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Commentary

Insulin sensitizers may prevent metabolic inflammation

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ABSTRACT

The relative decreased response of peripheral tissues to insulin (insulin resistance) is a key metabolic disturbance that predisposes a large percentage of individuals to the development of type 2 diabetes and to cardiovascular disease. As detailed in an extensive literature over the last two decades, insulin resistance co-exists in varying degrees with a variety of other key risk factors, including dyslipidemia, hypertension, and vascular inflammation, that contribute to poor cardiovascular outcomes of individuals with type 2 diabetes and metabolic syndrome. Whereas insulin resistance is generally thought of as pathology unto itself, this commentary suggests that insulin resistance is a physiological compensation to inappropriate oxidative metabolism that induces a metabolic inflammatory response. Via signaling of this inflammatory response, the protective compensation to excessive oxidative metabolism dampens metabolism by reducing insulin action, fatty acid oxidation, and eventually mitochondrial function and numbers. Such a scenario could explain the coexistence of these phenomena with obesity and reduced mitochondrial function. Recent evidence suggests that thiazolidinediones exert pharmacology through modifications of mitochondrial metabolism, preventing the metabolic inflammation and allowing the up regulation of mitochondrial biogenesis. A further understanding of these mechanisms, which are likely to involve key redox signaling events emanating from mitochondrial biochemistry, is needed to fuel new therapeutic advances for the treatment of metabolic syndrome.

1. Metabolic syndrome and insulin resistance

Luminaries from the diabetes field including Reaven, DeFronzo, Olefsky and their colleagues have made it clear that the reduction in the response of peripheral tissues to the action of insulin (or insulin resistance) is a key component in the etiology of type 2 diabetes [1–4]. In his seminal Banting Lecture in 1988 [1], Reaven pointed out that the observation that diabetes could occur with insulin insensitivity was made by Himsworth as early as 1936. However, it was the publication of the 1988 Banting Lecture that made clear that this insulin resistance occurred not only in type 2 diabetes, but also occurred in many individuals who are not diabetic and may never become diabetic. Reaven suggested that resistance to insulin and/or the hyperinsulinemia that occurs in compensation to the insulin resistance plays a key role in the ''...clinical course of three related diseases- NIDDM, hypertension, and CAD.'' This publication and other similar observations have spawned hundreds of studies over the last 15 years that have further defined how a collection of cardiovascular risk factors (hypertension, central obesity, dyslipidemia, prothrombotic conditions, and inflammation) variably cluster

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together in what has alternately been described as syndrome X or metabolic syndrome [5–10]. Recently several different formal definitions have been created to define metabolic syndrome [10–12] in the hope that the combination of an adequate definition together with adequate instruments of detection and treatment could result the ability to prevent the deadly related diseases that Reaven described.

Population studies demonstrate considerable variation in the clustering of the individual traits of metabolic syndrome such that there has been discussion as to what constitutes the syndrome [10–12] and indeed whether it in fact exists as a single entity [13]. There are two general hypotheses to account for this variation. The cardiovascular risk factors that cluster in metabolic syndrome could result from various combination of multiple defects (genetic/environmental hits on control of normal metabolic pathways) resulting in multiple phenotypes. Alternatively, these parameters may cluster in a variable way because, although they share a common etiology, genetic variability produces backgrounds that provide protection against individual symptoms (e.g. hypertension, obesity, lipid profiles, etc.) and/or exacerbation of others (e.g. Fig. 1). This commentary makes the case for a common etiology with the important implication that a single therapeutic could provide protection against multiple diseases. Support for this possibility is provided by the pharmacology of the insulin sensitizing thiazolidinediones.

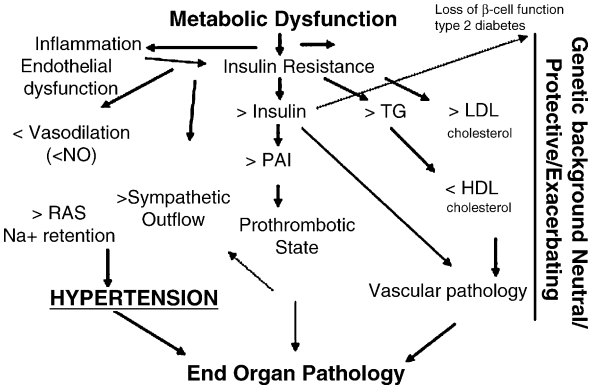

Fig. 1 – Diversity of metabolic syndrome. A single class of metabolic defects produces all of the signals to create the conditions that cluster as metabolic syndrome. Diversity of presentation of the clustering conditions (i.e., inflammation, prothrombotic state, hypertension, dyslididemia) occurs as a result of genetic backgrounds that can be neutral, protective, or exacerbating for each specific component. Inflammatory signals produce endothelial dysfunction, which contributes directly to hypetension and to vascular pathology. Insulin resistance is compensated for by an elevation of circulating insulin. These compensations contribute to hypertension, dyslipidemia (elevated triglycerides (TG), LDL cholesterol, and reduced HDL cholesterol) and a prothrombotic state with elevated plaminogen activator inhibitor (PAI). Activation of the rennin–angiotensin system (RAS) and sympathetic nervous system may also be involved. In the presence of a genetic background that limits compensation of the pancreatic β-cells, there is a progressive loss of β-cell function giving rise to type 2 diabetes. In any event, the common collection of sequelea contributes to end organ pathologies.

2. Insight provided by the insulin sensitizing thiazolidinediones (TZDs)

Thiazolidinediones were discovered empirically by scientists at the Takeda company over 30 years ago based on the ability of the compounds to lower circulating lipid and glucose levels in rodent models of insulin resistant diabetes [14–16]. A variety of subsequent studies suggested and then confirmed that the compounds acted as insulin sensitizers, treating and preventing diabetes in animal models [17–19]. As experimentation expanded into other animal models, and as the first three compounds from the class (troglitazone, rosiglitazone, and pioglitazone) were approved for clinical use, it soon became apparent the pleiotropic effects of the TZDs could include not only improved insulin sensitivity with reduction of circulating insulin levels, but also corrections of dyslipidemia (lowering triglycerides and LDL cholesterol and elevation of HDL cholesterol), lowering of blood pressure, reversal of the prothrombotic state, decreased inflammation, and a reduction of central obesity [20–22]. Interesting, therefore, the TZDs were capable of impacting *all* of the components of metabolic syndrome. Results were recently presented showing that long-term treatment with pioglitazone hydrochloride (Actos) significantly improved cardiovascular outcomes in high risk diabetic patients [23,24].

Troglitazone, the first TZD approved for treatment of diabetes was withdrawn because of a specific idiosyncratic hepatoxicity. A major draw back of the TZDs now in clinical use, however, is the propensity to cause edema and increased retention of fluid [25]. Furthermore, although the TZDs may reduce central obesity, they cause weight *gain* and not weight loss in practice. Recent evidence indicates that the fluid retention is driven by activation of the nuclear receptor PPARγ through an increased expression of a sodium channel in the kidney [26,27]. Increases in peripheral adipose stores are also thought to be driven by activation of the same receptor [28]. Unfortunately, this has also been proposed as the mechanism of action of the TZDs [29–31] and drug discovery has followed this path exclusively [32] resulting in compounds that retain or accentuate this liability. Recently published phases 2 and 3 clinical trials with a potent combined PPARγ/PPARα agonist demonstrated significant edema and an apparent *increase* in adverse cardiovascular outcomes [33].

Recent evidence, however, has called into question the hypothesis that the TZDs function purely as agonists for the nuclear transcription factor PPARγ. Thus, a variety of PPARγ knockout animals have been shown to still respond to TZDs in vivo [34–36]. Anti-inflammatory and cell-cycle effects of the TZDs can occur in vitro in complete absence of the transcription factor [37–39]. A recent review summarized data suggesting that the effects of these compounds may be mediated through effects on mitochondrial metabolism [40]. These data are interesting in that by taking an unbiased approach for location of cellular binding sites, we could only demonstrate specific binding of tritiated pioglitazone in the

mitochondria. Using tritiated pioglitazone and a photoaffinity crosslinker we identified a previously uncharacterized protein (mitoNEET) in the mitochondrial membrane that seems to be involved in TZD binding to mitochondria [41]. More recently we have shown that in a comparison of compounds with vastly different inherent PPARγ activating activity but similar binding to the mitochondria, the insulin sensitizing pharmacology correlated with the ability to increase the expression of mitochondrial proteins rather than with biomarkers for the activation of PPARγ-driven gene expression [42].

3. Mitochondrial function in diabetes, obesity, and hypertension

The fact that an unbiased approach lead to the discovery of a mitochondrial binding site for the TZDs is particularly interesting since recent evidence has demonstrated that alteration of mitochondrial function appears related to the metabolic syndrome. Thus, for example, the weight gain and dyslipidimea associated with combination treatments for AIDS is associated with mitochondrial damage [43]. A series of mitochondrial mutations are known to be associated with hypertension [44]. A mutation in mitochondrial tRNA has also been shown to result in metabolic syndrome [45]. Furthermore, a recent study of rats inbred for poor exercise capacity demonstrated that the offspring had all elements of the metabolic syndrome including insulin resistance, dyslipidemia, obesity, and hypertension and that this correlated with a decrease in mitochondrial function and mitochondrial protein expression [46].

There is also growing evidence that mitochondrial number and function are reduced in various tissues in human diabetes and obesity [47,48]. A recent study has shown that acute lipid infusion can decrease the expression of mitochondrial proteins in man [49]. Finally, treatment with pioglitazone produces *increased* expression of mitochondrial proteins in man [50]. This may involve an increased expression of PGC-1α, the master regulator of mitochondrial biogenesis [51,52]. Thus, the accumulating evidence suggests that mitochondrial metabolism plays a key role in whether or not the constellation of the symptoms of metabolic syndrome will be manifested. In this light, the collection of the evidence that suggests that pharmacology of the TZDs involves mitochondrial effects takes on new significance.

4. The involvement of IKK in insulin resistance

A major regulator of inflammatory signals in most tissues in the nuclear transcription factor NFkB. This system is regulated primarily by the redox-regulated kinase IKK-β. Shoelson and colleagues have made a strong case for the involvement of activation of this system in cases of insulin resistance. Interestingly, the ability of dietary interventions to impact insulin sensitivity is reduced in mice in which the activating kinase is inhibited or where the expression of the kinase is reduced by genetic means [53,54] and activation of the kinase results in insulin resistance [55]. The expression of this activating kinase in myeloid-derived cells may play a key role in connecting dietary excess and obesity with inflammation and insulin sensitivity [56]. It is also interesting that effects of the IKK-β inhibitor, sodium salicylate, and pioglitazone share some similar effects such as protection against inflammatory damage in the pancreatic islets [57].

5. Insulin resistance as a physiological response

Although we have come to view insulin resistance as a pathology unto itself, it important to note that insulin sensitivity is something that is physiologically regulated. That is, in a given individual the relative sensitivity to tissues to a given concentration of insulin varies throughout the day and in various states. It is easy to imagine that this is accomplished by variation in circulating counter-regulatory hormones and other neural and paracrine factors as well as by intracellular control mechanisms. Such complex regulation undoubtedly combines with regulation of hormone levels (insulin secretion and clearance) to provide fine tuning for a physiologically relevant response. By what is the physiological advantage of insulin resistance? It must serve a purpose. Perhaps some of the reduction of insulin action serves to protect against excessive oxidative metabolism. Perhaps the signal is a metabolic inflammatory response to protect against cellular oxidative damage that coordinates with a dampening of insulin action as well as in longer loop protection against reactive radicals in the face of continued supply of inappropriate oxidation.

6. Metabolic inflammation, a potential protection against reactive oxygen species

It is now clear that mitochondria are a major source of the generation of intracellular reactive free radicals including reactive oxygen species (ROS), hydroxyl radicals, and reactive nitrogen species [58]. Furthermore, mitochondrial DNA is unprotected and susceptible to damage by reactive radicals. If mitochondrial generation of ROS were continued unchecked, cumulative damage would lead to severe cellular dysfunction and death. It is also known the reactive radicals are a common mechanism for activation of a variety of inflammatory signals by redox reactions including activation of specific kinases or deactivation of specific phosphatases [59]. Thus, in the presence of excess oxidation for prevailing protective mechanisms (determined by genetic and environmental factors), an inflammatory response would ensue leading to a dampening of insulin action. An example of a biochemical link to the short loop correction would be phosphorylation of insulin signaling molecules [60]. Activation of this inflammatory system would also activate NFkB with generation of multiple changes in gene expression including mechanisms to increase inflammatory cytokines and chemokines. Excess nutrients would also tend to dampen fatty acid oxidation directly by reducing the activation of AMP kinase, increasing malonyl CoA levels and breaking fatty acid entry into the mitochondria [61]. The build up of long chain fatty acyl CoAs

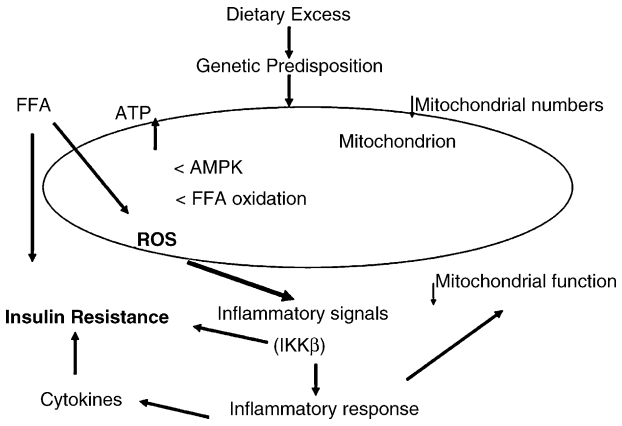

Fig. 2 – Metabolic inflammation. Excess nutrients, particularly free fatty acids (FFA), for the given genetic background/environmental conditions generate excessive oxidation resulting in reactive oxygen species (ROS) that trigger a metabolic inflammatory response. Protective mechanisms triggered include dampening of insulin action locally by phosphorylation of insulin signaling molecules as well as a propagated inflammatory response elevating circulating cytokines and chemokines. This is accomplished by ROS signals that activate IKKβ and perhaps other mechanisms controlling regulatory proteins. Increased release of cytokines and chemokines (e.g. TNFα and IL-8) exerts a longer loop reduction of insulin action. Protection against excessive oxidation is also achieved by a reduced number and function of mitochondria. As discussed in the text, reduced activation of AMP kinase under these conditions contributes to reduced oxidation of FFA and the build up of long chain acyl CoAs also contributes to local insulin resistance. Against this background, insulin sensitizing TZDs may protect by modification of mitochondrial signals, thus preventing the metabolic inflammation and impacting all of the connected components depicted in Fig. 1.

would also contribute directly to the insulin resistance [62]. There must also be signals involved to decrease overall mitochondrial function and mitochondrial numbers as a longer-term control loop. A schematic summary of this hypothesis is shown in Fig. 2.

According to this hypothesis, metabolic syndrome begins as a normal physiological response to excess nutrients for a specific situation. There is a known link between diet and inflammation [63,64]. As discussed above, the resulting phenotype will be determined by the complexity of the genetic and environmental background. This would explain the large incidence but diverse nature of presentation of metabolic syndrome. The thesis put forth in this commentary suggests that the TZDs are able to exert their pleiotropic effects by breaking this response at the level of their mitochondrial effects. This may include reduction the number of free radicals associated with the mitochondrial β-oxidation of fatty acids [65] and activation of AMP kinase by reducing mitochondrial coupling to energy production [42,66,67]. Activation of AMP kinase and perhaps other yet to be identified signals also increase the expression of PGC1-α resulting in an increase in the number of mitochondria [42,51,52].

7. Cellular specific effects produced by a mitochondrial effect of the TZDs

As discussed above, diabetes and insulin resistance are characterized by what appears to be a decrease in mitochondrial function and numbers in adipose tissue and skeletal muscle. There are clear and consistent experimental data demonstrating that TZDs can increase the expression of mitochondrial proteins in adipose tissue in animals and in man. It is not yet been shown that this happens in all tissues. It is possible that TZDs may have a similar biochemical action in mitochondria from all tissues but that the overall consequence of this may be cell specific perhaps depending on cellular function or mitochondrial coupling.

The effects of the TZDs on adipose tissue obviously result in changes in the output of potential regulatory factors including cytokines and hormones [68–70]. This may contribute to the overall metabolic effects and to balance of adipose stores and the interplay between adipose and macrophages [70]. In this respect, it is important to note that the overall pharmacology of these molecules may depend on the blend of the mitochondrial and non mitochondrial (e.g. PPARγ agonsim) characteristics of a given molecule. This may affect fluid balance as well as adipose stores. Using the powerful technique of metabolic profiling with stable labels and the SIDMAP technology, it was recently shown that marketed TZDs with more inherent PPARγ-activating activity result in much less anaplerosis, or return of carbon to mitochondrial metabolism, than a more mitochondrially specific analog in HepG2 cells [71]. The marketed drugs appeared to result in more peroxisomal oxidation of long chain fatty acids. It is not yet known if similar effects occur in other cell types, although these metabolic changes could be key to relative anti-inflammatory or metabolic actions of these compounds. As discussed above in "2", PPARγ agonism may also drive ectopic adipogensis and changes in fluid space. Therefore compounds that limit this interaction may have improved therapeutic characteristics.

The endothelium probably represents a key connection between metabolic inflammation and cardiovascular disease. Endothelial dysfunction is an early and common defect observed in essential hypertension and in metabolic syndrome [72]. Increased reactive oxygen radicals would reduce NO generation while resulting in the increased production of highly reactive and potentially damaging reactive nitrogen radicals. This not only reduces the ability of the endothelium to generate vasorelaxation contributing to hypertension, but also results in cellular damage by modification of key proteins [73]. The generation of ROS and the dampening of NO production can be demonstrated in the acute presence of excess extra cellular free fatty acids both in vitro [74] and in vivo [75] resulting in decreased endothelial-regulated vasorelaxation. The mechanism by which insulin's ability to produce vasorelation is down regulated by the metabolic inflammation likely follows the general mechanism as shown in Fig. 2. However, in addition to this physiological down

regulation of the response, reactive oxygen radicals also would remove NO generated with the consequence that the resulting peroxynitrite and reactive nitrogen radicals could lead to damage of the endothelium [73]. While it is generally accepted that endothelial damage contributes significantly to late stage complications [76], the suggestion here is that metabolic inflammation in the endothelium plays a point early on in the development of hypertension per se.

It is interesting to speculate that the metabolic inflammation to favor vasoconstriction may be part of an ancient invention that functions in conjunction with prothrombotic biochemistry to preserve vascular integrity in the face of a local injury.

8. Implications and future directions suggested by the metabolic inflammation and mitochondrial TZD action hypotheses

The hypotheses put forth in this commentary suggest that insulin resistance and propensity towards weight gain and obesity may be *caused* by metabolic dysfunction rather than vice versa. That is, as a protection against excessive or inappropriate oxidative metabolism, insulin action and mitochondrial function are dampened. According to this thesis, a metabolic inflammation is the signaling mechanism that accomplishes these tasks with the eventual effect to reduce mitochondrial function and numbers. One could imagine that this would contribute to a cycle of weight gain, but that the overall expression of individual phenotypes depends on many factors. The common result of the resulting metabolic syndrome, however, is an increase in many of the risk factors for cardiovascular disease.

This commentary also suggests that TZDs or molecules with similar mitochondrial interactions should be able to exert insulin sensitizer pharmacology (reversal of all metabolic syndrome abnormalities) without directly impacting the nuclear receptor PPARγ. This runs contrary to the prevailing view that activation, or partial activation, of PPARγ is part and parcel to the pharmacology of these compounds (e.g. [29–32]). As discussed above, avoidance of PPARγ activation should limit the major dose-limiting side effects encountered with the first generation of TZD insulin sensitizers. In support of this view, we have recently shown that PNU-91325, and analog with greatly reduced capacity to activate PPARγ in vitro and in vivo [42] exerts the entire insulin sensitivity pharmacology including more favorable effects on blood pressure in Dahl salt-sensitive rats than does the more potent PPARγ agonist, rosiglitazone [77].

An important issue to address at this point is the question of why so many of the analog programs designed to find nuclear receptor activators (e.g. [29–32,78,79]) exert overlapping pharmacology. It is reasonable to assume that all of these TZDs and related compounds are mimicking key endogenous fatty acid metabolites that interact with proteins containing similar binding sites. For example, Brunmair and colleagues noted that all known PPARγ agonists *and* antagonists, regardless of structure, affect mitochondrial coupling [66]. It will be important to determine whether these affects a mediated by a direct interaction with mitoNEET, the protein identified as being crosslinked by a TZD photoprobe, or perhaps to an associated protein (or proteins) that is (are) yet to be identified [41].

Finally, these considerations suggest several important questions that should be addressed by future research programs: (1) What is the molecular nature of the interactions with mitochondria that gives rise to insulin sensitizing pharmacology? (2) What is the function of mitoNEET, the protein crosslinked by the TZD photoprobe? (3) What is the nature and the key mediators (redox signals?) involved in the transmission of the metabolic inflammation signaling? (4) Can metabolic inflammation be controlled by dietary modifications and/or a combination of diet and therapeutic agents? (5) Are there other targets (perhaps affecting fatty acid oxidation [71]) that can be exploited to find novel therapeutic agents?

If progress can be made in these areas, it may be possible to create effective treatment strategies to impact the key related medical areas known as metabolic syndrome in a manner that will significantly impact our health care costs.

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