Is Oxidative Stress the Pathogenic Mechanism Underlying Insulin Resistance, Diabetes, and Cardiovascular Disease? The Common Soil Hypothesis Revisited

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Abstract—Type 2 diabetes is a worldwide increasing disease resulting from the interaction between a subject’s genetic makeup and lifestyle. In genetically predisposed subjects, the combination of excess caloric intake and reduced physical activity induces a state of insulin resistance. When beta cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance appears, characterized by excessive postprandial hyperglycemia. Impaired glucose tolerance may evolve into overt diabetes. These 3 conditions, ie, insulin resistance, impaired glucose tolerance, and overt diabetes, are associated with an increased risk of cardiovascular disease. Because all these conditions are also accompanied by the presence of an oxidative stress, this article proposes oxidative stress as the pathogenic mechanism linking insulin resistance with dysfunction of both beta cells and endothelium, eventually leading to overt diabetes and cardiovascular disease. This hypothesis, moreover, may also contribute to explaining why treating cardiovascular risk with drugs, such as calcium channel blockers, ACE inhibitors, AT-1 receptor antagonists, and statins, all compounds showing intracellular preventive antioxidant activity, results in the onset of new cases of diabetes possibly being reduced. (Arterioscler Thromb Vasc Biol. 2004;24:816-823.)

Key Words: oxidative stress ■ insulin resistance ■ impaired glucose tolerance ■ diabetes ■ cardiovascular disease

In the past few decades, type 2 diabetes mellitus (T2DM) has rapidly increased in the world. It has been estimated that the number of diabetic patients will more than double within 15 years.1 Moreover, although T2DM was previously considered a slow-onset disease of middle-aged and older subjects, an emerging issue is the recent increase in diagnoses of T2D and prediabetic conditions in children.2 T2DM is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease (CVD),3 so that it has been suggested that diabetes may be considered a cardiovascular disease.4 However, CVD risk is elevated long before the development of diabetes.4 The close relationship between T2DM and CVD has led to the "common soil" hypothesis,5 postulating that T2DM and CVD share common genetic and environmental antecedents. One of the most important of these possible antecedents is considered insulin resistance. In genetically predisposed subjects, the combination of excess caloric intake and relatively scarce physical activity, with the likely consequence of obesity, can induce a state of resistance to the action of insulin.7 Insulin resistance is an important component of the metabolic syndrome, first described as a clinical syndrome in which the clustering of factors such as obesity, dyslipidemia, and hypertension leads to a substantial increase in CVD risk.8 Insulin resistance is also a crucially important metabolic abnormality in T2DM, and overt diabetes is thought to be preceded by a long period of insulin resistance, during which blood glucose is maintained near normal levels by compensatory hyperinsulinemia.7 When β cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance (IGT) appears.7 This condition is characterized by an excessive blood glucose concentration in the postprandial phase, with fasting glucose being in the normal range.7 Persistence of imbalance between caloric intake and expenditure eventually leads to overt diabetes, characterized by high glycemia in any condition whether fasting or postprandial.7 Studies currently performed in children point out the co-existence of obesity, insulin resistance, and β-cell dysfunction, as occurs in classic older T2DM-prone subjects.9 The mix of overfeeding and sedentary habits has extended to children.

These 3 conditions, ie, insulin resistance, IGT, and overt diabetes, appear to be associated, although to a variable degree, with an increased risk of CVD.10,11 Recent trials have confirmed the hypothesis that lifestyle modifications, in terms of reduced caloric intake and increased physical activity, can reduce the incidence of new cases of diabetes.12,13 In other intervention trials, the same goal has been attained by means of several drugs.

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There have been studies specifically aimed to demonstrate the ability of known antidiabetic drugs, ie, metformin, troglitazone, and acarbose, to hamper the evolution from IGT to diabetes. Each of the 3 drugs was successful in that regard. Interestingly, although metformin and troglitazone were expected to act by abating insulin resistance, and secondarily hyperglycemia, acarbose lowers postprandial hyperglycemia by impairing carbohydrate absorption from the intestinal lumen without any direct effect on insulin resistance. It appears, then, that prevention of the development of diabetes is obtainable by simply lowering postprandial glycemic peaks. However, the picture is more intricate. In studies aimed to reduce the incidence of cardiovascular disease in high-risk populations by means of calcium channel blockers (CCBs), angiotensin-converting enzyme (ACE) inhibitors, angiotensin-1 (AT-1) receptor antagonists, and statins, all of which are devoid of any effect on glycemia, a significant reduction of new cases of diabetes has been incidentally discovered. It appears that in the prevention of diabetes, a direct action on insulin resistance is not a requisite, nor is the reduction of postprandial hyperglycemia. The question now is what type of effect do CCBs, ACE inhibitors, AT-1 receptor antagonists, and statins have that are responsible for the prevention of diabetes? Do these compounds share any mechanism of action with the aforementioned antidiabetic drugs? It has been shown that CCBs, statins, ACE inhibitors, and AT-1 receptor antagonists have a strong intracellular “preventive” antioxidant activity, and it has been suggested that many of their beneficial ancillary effects, such as a decrease in cardiovascular mortality not fully accounted for by hypotensive or lipid-lowering effects, may be caused by this property.

If we consider that glitazones are intracellular antioxidants, too, and that postprandial hyperglycemia itself produces an oxidative stress, so that acarbose (an inhibitor of intestinal glucose absorption) and glinides (ie, repaglinide, nateglinide, and metlinide, which restore the first phase of insulin secretion) may be expected to reduce oxidative stress by specifically lowering postprandial hyperglycemia, then the antioxidant effect is the only known property that all of these drugs have in common.

Because evidence suggests that overnutrition, insulin resistance, IGT, diabetes, and CVD share in common the presence of an oxidative stress, in this article oxidative stress generation is proposed as the common persistent pathogenic factor mediating the appearance of insulin resistance as well as the passage from insulin resistance to overt diabetes, via IGT, while producing the increased cardiovascular risk condition typical of prediabetic and diabetic subjects by favoring atherosclerotic complications. This hypothesis may help us understand why diverse therapeutic interventions, which have in common the ability to reduce oxidative stress, can impede or delay the onset of diabetes and CVD.

From Overfeeding to Insulin Resistance: The Role of Oxidative Stress

The most important tissues involved in the pathogenesis of insulin resistance are muscle and adipose tissue. When caloric intake exceeds the energy expenditure, the substrate-induced increase in citric acid cycle activity generates an excess of mitochondrial NADH (mNADH) and reactive oxygen species (ROS). To protect themselves against harmful effects of ROS, cells may reduce the formation of ROS and/or enhance ROS removal. Prevention of ROS formation is accomplished by preventing the build-up of mNADH by inhibiting insulin-stimulated nutrient uptake and preventing the entrance of energetic substrates (pyruvate, fatty acids) into the mitochondria.

Controversy exists as to whether free fatty acid (FFA) or glucose is the primary fuel source in the overnourished muscle and adipose tissue. In either case, an influx of substrates into the citric acid cycle generates mitochondrial acetyl-CoA and NADH. Acetyl-CoA, derived either from glucose through pyruvate or from beta-oxidation of FFA, combines with oxaloacetate to form citrate, which enters the citric acid cycle and is converted to isocitrate. NAD-dependent isocitrate dehydrogenase generates NADH. When excessive NADH cannot be dissipated by oxidative phosphorylation (or other mechanisms), the mitochondrial proton gradient increases and single electrons are transferred to oxygen, leading to the formation of free radicals, particularly superoxide anion (Figure 1). The generation of excessive NADH may be prevented in several ways, one of which is the inhibition of FFA oxidation. An increase in intracellular FFA, in turn, leads to reduced GLUT4 translocation to the plasma membrane, resulting in resistance to insulin-stimulated glucose uptake in muscle and adipose tissue.

In this setting, insulin resistance may be considered a compensatory mechanism that protects the cells against further insulin-stimulated glucose and fatty acid uptake and therefore oxidative damage.

Many studies support this hypothesis: in vitro studies and in animal models, antioxidants have been shown to improve insulin sensitivity. Several clinical trials have demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals, although there is evidence from molecular biology studies to support the possibility that oxidative stress alters the intracellular signaling pathway inducing insulin resistance. The recent finding that insulin resistance is associated in humans with reduced intracellular antioxidant defense also support this hypothesis.

Oxidative Stress as a Common Pathogenic Factor for the Dysfunction of Beta and Endothelial Cells

It is a reasonable hypothesis that what happens in muscle and fat cells may also occur in other cells, particularly in β cells and endothelial cells. Moreover, these cell types may be particularly affected by overfeeding. These cells are notably not dependent on insulin for glucose uptake, which here is via facilitative diffusion instead of insulin-regulated glucose transporters. Therefore, if overfed, they cannot downregulate the influx of nutrients by means of insulin resistance, and must allow intracellular concentrations to increase further.

Many studies have suggested that β-cell dysfunction results from prolonged exposure to high glucose, elevated FFA levels, or a combination of both. β Cells are particularly sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, gluthathione per-
There is a strong evidence linking high glucose and free fatty acids (FFA) with increased oxidative stress in diabetes. In diabetes, oxidative stress leads to mitochondrial dysfunction, which is a key event in the activation of all other pathways involved in diabetic complications. The role of free radical generation in producing the initial damage affects the arterial endothelium in terms of endothelial dysfunction. Notably, today's evidence confirms that endothelial dysfunction, associated with oxidative stress, predicts cardiovascular disease. Insulin resistance is associated with impaired endothelial function. Glucose and FFA overload may be supposed to influence endothelial cells, as well as β cells, producing an endothelial dysfunction through an oxidative stress.

The response-to-injury hypothesis of atherosclerosis states that the initial damage affects the arterial endothelium in terms of endothelial dysfunction. Notably, today’s evidence confirms that endothelial dysfunction, associated with oxidative stress, predicts cardiovascular disease. Insulin resistance is associated with impaired endothelial function. Glucose and FFA overload may be supposed to influence endothelial cells, as well as β cells, producing an endothelial dysfunction through an oxidative stress.

Indeed, many studies show that high glucose concentrations induce endothelial dysfunction. In vitro, the direct role of hyperglycemia has been suggested by evidence that arteries isolated from normal animals and subsequently exposed to exogenous hyperglycemia exhibit attenuated endothelium-dependent relaxation. Consistently, in vivo studies have also shown that hyperglycemia directly induces, in diabetic subjects and nondiabetic subjects, endothelial dysfunction.

The role of free radical generation in producing the hyperglycemia-dependent endothelial dysfunction is suggested by studies showing that in vitro and in vivo the acute effects of hyperglycemia are counterbalanced by antioxidants.

Recent studies demonstrate that a single hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron transport chain seems to be the first and key event in the activation of all other pathways involved.
in the pathogenesis of endothelial dysfunction in the case of hyperglycemia. Superoxide overproduction is accompanied by increased nitric oxide generation, caused by eNOS and iNOS uncoupled state. A phenomenon favoring the formation of the strong oxidant peroxynitrite, which in turn damages DNA. DNA damage is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP-ribose) polymerase. Poly(ADP-ribose) polymerase activation in turn depletes the intracellular concentration of its substrate NAD⁺, slowing the rate of glycolysis, electron transport, and ATP formation, and produces an ADP-ribosylation of the GAPDH. These processes result in acute endothelial dysfunction. Convincingly, FFA may work the same way: FFA increases oxidative stress generation in humans and induces endothelial dysfunction, which can be reversed by antioxidants.

From Insulin Resistance to IGT: The Role of Oxidative Stress
Initially, insulin resistance is compensated by hyperinsulinemia through which a normal glucose tolerance is preserved. Deterioration to IGT occurs when insulin resistance increases further and/or the compensatory insulin secretory response decreases. An increase in insulin, FFA, and/or glucose levels can increase ROS production and oxidative stress, as well as activate stress-sensitive pathways. This, in turn, can worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes.

IGT, ie, postprandial hyperglycemia with fasting glycemia in the normal range, is a risk factor for increased cardiovascular mortality. Many studies show that postprandial hyperglycemia is associated with oxidative stress generation.

A loss of early-phase insulin response is a common event in subjects with impaired glucose metabolism. This alteration may not simply be a marker of the risk of diabetes, but rather an important pathogenic mechanism causing excessive postprandial hyperglycemia.

In response to intravenous glucose, insulin secretion is biphasic. The first phase is a rapid release of insulin into the bloodstream in response to the ingestion of carbohydrates or a mixed meal. The rapid increase in portal blood insulin concentration and the avid binding of the hormone to its receptors on liver cell membranes account for a prompt suppression of endogenous glucose production and a reduced rate of increase in plasma glucose concentrations. In experiments performed in animals and humans, the selective abolition of early insulin secretion in healthy subjects resulted in IGT, excessive glycemic excursions, and possible hampering of the thermic effects of ingested carbohydrates. In nondiabetic subjects, the loss of early insulin secretion is a determinant for the subsequent development of diabetes. The critical role of the early-phase insulin response in determining postprandial hyperglycemia is supported by the demonstration that glucose tolerance is improved by restoring the acute increase in plasma insulin concentrations after the ingestion of both glucose and a mixed meal. This amelioration of the glycemic profile can prevent late hyperglycemia and hyperinsulinemia. Oxidative stress contributes, in vivo, to specifically alter the early phase of insulin secretion because the latter can be restored by antioxidants. Moreover, it has been proposed that mitochondrial overproduction of free radicals is a potential mechanism causing impaired first phase of glucose-induced insulin secretion.

Evidence indicates that postprandial hyperglycemia is directly implicated in the development of cardiovascular disease, whereas evidence linking fasting glycemia to diabetic complications is inconclusive as yet. Moreover, in many studies postprandial glycemia is a better predictor of the cardiovascular risk than Hba1c, which reflects both fasting and postprandial blood glucose levels. Postprandial glucose may be directly involved in cardiovascular complications through a toxic effect on the vascular endothelium, mediated by oxidative stress. This atherogenic effect appears to be independent of other cardiovascular risk factors such as hyperlipidemia.

From IGT to Diabetes and Endothelial Dysfunction
Repeated exposure to hyperglycemia and increased levels of FFA can lead to β-cell dysfunction that may become irreversible over time.

In its initial stages, this damage is characterized by a reversible defective insulin gene expression. Glucose and lipid toxicity induce the gradual, time-dependent establishment of irreversible damage to cellular components of insulin production, and, therefore, to insulin content and secretion. Oxidative stress is convincingly the mediator of such damage.

Recent studies in type 2 diabetic animal models report that the progressive reduction of islet β cells is associated with excessive oxidative stress. In these animal models, when hyperglycemia is allowed to continue, a so-called glucotoxicity to β cells impairs insulin secretion and eventually causes fatal islet cell injury, accelerating β cell loss. Consistently, Japanese type 2 diabetic patients show a reduction of β-cell mass and evidence of increased oxidative stress-related tissue damage that is correlated with the extent of the β-cell lesions.

Vascular function in diabetes mellitus has been studied extensively in both animal models and humans. Impaired endothelium-dependent vasodilation has been a consistent finding in animal models of diabetes induced by alloxan or streptozotocin. Similarly, studies in humans with insulin-dependent and non-insulin-dependent diabetes have found endothelial dysfunction when compared with vascular function in nondiabetic subjects. Strong evidence suggests that oxidative stress is the mediator of impaired endothelial function in diabetes.

The Possible Link Between Oxidative Stress and Inflammation in Insulin Resistance, Diabetes, and CVD
Although the concept of atherosclerosis as an inflammatory disease is now well established, line of evidence suggests that chronic inflammation may be involved in the pathogenesis of insulin resistance and T2DM. This lead to the hypothesis that inflammatory changes may be considered a common pathogenic step in all of these conditions.
The concept that oxidative stress is the common factor underlying insulin resistance, T2DM, and CVD, and may explain the presence of inflammation in all these conditions. It is well recognized that inflammation is one manifestation of oxidative stress, and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress.

Interestingly, it has recently been proposed that the subclinical pro-inflammatory state observable in many conditions including atherosclerosis, cancer, and aging is caused by a mitochondrial overgeneration of free radicals. Moreover, the hypothesis is supported by in vivo studies, showing that FFA and glucose induce inflammation through oxidative stress, have a cumulative and independent effect, and that antioxidants reverse the phenomenon.

Oxidative Stress as the Connection Between Nutrition Overload and Diabetes and Related Cardiovascular Complications:

Therapeutic Implications

Available evidence leads to the hypothesis, summarized in Figure 2, that oxidative stress can be considered the clue to the association of overnutrition with the development of overt diabetes. It may also link the progressive β-cell failure to an increased cardiovascular risk, a prominent association in the clinical setting.

However, this hypothesis can also contribute to understand why different therapeutic strategies, apparently having in common only the ability to reduce oxidative stress, appear to simultaneously lead to decreased cardiovascular mortality and lower incidence of diabetes. If oxidative stress is the pathogenic mechanism leading from insulin resistance to overt diabetes, the ability of a drug to prevent or reverse oxidant stress can account for its clinical usefulness.

Furthermore, the beneficial effect of controlling postprandial hyperglycemia on both the development of diabetes and the prevention of cardiovascular disease also supports this hypothesis, because it has been shown that in the postprandial state there is an oxidative stress generation, which is strictly dependent on the level of glycemia reached.

However, even convincing evidence is now available supporting the hypothesis that oxidative stress may play a key role in the development of both diabetes and CVD clinical trials with antioxidants, in particular with vitamin E, have failed to demonstrate any beneficial effect.

On this matter, it has recently been suggested that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already formed oxidants and may, therefore, be considered a more “symptomatic” rather than a causal treatment for oxidative stress.

According to the evidence discussed in this article, it is suggested that interrupting the overproduction of superoxide...
by the mitochondrial electron transport chain would normalize the pathways involved in the development of the oxidative stress.\textsuperscript{86} It might, however, be difficult to accomplish this using conventional antioxidants, because these scavenge ROS in a stoichiometric manner. However, while waiting for more focused tools,\textsuperscript{87} CCBs,\textsuperscript{21} statins,\textsuperscript{22} ACE inhibitors,\textsuperscript{23} and AT-1 receptor antagonists\textsuperscript{24} and glitazones,\textsuperscript{25} which have a strong ability to prevent intracellular oxidant activity,\textsuperscript{86} seem to be valid options already available. This topic has been extensively reviewed.\textsuperscript{21,85,86} This concept is also summarized in Figure 3.

In conclusion, a puzzle of many pieces of evidence suggests that free radical overgeneration may be considered the key in the generation of insulin resistance, diabetes, and cardiovascular disease. Even if a change in lifestyle remains the best preventive and therapeutic approach, many new specific and causal antioxidants are being developed\textsuperscript{85,86} and may become important tools to oppose the increasing epidemic of diabetes, a real emergency in our future. Moreover, this concept can explain why treating cardiovascular risk with drugs such as CCBs, ACE inhibitors, AT-1 receptor antagonists, and statins may also prevent diabetes. Last but not least, because it has been demonstrated that insulin resistance is associated in humans with reduced intracellular antioxidant defense\textsuperscript{88} and that diabetic subjects prone to complications may have a defective intracellular antioxidant response,\textsuperscript{88,89} even what we call genetic predisposition to diabetes, as well as liability to its late complications, might be based on a deficient ROS-scavenging ability in $\beta$ cells and/or in target tissues such as endothelium.

References


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