Diabetes mellitus is a major source of morbidity in developed countries and, among its comorbid conditions, atherosclerosis is perhaps the most important. Since the availability of insulin, up to three fourths of all deaths among diabetics can be directly attributed to coronary artery disease (CAD). In adult patients with diabetes, the risk of CAD is 3- to 5-fold greater than in nondiabetics despite controlling for other known CAD risk factors. In patients with IDDM, up to one third will die of CAD by the age of 50 years. A number of known risk factors for CAD, such as hypertension, central obesity, and dyslipidemia, are more common in diabetics than in the general population. Despite this prevalence of risk factors, no more than 25% of the excess CAD risk in diabetes can be accounted for by known risk factors. Thus diabetes represents a major contributing factor to the CAD burden in the developed world, and most of the excess attributed risk of CAD in diabetics cannot be readily quantified with the use of traditional risk factor analysis.

Diabetes is associated with a variety of metabolic abnormalities, principle among which is hyperglycemia. The relation between hyperglycemia and CAD is the subject of considerable debate because serum glucose does not consistently predict the existence of CAD. Presumably, this confusion stems from the reliance on a single blood glucose measurement, as recent prospective data have clearly established a link between a marker for chronic average glucose levels (HbA1C) and cardiovascular morbidity and mortality. There is considerable controversy with respect to the precise mechanism by which hyperglycemia may contribute to the development of CAD in diabetes. Established sequelae of hyperglycemia, such as cytotoxicity, increased extracellular matrix production, and vascular dysfunction, have all been implicated in the pathogenesis of diabetes-induced vascular disease.

Among the sequelae of hyperglycemia, excessive oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. The established association between atherosclerosis and lipid peroxidation within the vascular wall has led to a renewed interest in the oxidative stress of hyperglycemia as a potential mechanism for diabetic vascular disease. Early evidence to support the association of oxidative stress and hyperglycemia was largely indirect. For example, the plasma from diabetic subjects contains increased levels of thiobarbituric acid-reactive substances and lipid hydroperoxides, two markers of lipid peroxidation. A major problem with such studies is assay specificity because the determination of thiobarbituric acid-reactive substances or lipid hydroperoxides in biological samples is prone to artifact.

The molecular mechanism of biological oxidation by glucose was first identified in 1912 by Louis Maillard. This French chemist described a brown color that formed from heating solutions of carbohydrates and amines and termed this process the “reaction du Maillard.” The Maillard reaction accounts for the glucose-dependent, nonenzymatic covalent modification of proteins that accompanies hyperglycemic states. Initially, the Maillard reaction involves the combination of the aldehyde group of glucose in the open-chain form with amine groups on proteins to form a Schiff base followed by Amadori rearrangement to form fructoselysine. This reversible glycosylation of amino groups, or glycation, underlies the formation of HbA1c, the well-recognized marker of chronic glycemic control in diabetes mellitus, and is not of any direct pathophysiological significance for the complications of diabetes. By contrast, the final stage of the Maillard reaction involves the irreversible oxidation, or glycoxidation, of fructoselysine to yield a host of advanced glycation end products (AGEs) such as N-(carboxymethyl)lysine, pentosidine, and pyrroline, the formation of which correlate directly with the vascular and renal complications of diabetes mellitus. These carbohydrate-derived protein oxidation products are readily quantified with gas chromatography/mass spectroscopy and are more abundant in diabetics than inagematched control subjects.

Unlike the quantitation of AGEs and AGE-modified proteins, the quantitation of lipid peroxidation in the setting of hyperglycemia has been more problematic. Recently, a novel class of prostanoid-like compounds has been described by Morrow and Roberts (for review, see Reference 10). These compounds, known collectively as F2-isoprostanes, are specific nonenzymatic oxidation products of arachidonic acid that form in situ on esterified phospholipids and are subsequently released in the free form, presumably through the action of phospholipases. In the quantification of oxidative stress, the determination of F2-isoprostanes has proven quite useful as a marker of lipid peroxidation both in vitro and in vivo. Pathological conditions known to involve a heightened state of oxidative stress, such as carbon tetrachloride poisoning and smoking, are characterized by increased production of F2-isoprostanes. With respect to diabetes, Gopaul and...
have found that plasma levels of esterified 8-epi-PGF$_{2\alpha}$, a prototypical F$_2$-isoprostane, are 3-fold higher in patients with NIDDM than in normal control subjects, unequivocally demonstrating an association between NIDDM and increased lipid peroxidation. However, the precise role of enhanced lipid peroxidation, or F$_2$-isoprostanes in particular, in the vascular pathology associated with diabetes mellitus remains to be determined.

In this issue of Circulation, Davi and colleagues have provided an important first step in understanding the implications of lipid peroxidation and increased production of 8-epi-PGF$_{2\alpha}$ in diabetic vascular disease. Using a specific radioligand assay, these investigators found that urinary excretion of 8-epi-PGF$_{2\alpha}$ in NIDDM and IDDM patients was essentially twice that of healthy age-matched control subjects. This increase in 8-epi-PGF$_{2\alpha}$ was significantly correlated with increased platelet activation as determined by urinary levels of TXB$_{2}$, the major metabolite of thromboxane A$_2$, and with blood glucose in NIDDM patients. On the basis of prior studies indicating that 8-epi-PGF$_{2\alpha}$ amplifies agonist-induced platelet aggregation, Davi and colleagues concluded that increased lipid peroxidation in NIDDM leads to the formation of 8-epi-PGF$_{2\alpha}$ which, in turn, leads to platelet activation. In support of this hypothesis, the authors found that reducing 8-epi-PGF$_{2\alpha}$ formation through improved metabolic control or vitamin E supplementation also reduced platelet activation as reflected by a reduction in urinary excretion of TXB$_2$. Importantly, they also showed that by inhibiting platelet function with aspirin or indobufen, there was no change in the 8-epi-PGF$_{2\alpha}$ levels, indicating that the oxidative stress in these diabetics was likely to be a cause and not a consequence of platelet activation. Taken together, these data indicate that increased lipid peroxidation in NIDDM has important implications for vascular disease in diabetes.

Despite the accumulating evidence for increased lipid peroxidation in diabetes, the source of this oxidative stress in not known. Both plasma and urinary levels of 8-epi-PGF$_{2\alpha}$ are increased in patients with NIDDM. With regard to urinary 8-epi-PGF$_{2\alpha}$ excretion, a similar finding was observed for IDDM patients as well. These concordant observations between NIDDM and IDDM patients would tend to implicate hyperglycemia as the culprit metabolic derangement, since this is a major common feature of both patient populations. Consistent with this notion, Davi and colleagues found that improved metabolic control of NIDDM patients significantly reduced urinary 8-epi-PGF$_{2\alpha}$ levels by 32%. Reports that improved glycemic control by pancreatic islet transplantation reduces vascular oxidative stress and reverses antioxidant enzyme upregulation in rats with streptozotocin-induced diabetes are consistent with hyperglycemia as a source of oxidative stress. Taking together, these data suggest that the extent of metabolic control has a profound influence on the degree of oxidative stress in patients with diabetes.

In addition to AGE formation by oxidation of fructosel-yse, there are a number of other putative mechanisms that link hyperglycemia to oxidative stress. Among the most direct is the autoxidation of glucose. Monosaccharides with an ß-hydroxyaldehyde structure, like glucose, are subject to enediol rearrangement that results in the formation of an enediol radical ion. The formation of this radical anion has two important implications. First, this species is capable of reduced molecular oxygen to form superoxide anion which, under certain circumstances, may contribute to the oxidation of lipids or the activation of platelets. Second, the dicar-bonyl products formed by this pathway are quite reactive and may modify adjacent lysine groups to form AGEs such as N$^\epsilon$-(carboxymethyl)lysine directly. These reactions derived from glucose enolization are, however, dependent on transition metal ions, and the availability of free, redox-active transition metal ions in vivo is controversial. Recent data demonstrating glycation-induced ceruloplasmin fragmenta-tion and free copper release offer one possible mechanism for a source of extracellular transition metals. As an alternative mechanism of AGE-mediated oxidative stress, AGEs have also been shown to induce cellular lipid peroxidation through interacting with their specific surface receptor (RAGE), and this effect can be attenuated by vitamin E.

Regardless of the mechanism for the synthesis of AGEs, several features of AGE action have direct bearing on the findings of Davi and colleagues. In addition to their role in lipid peroxidation, AGEs enhance the aggregation of human platelets ex vivo. AGE-modified albumin has also been shown to induce monocyte tissue factor expression and procoagulant activity. Thus AGE formation on proteins and lipids appears to contribute to both lipid peroxidation and platelet activation and may therefore contribute to the findings of Davi and colleagues.

Although there is considerable evidence at hand for increased lipid peroxidation in diabetes, arguments for a more generalized increase in oxidative stress are not secure. In vitro, glycoxidation of collagen results in the simultaneous formation of AGEs as well as the protein oxidation products ortho-tyrosine and methionine sulfoxide. Diabetic patients demonstrate an increase in AGE formation compared with age-matched control subjects but no increase in the noncarbohydrate-derived protein oxidation products ortho-tyrosine and methionine sulfoxide. These data underscore the need for further investigation into the precise molecular nature of oxidative stress in diabetes mellitus and the impact of such stress on diabetic vascular complications.

References


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