pharmacother* **9**: 1087–1108.

Samaha FF, Szapary PO, Iqbal N, Williams MM, Bloedon LT, Kochar A *et al*. (2006). Effects of rosiglitazone on lipids, adipokines, and inflammatory markers in nondiabetic patients with low high-density lipoprotein cholesterol and metabolic syndrome. *Arterioscler Thromb Vasc Biol* **26**: 624–630.

Schenk S, Saberi M, Olefsky JM (2008). Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* **118**: 2992–3002.

Senn JJ, Klover PJ, Nowak IA, Mooney RA (2002). Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* **51**: 3391–3399.

Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW *et al*. (2003). Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* **278**: 13740–13746.

Yoshimoto T, Naruse M, Nishikawa M, Naruse K, Tanabe A, Seki T *et al*. (1997). Antihypertensive and vasculo- and renoprotective effects of pioglitazone in genetically obese diabetic rats. *Am J Physiol* **272**: E989–E996.

Zhao X, Strong R, Zhang J, Sun G, Tsien JZ, Cui Z *et al*. (2009). Neuronal PPARgamma deficiency increases susceptibility to brain damage after cerebral ischemia. *J Neurosci* **29**: 6186–6195.