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Evan D. Rosen, Bruce M. Spiegelman

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Commentary

One of the great ironies of the present-day industrialized world is serious disease and death brought about by too much rich food and too little physical exertion. The incidence of obesity has increased to the point that one in two American adults is now considered overweight (1). Pathologies linked to obesity, such as type 2 diabetes, hypertension, and cardiovascular disorders, are also increasingly prevalent in our society. The tight linkage of obesity, insulin resistance (and frank diabetes), dyslipidemia, and hypertension has been widely observed and has been dignified with a label – syndrome X, or the metabolic syndrome (2). The exact pathogenic relationships between the component conditions of the metabolic syndrome are complex and incompletely understood, despite significant and ongoing efforts to identify susceptibility genes in human populations and animal models. The convergence of these conditions in the metabolic syndrome is not an area isolated to mere academic interest: Coronary and peripheral vascular disease leading to myocardial infarction and stroke is the unhappy fate of many affected individuals. In an ideal world, the metabolic syndrome would be treated by diet and exercise, leading to weight loss. Even relatively modest degrees of weight loss have been shown to improve markers of the metabolic syndrome, such as blood pressure, serum cholesterol, and insulin levels. Unfortunately, most patients find the necessary dietary and […]

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Evan D. Rosen[1,2] and Bruce M. Spiegelman[1]

[1]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA
[2]Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts, USA

Address correspondence to: Bruce Spiegelman, Department of Cancer Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, USA.
Phone: (617) 632-3567; Fax: (617) 632-4655; E-mail: bruce_spiegelman@dfci.harvard.edu.

One of the great ironies of the present-day industrialized world is serious disease and death brought about by too much rich food and too little physical exertion. The incidence of obesity has increased to the point that one in two American adults is now considered overweight (1). Pathologies linked to obesity, such as type 2 diabetes, hypertension, and cardiovascular disorders, are also increasingly prevalent in our society.

The tight linkage of obesity, insulin resistance (and frank diabetes), dyslipidemia, and hypertension has been widely observed and has been dignified with a label – syndrome X, or the metabolic syndrome (2). The exact pathogenic relationships between the component conditions of the metabolic syndrome are complex and incompletely understood, despite significant and ongoing efforts to identify susceptibility genes in human populations and animal models. The convergence of these conditions in the metabolic syndrome is not an area isolated to mere academic interest: Coronary and peripheral vascular disease leading to myocardial infarction and stroke is the unhappy fate of many affected individuals.

In an ideal world, the metabolic syndrome would be treated by diet and exercise, leading to weight loss. Even relatively modest degrees of weight loss have been shown to improve markers of the metabolic syndrome, such as blood pressure, serum cholesterol, and insulin levels. Unfortunately, most patients find the necessary dietary and exercise regimens to be difficult. Even if the difficulty is surmounted, they find that their bodies resist any deviation from the "set-point" of their elevated weight. Much emphasis, therefore, has been placed on treating the component conditions of the metabolic syndrome pharmacologically. Indeed, these efforts have been successful, and new medications for hypertension and dyslipidemia are now available that can reduce morbidity and mortality from cardiovascular disease in these patients.

The treatment of insulin resistance and diabetes has until recently been restricted to the administration of exogenous insulin or to sulfonylureas, which promote the release of endogenous insulin. Although effective at reducing serum glucose levels in diabetic patients, neither of these agents addresses the underlying insulin resistance at the core of the metabolic syndrome. In the last five years, however, metformin became available in the US. Metformin reduces insulin resistance primarily in the liver, although its precise molecular targets are not known. Unfortunately, the use of metformin in patients with significant renal, hepatic, or cardiac impairment can lead to life-threatening lactic acidosis, reducing the utility of this agent in many people with diabetes (3).

It was with considerable excitement, therefore, that the thiazolidinedione (TZD) drugs were introduced into the US market in the last five years. Like metformin, these antidiabetic agents, such as troglitazone (Rezulin™), rosiglitazone (Avandia™), and pioglitazone (Actos™), were originally developed without knowledge of their mechanism of action. Several lines of evidence, however, have now converged to identify the peroxisome proliferator–activated receptor γ (PPARγ) as the relevant molecular target of these compounds (4). Perhaps most convincing is the fact that non-TZD synthetic compounds isolated solely on the basis of binding to PPARγ, a ligand-activated transcriptional regulator, exert antidiabetic effects similar to those of the TZDs (5).

As discussed by Olefsky (6) and others in the recent *JCI* Perspective series on insulin resistance, TZDs reduce insulin resistance and improve glucose homeostasis in diabetic rodents and humans. Nevertheless, concerns remain about possible deleterious side effects of these drugs. Troglitazone, the first TZD approved, was shown to have hepatotoxic effects in some patients during post-marketing analysis (7). Unfortunately, this reaction was severe enough in a few patients to cause death or a requirement for liver transplantation, leading to the withdrawal of this drug from the US market. Thus far, monitoring of patients taking rosiglitazone and pioglitazone has not revealed significant hepatotoxicity suggesting that this undesired effect may be idiosyncratic to troglitazone and not related to the activation of PPARγ per se. A more precise understanding of the spectrum of side effects of rosiglitazone and pioglitazone must await more studies in larger numbers of patients.

One complicating feature in the use of PPARγ agonists is that the precise tissue targets relevant to metabolic disease are not fully understood. PPARγ is expressed at its highest levels in adipose tissue, with lower levels expressed in many cell types, including monocytes, skeletal muscle, vascular endothelial cells, and breast, colon, and prostate epithelium. PPARγ is a dominant regulator of many aspects of fat cell biology, including adipose cell differentiation, fatty acid uptake, and lipogenesis, raising the possibility that the insulin-sensitizing effects of TZDs reflect the increased performance of TZD-treated adipose tissue as a sink for both fat and glucose. On the other hand, it is entirely possible that TZDs act primarily through PPARγ in other tissues such as muscle, liver, or the β cells of the pancreas. Global deletion of the *PPARγ* gene in mice results in placental dysfunction and embryonic lethality (8, 9), so resolution of this question in a definitive way will require the construction of tissue-specific knockouts.

PPARγ and atherosclerosis: framing the debate

The presence of PPARγ in monocytes and macrophages led to investigations into the effects of TZDs in those cells. Some of these studies revealed that activation of PPARγ by several ligands could induce expression of the scavenger receptor CD36 and promote the differentiation of macrophages from monocytic cell lines (10). Furthermore, treatment of monocytic cells with PPARγ agonists could induce lipid accumulations reminiscent of those found in foam cells of the atherosclerotic plaque. Also endogenous ligands of PPARγ were identified within atherogenic serum lipid particles (oxidized LDL [oxLDL]) and these agents could increase the expression of PPARγ itself (11). A pathological cycle was suggested in which atherogenic oxLDL particles could induce their own uptake through activation of PPARγ and expression of CD36, leading to foam cell formation. This hypothesis was consistent with the observation that PPARγ is expressed at moderately high levels within the atherosclerotic plaques of mice (10) and humans (12, 13), and with the demonstration that mice carrying targeted deletions of CD36 are relatively protected from atherosclerosis (14). These studies, then, opened the possibility that TZDs, acting through PPARγ, promote development of atherosclerosis in precisely the group of patients at most risk of this disease.

Atherosclerosis, however, is a complex phenotype with multiple inputs. Physiological parameters like blood pressure and serum lipid levels interact with factors intrinsic to the vessel wall, such as vascular smooth muscle migration and the production of thrombotic proteins by vascular endothelium, to influence the development of atheromas. Atherosclerosis is also believed to have a large inflammatory component, and factors specific to macrophages are likely to be critical as well. TZDs have been shown to favorably affect many of these parameters. For example, these drugs are known to reduce blood pressure in several mammalian models, in a manner that does not always correlate with improvements in insulin sensitivity (15, 16). TZDs are also known to inhibit vascular smooth muscle proliferation and migration in vitro (17) and in vivo (18), events which are believed to be essential for the development of atherosclerotic lesions. This effect is at least partially mediated by inhibition of mitogen-activated protein kinases that transduce activating signals from angiotensin II and TNF-α or other hormones or cytokines (19). Even within the macrophage, PPARγ has been shown to confer anti-inflammatory effects including repression of phorbol ester–stimulated expression of IL-6, IL-1β, TNF-α, gelatinase, and scavenger receptor-A (SR-A) (20, 21). In one interesting study, the homing of cultured monocytic cells to atherosclerotic lesions of *ApoE* knockout mice was inhibited by TZD treatment (22).

PPARγ agonists and atherosclerotic lesions in vivo

The analysis of these surrogate markers of atherosclerosis, therefore, reveals evidence of both pro- and antiatherogenic effects of TZDs. Fortunately, data are also available, including from the work of Li et al. (23) in a recent issue of the *JCI*, that address the endpoint that really matters: the physical development of atheromatous lesions. Li and colleagues used the LDL receptor knockout mouse as a model of atherogenesis. The LDL receptor provides the major means by which the cholesterol-rich LDL particle is removed from the circulation, and LDL receptor–deficient mice, like their human counterparts, are hypercholesterolemic and prone to atherosclerosis, particularly when fed a "Western-style" diet rich in fats and cholesterol. To assess the effects of PPARγ activation on the development of vascular lesions, the authors employed two different classes of synthetic PPARγ ligands — the TZD rosiglitazone, and GW7845, a non-TZD tyrosine analog. Both of these drugs improved atherosclerotic lesions in the male mice. These effects were striking and revealed a reduction in both number and size of lesions. Surprisingly, no change was noted in the extent of atherosclerosis in the females, a confusing feature of this study, discussed below.

The findings of Li et al. (23) extend and confirm observations made previously in another model of atherosclerosis, the Watanabe heritable hyperlipidemic (WHHL) rabbit. The development of atherosclerotic lesions in this animal was reduced by treatment with troglitazone, to the same degree as treatment with an inhibitor of HMG CoA reductase. Combining the two agents led to an additional suppression of atherogenesis (24). The current study has the advantage of using two different and structurally unrelated PPARγ ligands, which reduces the possibility that the compounds are working through some unexpected molecular pathway. Additionally, Li et al. (23) show that the PPARγ ligands affect gene expression within the atherosclerotic lesion itself. In particular, TNF-α and gelatinase B were consistently reduced in male mice treated with PPARγ ligands, whereas CD36 was elevated. Although the reduction in the expression of TNF-α and gelatinase B may or may not contribute to the antiatherogenic effects of these drugs, their proinflammatory action and the fact that they have been shown to be direct PPARγ targets in monocytic cells makes this idea plausible.

These studies provide direct experimental evidence that PPARγ activation can improve atherosclerosis in a well-controlled model system. Interestingly, a preliminary study in Japanese patients with type 2 diabetes showed a significant reduction of carotid artery wall thickness after treatment with a TZD, suggesting that these results may be applicable to humans as well (25).

One possibility to consider is that the reduction in atherosclerosis was an indirect effect of PPARγ activation, secondary to improvements in insulin sensitivity (see Ginsberg [ref. 26]). This hypothesis is consistent with the observation that the only mice in the study by Li et al. (23) that showed improvement in atherosclerosis (i.e., male mice) also exhibited reductions in insulin resistance, while female mice did not demonstrate either effect. Similarly, the prevention of vascular lesions in WHHL rabbits by TZD treatment was associated with improved insulin sensitivity (24). While the authors correctly point out that there is no direct evidence linking improved glycemic control to reductions in macrovascular disease in diabetes, neither is there direct evidence against such a scenario. Randomized clinical trials in humans with type 1 (27) and type 2 diabetes (28) have not settled the issue, because of insufficient statistical power or because of insufficient differences in achieved glycemic control between intensively and conventionally treated patients.

The aforementioned differences between males and females noted by Li et al. (23) also currently defy explanation. There are many studies in both animal models and premenopausal women

that indicate that TZDs improve insulin sensitivity in females (24, 29, 30). The fact that Li et al. state that ovariectomized females behave like males indicates that the ratio of serum estrogens to androgens likely plays a dominant role in the phenomenon; why the responses of LDL receptor–deficient mice should differ from those of other mammalian (including murine) models remains a mystery.

Quandaries and opportunities

Both the current paper and the earlier work of Tontonoz et al. (10) demonstrate the induction of CD36 by PPARγ and its ligands. Because this scavenger receptor has been shown to be required for atherogenesis in mice, the induction of this mRNA by PPARγ could be interpreted as an ominous sign. The lack of correlation now shown in vivo between CD36 induction and atherogenesis suggests several possible explanations. First, PPARγ could differentially regulate various macrophage-expressed scavenger receptors. Indeed, PPARγ ligands can suppress the induction of SR-A by phorbol esters (21) while promoting expression of CD36. Another possibility is that CD36 expression is not ordinarily rate-limiting in the development of foam cells. While CD36 is required for foam cell formation, there is no evidence that increased expression of CD36 can further accelerate this process. More likely, levels of LDL cholesterol or the conversion to oxLDL limit the rate of foam cell conversion. In this regard, it is worth noting that PPARγ ligands induce CD36 in multiple tissues, including fat (our laboratory, unpublished observations). Hence, the removal of oxLDL from the circulation into adipose or other tissues may provide a net total benefit on atherogenesis that is not diminished by elevated CD36 in macrophages. In fact, heterozygous *LDL receptor* knockout mice are protected from atherosclerosis by overexpression of CD36 in the liver, demonstrating the validity of this idea (31).

While the data from Li et al. (23) should clearly reassure us about the value of treating patients with type 2 diabetes or the metabolic syndrome with PPARγ agonists, it still remains possible that PPARγ in the macrophage is required for atherosclerosis. Put another way, macrophage CD36, induced by PPARγ, may yet prove to be a critical determinant of foam cell formation despite the fact of net reduction of atherosclerosis in TZD-treated mice. In order to actually address this issue, studies will need to be performed with macrophage-specific PPARγ knockout mice, or with *LDL receptor* knockout mice that have had their bone marrow ablated and replaced with PPARγ-deficient cells. Regardless of the outcome of such experiments, there is clearly no reason to discontinue the use of PPARγ activators in patients with insulin resistance because of fears of promoting atherosclerosis. On the contrary, one hopes that future generations of PPARγ ligands will be able to exploit the considerable antiatherogenic properties of this receptor to the fullest.

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