Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm

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Abstract

Long-term vascular complications still represent the main cause of morbidity and mortality in diabetic patients. Although randomized long-term clinical studies comparing the effects of conventional and intensive therapy have demonstrated a clear link between hyperglycemia and the development of complications of diabetes, they have not defined the mechanism through which excess glucose results in tissue damage. Evidence has accumulated indicating that oxidative stress may play a key role in the etiology of diabetic complications. Isoprostanes are emerging as a new class of biologically active products of arachidonic acid metabolism of potential relevance to human vascular disease. Their formation in vivo seems to reflect primarily, if not exclusively, a nonenzymatic process of lipid peroxidation. Enhanced urinary excretion of 8-iso-PGF$_{2\alpha}$ has been described in association with both type 1 and type 2 diabetes mellitus, and correlates with impaired glycemic control. Besides providing a likely noninvasive index of lipid peroxidation in this setting, measurements of specific F$_{2\alpha}$ isoprostanes in urine may provide a sensitive biochemical end point for dose-finding studies of natural and synthetic inhibitors of lipid peroxidation. Although the biological effects of 8-iso-PGF$_{2\alpha}$ in vitro suggest that it and other isoeicosanoids may modulate the functional consequences of lipid peroxidation in diabetes, evidence that this is likely in vivo remains inadequate at this time.

Keywords: Cholesterol; Coronary disease; Diabetes; Free radicals; Lipid metabolism

1. Introduction

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) [1]. 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 [1]. In patients with type 1 diabetes, up to one third will die of CAD by the age of 50 years [2]. 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 [1]. 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 [2]. 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, principal among which are insulin resistance and hyperglycemia. Insulin resistance develops from obesity and physical inactivity, acting on a substrate of genetic susceptibility [3,4]. Insulin secretion declines with advancing age [5,6], and this decline may be accelerated by genetic factors [7,8]. Insulin resistance typically precedes the onset of type 2 diabetes and is commonly accompanied by other cardiovascular risk factors: dyslipidemia, hypertension, and prothrombotic factors [9,10]. There has
been much debate as to whether or not hyperinsulinemia in subjects with type 2 diabetes may increase the coronary artery disease risk via potential adverse effects of insulin on the myocardium or coronary arteries due to growth stimulating effects or adverse effects on blood pressure, weight or lipids. In fact, CAD is often seen in subjects with newly diagnosed diabetes who could not have had a prolonged period of hyperglycemia [11]. However, in a feasibility study of intensive therapy with insulin compared with standard care in 153 men with type 2 diabetes on cardiovascular events, no differences in the rate of new events were detected between groups over a 2-year follow-up period [12]. Furthermore, the Diabetes Insulin-Glucose in Acute Myocardial Infarction (DIGAMI) study [13], with 620 diabetic subjects, showed that intensive insulin treatment was associated with a lower mortality rate than standard treatment in subjects with acute myocardial infarction (18.6% vs. 26.1%, \( P = 0.03 \)). The United Kingdom Prospective Diabetes Study [14] also showed that intensive treatment with insulin or oral sulphonylureas reduced the risk of myocardial infarction (15.8% vs. 18.1%, \( P = 0.052 \)), a reduction of borderline statistical significance. Insulin treatment did not increase the risk of cardiovascular disease. These studies suggest that the macrovascular complications of diabetes are more likely related to the degree of prevailing hyperglycemia or its associated cardiovascular risk factors and are not due to the administration of exogenous insulin.

The relation between hyperglycemia and CAD is the subject of considerable debate because serum glucose does not consistently predict the existence of CAD [2,15], especially in type 2 diabetes [15,16]. 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 [17]. There is considerable controversy with respect to the precise mechanism by which hyperglycemia may contribute to the development of CAD in diabetes. A number of equally tenable hypotheses have been suggested, including but not limited to, the Maillard, or advanced glycation end product (AGE) hypothesis [18,19], the aldose reductase hypothesis [20], oxidative stress [21–23], reductive stress (pseudohypoxia) [24,25], true hypoxia [26], carbonyl stress [27,28], altered lipoprotein metabolism [27,29,30], increased protein kinase C activity [31], and altered growth factor [32] or cytokine [33] activities. All of these hypotheses have strong proponents in academe, in medicine, and in the pharmaceutical industry. The list is long, perhaps because each hypothesis is a different reflection of an underlying common pathogenic mechanism, or perhaps because different tissues are sensitive to different mechanisms. The various hypotheses overlap and intersect with one another: AGE formation and altered polyol pathway activity may lead to oxidative stress, oxidative stress may accelerate AGE formation, reductive stress may lead to activation of protein kinase C, AGEs may induce oxidative stress and growth factor expression, and so on. The long list is a strong indication of the uncertainties in our understanding of the pathogenesis of diabetic complications.

In this article, we will present a current perspective on one of the above hypotheses, the oxidative stress hypothesis, and will discuss the relevance of F2-isoprostanes as new markers of in vivo oxidative stress.

2. Oxidative stress in diabetes mellitus

Among the sequelae of hyperglycemia, excess 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 in plasma [34] and within the vascular wall [35] has led to a renewed interest in the oxidative stress of hyperglycemia as a potential mechanism for diabetic vascular disease. Early experimental evidence have supported the association between oxidative stress and hyperglycemia, showing that plasma from diabetic subjects contains increased levels of thiobarbituric acid-reactive substances [36] and lipid hydroperoxides [37], two classic markers of lipid peroxidation.

Consistent with the concept of enhanced lipid peroxidation in diabetes, Gopaul et al. [38] have reported that the average concentration of esterified 8-iso-PGF2α (a major F2-isoprostane) in plasma from 39 patients with type 2 diabetes was approximately threefold higher than in healthy individuals. Catella-Lawson et al. [39] have reported a trend toward increased urinary 8-iso-PGF2α excretion in a group of 18 diabetics, with statistically significant elevations in patients presenting with diabetic ketoacidosis. Finally, in our recent study [40], urinary immunoreactive 8-iso-PGF2α was significantly higher in a group of 62 type 2 and 23 type 1 patients than in age-matched control subjects by approximately twofold (Fig. 1).

3. Putative mechanisms of oxidative stress in diabetes

The molecular mechanism of biological oxidation by glucose was first identified in 1912 and referred as the ‘Maillard reaction’ [41]. 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,
The most direct is the autoxidation of glucose. Monosaccharides with an α-hydroxyaldehyde structure, like glucose, are subject to enediol rearrangement that results in the formation of an enediol radical ion [43]. 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 [44] or the activation of platelets [45]. Second, the dicarbonyl products formed by this pathway are quite reactive and may modify adjacent lysine groups to form AGEs such as N\(^{-}\)-(carboxymethyl)lysine directly. These reactions derived from glucose enolization are, however, dependent on transition metal ions [43], and the availability of free, redox-active transition metal ions in vivo is controversial. Recent data demonstrating glycation-induced ceruloplasmin fragmentation and free copper release offer one possible mechanism for a source of extracellular transition metals [46]. 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) [47], and this effect can be attenuated by vitamin E [48]. Regardless of the mechanism for the synthesis of AGEs, several features of AGE action can have direct influence on the progression of atherosclerosis. In fact, in addition to their role in lipid peroxidation, AGEs enhance the aggregation of human platelets ex vivo [49]. Moreover, AGE-modified albumin has also been shown to induce monocyte tissue factor expression and procoagulant activity [50]. Thus, AGE formation on proteins and lipids appears to contribute to both lipid peroxidation and platelet activation and may therefore contribute to the rapid progression of CAD in diabetic patients.

One interesting point raised by several studies is the enzyme(s) responsible for free radical production in arterial vessel. NADH oxidase has been proposed to be a major source of superoxide anion (O\(_2^−\)) in normal and diseased blood vessels [51]. To test this hypothesis, Lund et al. [52] assessed O\(_2^−\) production in vessels from normal or diabetic rabbits that were stimulated by NADH. Superoxide production in response to NADH was ≥2-fold greater in carotid arteries from diabetic rabbits than in normals. These data suggest increased propensity to generate O\(_2^−\) levels in arterial vessels from diabetic animals during treatment with NADH. Because numerous studies suggest that the mechanism of impaired endothelium-dependent relaxation in diabetes may involve inactivation of nitric oxide (NO) by oxygen-derived free radicals [53,54], increased activity of NAD/NADH oxidase in diabetic vessel could play a pivotal role in the early stage of vascular complications in diabetes.

In tissues where glucose uptake is independent of insulin, including retina, lens, kidney, and peripheral nerves, all tissue sites of diabetic complications, exposure to elevated glucose levels causes an increase in intracellular sorbitol and fructose levels due to increased activity of
aldose reductase (AR) and sorbitol dehydrogenase (SDH) [55]. These two enzymes constitute the polyol pathway. Increased substrate flux through the polyol pathway not only increases cellular levels of sorbitol and fructose but also decreases the ratio of NADPH to NADP⁺ and increases the cytosolic NADH-to-NAD⁺ ratio [56].

The depletion of NADPH cell stores by AR may inhibit the activity of other NADPH-requiring enzymes. This possibility is supported by the finding that human umbilical vein endothelial cells exposed to 33 mmol/l glucose show a 42% decrease in NADPH concentration and a 34% reduction of glutathione release into the medium [57]. Moreover, the same endothelial cells cultured under conditions of high glucose and oxidative stress (200 μmol/l H₂O₂) reduce the supply of NADPH to various NADPH-dependent pathways, including the reduced glutathione redox cycle [58]. Decreased levels of reduced glutathione increase both the susceptibility of endothelial cells to damage by H₂O₂ [57] and the cytotoxicity by a xenobiotic (1-chloro-2,4-dinitrobenzene) in K502 cells that possess characteristics for erythroid cells [59]. On the other hand, there is no direct proof that tissue levels of NADPH are depleted in vivo in diabetes.

The hyperglycemia-induced increase in the NADH-to-NAD⁺ ratio is referred to as hyperglycemic pseudohypoxia [56] and is thought to play a role in diabetic complications. Part of this idea is based on similarities existing between the metabolic consequences of pseudohypoxia (raised NADH-to-NAD⁺ ratio) and true hypoxia. Impaired blood flow leading to ischemia has been noted in tissues exposed to diabetic complications, such as nerve, which may explain the benefit of hyperbaric oxygen [60]. Both true hypoxia and pseudohypoxia may generate free radicals: the latter via an increased synthesis of prostaglandin H₂ from prostaglandin G₂ as the enzyme hydroperoxidase uses NADH as cofactor [61], the former via ischemia/reperfusion injury [62]. A vicious cycle might be envisaged in which free radicals are both cause and consequence, at least in part, of ischemia.

Because of mutual facilitatory interactions between the main mediators of hyperglycemic damage to tissues (polyol pathway, oxidative stress, AGEs, prostanoid metabolism), it is not surprising that correction of any of them may result in amelioration of endothelium-dependent vasoconstriction. In experimental diabetic neuropathy, for example, aminoguanidine, inhibitors of AR, and antioxidants are all effective in ameliorating or preventing early neurovascular dysfunction [63].

4. Isoprostanes as new markers of in vivo lipid peroxidation

Unlike the quantitation of AGEs and AGE-modified proteins, the quantitation of lipid peroxidation in the setting of hyperglycemia has been more problematic. Recently F₂ isoprostanes, a novel class of prostanoid-like compounds has been described by Morrow and Roberts [64]. F₂ isoprostanes are a family of Prostaglandin (PG) F₂α isomers (Fig. 2) first described as products of noncyclooxygenase oxidative modifications of arachidonic acid that have resulted from free-radical attack of cell membrane phospholipids [65] or circulating LDLs [66]. In contrast to classic prosta glandins, which are formed through the action of PGH synthase isozymes from free arachidonic acid [67], F₂ isoprostanes are formed in situ from the fatty acid backbone esterified in membrane phospholipids. Isoprostanes maybe formed by either of two routes of peroxidation [68,69], an endoperoxide mechanism (Fig. 2, upper panel) or a dioxetane/endoperoxide mechanism (Fig. 2, lower panel). In the former, the first oxygen molecule is incorporated into the endoperoxide ring to form the two hydroxyl groups on the PGF ring. In the latter, by contrast, it is the second oxygen molecule that is incorporated into the PGF ring. Also 5- and 15-hydroperoxy radicals can only form Groups VI and III by the dioxetane mechanism. The radical at position 10 of arachidonic acid, by contrast, can yield isoprostanes by both mechanisms. Thus, hydroperoxy radicals formed at 8 and 12 have the option to proceed to form a dioxetane ring (Fig. 2, upper panel) or a dioxypentane ring (Fig. 2, lower panel) on a competitive basis, although it is not yet clear which is favored. Recent attention has focused upon Group VI isoprostanes. These compounds may be derived from a 9-hydroperoxy radical by the endoperoxide mechanism or from a 5-hydroperoxy radical by the dioxetane mechanism. However, both are derived from an initial hydrogen atom abstraction at position 7 of arachidonic acid. Abstraction at carbon 13 can give rise to 11- and 15-hydroperoxy radicals, yielding only one series (Group III) of isoprostanes. A radical at position 10 of arachidonic acid gives a radical at 8 and 12, which yields groups V and IV, respectively. If the dioxetane mechanism is operative, the same 8- and 12-hydroperoxy radicals will yield Groups IV and V, respectively. After their formation, isoprostanes are released from the membrane phospholipids in response to cellular activation, presumably through a phospholipase-mediated mechanism; they circulate in plasma and are excreted in the urine [70,71]. They may circulate as the free form or as esters in phospholipids in plasma. The factors that regulate release of endogenous isoprostanes from cell membranes and interconversion between the free and esterified forms are presently poorly understood. It is conjecturable that hyperglycemia could influence the release of isoprostanes from cell membranes via increased activation of protein kinase C and phospholipase A₂.

Given the ubiquitous distribution of the precursor arachidonic acid, isoprostane biosynthesis can occur in virtually all of the cellular players of the atherosclerotic lesion, including monocytes [72]. Indeed, coinubcation of activated human monocytes with LDL resulted in a time-
Fig. 2. Formation of four types of isoprostanes by the two routes of peroxidation, an endoperoxide (upper panel) or dioxetane/endoperoxide (lower panel) mechanism (modified from Lawson JA. et al., J Biol Chem 1999;274:24441–24444).
dependent formation of the F₃ isoprostane 8-iso-prostaglandin (PG) F₃₂₀ (also referred to as iPF₃₂₀-III [73]), but not of PGE₂ or thromboxane (TX) B₂, coincident with LDL oxidation [72]. The increase in 8-iso-PGF₂₀a formation was associated with an increase in thiobarbituric acid-reactive substance (TBARS) and hydroperoxide levels. This phenomenon was prevented by the oxygen free-radical scavengers superoxide dismutase (SOD) and butylhydroxytoluene (BHT) but not by cyclooxygenase inhibitors [72]. Consistent with these in vitro studies, immunolocalization of 8-iso-PGF₂₀a in the monocytes/macrophages and vascular smooth muscle cells of human atherosclerotic tissue has recently been described in specimens obtained during carotid endarterectomy [74].

In addition to a cyclooxygenase-independent mechanism of formation involving peroxidative attack on arachidonic acid, in which bicyclic endoperoxide intermediates are formed that are then reduced to give rise to isoprostanes such as 8-iso-PGF₂₀a, there is recent evidence that 8-iso-PGF₂₀a, unlike other F₂ isoprostanes, can be produced as a minor product of the cyclooxygenase activity of platelet PGH synthase-1 in response to platelet stimulation with collagen, thrombin, or arachidonate [71,75]. Activated platelets can generate 8-iso-PGF₂₀a and TXB₂ in a molar ratio of ~1:1000 [71,75]. A second enzyme endowed with cyclooxygenase activity, PGH synthase-2, can be expressed in different cell types in response to inflammatory and mitogenic stimuli (reviewed in Reference [67]). Induction of PGH synthase-2 in human monocytes by concanavalin A, phorbol ester, or bacterial lipopolysaccharide was associated with cyclooxygenase-dependent formation of 8-iso-PGF₂₀a and PGE₂ in a molar ratio ranging from 1:5 to 1:30 [72,76]. Dexamethasone, an inhibitor of PGH synthase-2 induction, and L-745337, a selective inhibitor of the cyclooxygenase activity of monocyte PGH synthase-2 [77], dose-dependently suppressed 8-iso-PGF₂₀a and PGE₂ formation with similar potency [72,76]. However, the contribution of cyclooxygenase-dependent mechanisms to the formation of 8-iso-PGF₂₀a in vivo appears to be negligible [71], and the general assumption that measurements of this F₂-isoprostane in plasma or urine are a reflection of nonenzymatic lipid peroxidation has been validated in clinical settings of platelet and/or monocyte activation (see below).

5. Biological effects of isoprostanes

Several isoprostanes are known to have biological effects in vitro via membrane receptors for prostaglandins. Their formation in lipid bilayers by a free radical-catalyzed mechanism may contribute importantly to alterations in the fluidity and integrity of cellular membranes. In addition, 8-iso-PGF₂₀a is a potent vasoconstrictor [78] and induces DNA synthesis in vascular smooth muscle cells, through interaction with TXA₂ receptor [79] or perhaps with specific receptors [80]. Similarly, another F₂ isoprostane 12-epi-PGF₂₀a, activates PGF₂₀a receptors and stimulates proliferative responses in fibroblasts [81].

Formation of isoprostanes in situ in the phospholipid bilayer may modify cellular function. Subsequent cleavage may release products, such as 8-iso-PGF₂₀a, that modify aspects of platelet function such as adhesive reactions and activation by low concentrations of other agonists. Formation of isoprostanes in monocytes may modify aspects of their function, such as expression of tissue factor [82]. Similarly, formation of isoprostanes in oxidized LDL may result in their uptake by macrophages, resulting in the formation of foam cells. Isoprostanes may also modify vascular smooth muscle cell function and accumulate in these cells in proximity to atherosclerotic plaques (Fig. 3).

Concentration of 8-iso-PGF₂₀a in the range of 1 nmol/l to 1 μmol/l induces a dose-dependent increase in platelet shape change, calcium release from intracellular stores, and inositol phosphates [80,83]. Moreover, 8-iso-PGF₂₀a causes dose-dependent, irreversible platelet aggregation in the presence of concentrations of collagen, ADP, arachidonic acid, and PGH₂/TXA₃ analogues that, when acting alone, fail to aggregate platelets [80]. These effects are prevented by PGH₂/TXA₃ receptor antagonists and 8-iso-PGF₂₀a may cross-desensitize biochemical and functional responses to thromboxane mimetics [84,85]. Moreover, recent evidences based on genetically modified mice have confirmed that the effects of bioactive isoprostanes 8-iso-PGF₂₀a and iPE₂-III on vascular tone and platelet function are transduced via activation of the thromboxane A₂ receptor in vivo and do not depend on the existence of related, but distinct isoprostane receptors [86]. Thus, although 8-iso-PGF₂₀a fails to activate either of the TX receptor isomers described in platelet at concentrations that typically circulate during syndromes of oxidant stress [80], evidences suggesting a distinct receptor for this molecule are inconclusive. Furthermore, although isoprostanes may act as incidental ligands for prostanooid receptors, their effects may differ from the cognate ligand. For example, 8,12-iso-iPF₂₀-III, which ligates the PGF₂₀a receptor and causes a hypertrophic response in cardiomyocytes, activates distinct as well as overlapping downstream signaling pathways when compared with PGF₂₀a [87].

Whether the local concentrations of isoprostanes achieved in vivo are sufficient to allow them to act as a ligand for prostanooid receptors remains unclear. However, the ability of 8-iso-PGF₂₀a to amplify the aggregation response to subthreshold concentrations of platelet agonists might be relevant to setting, such as diabetes mellitus, where persistent platelet activation and enhanced free-radical formation coincide. In fact, diabetes is characterized by platelet activation in vivo, as reflected by enhanced thromboxane metabolite excretion [88]. Immunoreactive 8-iso-PGF₂₀a is altered in patients with diabetes and appears to contribute to platelet activation in this setting [40]. Paired urinary measurements of 8-iso-PGF₂₀a and 11-dehydro-TXB₂ (a major metabolite of TXA₂) in dia-
Fig. 3. Potential sites of isoprostane formation and cellular targets of 8-iso-PGF$_{2\alpha}$ of relevance to atherothrombosis. ELAM indicates endothelial leukocyte adhesion molecule; PGI$_3$, prostacyclin; PAF, platelet-activating factor; TX, thromboxane; and VCAM, vascular cell adhesion molecule (published by the permission of Patrono et al., Arterioscler Thromb Vasc Biol 1997;17:2309–2315).

Betic patients revealed a highly significant linear correlation between the two. Urinary immunoreactive 8-iso-PGF$_{2\alpha}$ was unchanged following a 2-week dosing with aspirin or indobufen, a reversible cyclooxygenase inhibitor, despite complete suppression of thromboxane metabolite excretion, indicating that the oxidative stress in diabetics is likely to be a cause and not a consequence of platelet activation [40]. Consistent with the hypothesis of enhanced 8-iso-PGF$_{2\alpha}$ formation’s contribution to platelet activation in this setting, dose-dependent suppression of the former by vitamin E supplementation was associated with comparable reductions in 11-dehydro-TXB$_2$ excretion. Thus, enhanced nonenzymatic peroxidation of arachidonic acid may provide a biochemical link between oxidant stress and platelet activation in the setting of diabetes mellitus.

The studies of 8-iso-PGF$_{2\alpha}$ formation conducted so far appear to exclude a significant contribution from a cyclooxygenase-dependent mechanism of biosynthesis in vivo. This is particularly supported by no effects of aspirin and other nonspecific cyclooxygenase inhibitors on urinary 8-iso-PGF$_{2\alpha}$ in diabetic patients. The availability of selective inhibitors of PGH synthase-2 will permit further definition of the potential contribution of this pathway to formation of 8-iso-PGF$_{2\alpha}$, particularly in the setting of complicated diabetes, were induction of this enzyme might be anticipated.

6. Hyperglycemia and isoprostane generation

It is debated if hyperglycemia per se is causally related to cardiovascular disease or is merely a marker for some underlying factor that causes both diabetes and CAD. While there is a definite association between the level of glycemia both above and below the threshold for diabetes and cardiovascular risk, proof that hyperglycemia causes CAD was for long time less convincing. Establishing a causal link between hyperglycemia and CAD is likely to be difficult given that both are complex conditions. Recently, it has been provided an important first step in understanding the implications of hyperglycemia-induced lipid peroxidation and increased production of 8-iso-PGF$_{2\alpha}$ in diabetic vascular disease [40]. Using a specific radioimmunoassay, it has been found that urinary excretion of 8-iso-PGF$_{2\alpha}$ in diabetic patients was essentially twice that of healthy age-matched control subjects (Fig. 1). With regard to urinary 8-iso-PGF$_{2\alpha}$ excretion, similar findings were observed both for type 2 and type 1 diabetic patients [40] (Fig. 1). These concordant observations between type 2 and type 1 diabetic 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, we have reported that improved metabolic control of type 2 diabetic patients significantly reduced urinary 8-iso-PGF$_{2\alpha}$ levels by 32% [40] (Fig. 4). Furthermore, 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 [89]. This increase in 8-iso-PGF$_{2\alpha}$ was significantly correlated with blood glucose and increased platelet activation. On the basis of prior studies indicating that 8-iso-PGF$_{2\alpha}$ amplifies agonist-induced platelet aggregation [80], these results strongly suggest that increased lipid peroxidation in diabetic patients leads to the formation of 8-iso-PGF$_{2\alpha}$, which, in turn, leads to platelet activation. In support of this hypothesis, the reduction of 8-epi-PGF$_{2\alpha}$ formation...
A limited number of studies have been completed during the past 5 years addressing the formation of $F_2$-isoprostanes in clinical settings putatively associated with oxidative damage, often as a result of long-standing metabolic abnormalities.

Persistently enhanced formation of $F_2$-isoprostanes has been reported in association with several clinical conditions, including diabetes mellitus [38–40], hypercholesterolemia [94,95], coronary ischemia-reperfusion syndromes [96,97], unstable angina [98], chronic obstructive pulmonary disease [99], cystic fibrosis [100] and cigarette smoking [101,102], conditions putatively characterized by increased lipid peroxidation in response to the complex metabolic abnormalities or various constituents of cigarette smoke. Accelerated LDL oxidation [103] as well as platelet activation [104], are common features of these conditions.

Although diabetes mellitus is a clearly established risk factor for cardiovascular disease, the mechanism(s) responsible for accelerated atherogenesis remain elusive. Altered PGF excretion are represented for 17 patients who achieved improved metabolic control (ie, blood glucose<200 mg/dL and HbA$_1c$ <9%) after intensive monitoring and treatment (published by the permission of Davi et al., Circulation 1999;99:224–229). Although diabetes mellitus is a clearly established risk factor for cardiovascular disease, the mechanism(s) responsible for accelerated atherogenesis remain elusive. Altered PGF excretion are represented for 17 patients who achieved improved metabolic control (ie, blood glucose<200 mg/dL and HbA$_1c$ <9%) after intensive monitoring and treatment (published by the permission of Davi et al., Circulation 1999;99:224–229).

Whether $F_2$-isoprostanes can also influence insulin resistance in diabetic patients is presently poorly understood. Recently, Laight et al. [90] have investigated oxidant stress, glucose tolerance and glucose-stimulated insulin responses in the obese Zucker rat, a widely used model of insulin resistance. The plasma level of 8-iso-PGF$_{2\alpha}$ was elevated approximately 5-fold in old obese relative to age-matched, insulin-sensitive. Supplementation of the diet with vitamin E reduced plasma 8-iso-PGF$_{2\alpha}$ and concomitantly reversed glucose-stimulated hyperinsulinemia in this experimental model. This could explain, at least in part, the beneficial effects of antioxidants on insulin action previously reported in literature [91]. However, whether 8-iso-PGF$_{2\alpha}$ plays a pivotal role also in patients with hyperinsulinemia and insulin resistance but without overt hyperglycemia is still unclear at this time.

7. Potential role of isoprostanes in atherosclerosis

Given the abundant information that oxidation of LDL confers properties thought relevant to atherogenesis [92], there has been interest in the potential of isoprostane measurements both to identify patient populations for interventional studies and for dose selection for antioxidants employed in such clinical trials [93].

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isoprostanes in atherogenesis remains to be established, discriminant production of isoprostanes by both reactive oxygen species and monocyte PGH synthase 2 within atherosclerotic plaques renders it a candidate molecule to transduce, at least in part, the effects of lipid peroxidation and inflammation on vascular dysfunction.

To address this hypothesis, Pratico et al. [113] have recently studied isoprostane generation before and after vitamin E supplementation in the hypercholesterolemic, ApoE-deficient mouse that develops atherosclerosis-type lesions on a chow diet. Supplementation with vitamin E significantly reduced isoprostane generation, but had not effect on plasma cholesterol levels in apoE knock-out mice. This intervention also suppressed the elevated levels of 8-iso-PGF₂α-VI esterified in LDL and in vascular tissue and retarded the development of atherosclerosis, despite persistent hypercholesterolemia [113].

Despite these observations, it is difficult to relate the concentrations of isoprostanes used to evoke biological effects in vitro to what might persist in vivo. First, a myriad of products is formed under conditions of oxidant stress, and yet, to date, studies have concentrated on single isomers. Second, these compounds may be subject to rapid reesterification after release from membrane phospholipids. Finally, the mechanisms and regulation of their release are poorly understood. Nonetheless, there are hints that they might have some relevance in vivo. A thromboxane receptor antagonist is more effective at preventing platelet-dependent coronary occlusion in dogs after thrombolysis than aspirin, despite complete inhibition of thromboxane formation by the latter [114]. This observation suggests activation of the thromboxane receptor by a ligand distinct from TXA₂ and 8-isopGF₂α is known to increase during coronary reperfusion in this model [96]. Moreover, in patients with unstable angina, roughly 50% of the individual variation in aspirin-insensitive TXA₂ biosynthesis could be accounted for by the individual variation in 8-isopGF₂α formation [98]. Finally, vitamin E suppresses not only 8-isopGF₂α in patients with diabetes but also the elevated levels of a thromboxane metabolite [40]. Perhaps, as suggested above, platelet active isoprostanes, such as 8-isopGF₂α, contribute to the enhanced platelet activation in diabetic patients at risk of cardiovascular events.

8. Effects of antioxidants

Numerous epidemiological studies have shown that dietary intake of vitamin E is inversely associated with the risk of cardiovascular disease, though randomized intervention trials have uncertain proved the cardiovascular benefit from vitamin treatment [115]. In general, the epidemiological studies have been interpreted to suggest that prevention of cardiovascular disease requires large amounts of vitamin E, in excess of the conventional dietary intake. However, there is substantial uncertainty as to the optimal dose of vitamin E supplementation, largely because of inadequate biological end points for its efficacy. This is reflected in a wide range of vitamin E doses tested in randomized clinical trials, from as low as 50 mg daily to as high as ~500 mg daily [115–117], and in a broad spectrum of clinical benefits, from a 60% reduction in nonfatal myocardial infarction in the CHAOS study [116] to the absence of benefits in the recent HOPE trial [117]. A number of mechanisms have been suggested to contribute to the putative beneficial effects of vitamin E, including inhibition of LDL oxidation and prevention of fatty streak formation, stabilization of coronary lesions, a direct antiplatelet effect, or improvement of endothelium-dependent vasodilatation.

Limited information is available on the effects of vitamin E on F₂ isoprostane formation. Thus, Reilly et al. [102] have reported no significant changes in urinary 8-isopGF₂α excretion following a 5-day course of vitamin E supplementation in a limited number of moderate (100 U/d) or heavy (800 U/d) smokers. In contrast, vitamin C (2 g/d) alone or in combination with vitamin E significantly depressed urinary 8-isopGF₂α in heavy smokers to a comparable level achieved by smoking cessation [102]. Similarly, we found that supplementation with vitamin E up to 1200 mg did not significantly affect the urinary excretion of 8-isopGF₂α in chronic smokers [118]. In contrast, two-week dosing with vitamin E (100 to 600 mg daily) was found to reduce immunoreactive 8-isopGF₂α in heavy smokers compared to a comparable level achieved by smoking cessation [40]. Failure of vitamin E supplementation to reduce thromboxane metabolite excretion in healthy cigarette smokers [118] provides important support to the contention [40,94] that vitamin E may blunt F₂-isoprostane-mediated amplification of platelet activation in several pathological clinical setting, such as those depicted in Fig. 5, rather than exerting a direct antiplatelet effect.

The overall picture emerging from a series of studies of vitamin E supplementation using the in vivo formation of F₂ isoprostane as the primary biochemical end-point [40,94,102,113,118] suggests that the effect of vitamin E on lipid peroxidation can not be equated to that of a conventional drug blocking an enzyme or receptor in a reproducible fashion in the vast majority of patients exposed to treatment. Most likely, both the mechanism(s) responsible for enhanced oxidant stress and the rate of lipid peroxidation are important determinants of the antioxidant effects of vitamin E supplementation. This hypothesis may help interpreting the conflicting and largely disappointing results of recently completed trials of vitamin E supplementation in patients with ischemic heart disease [116,117,119]. Any protective effect of antioxidant intervention, that is readily apparent in the setting of genetically determined enhanced lipid peroxidation [113], is likely to be diluted by inclusion of a large proportion of
patients with low levels of lipid peroxidation because of dietary habits (such as in the GISSI-Prevenzione Study carried out in the setting of a largely Mediterranean diet: Ref. [119]) or lack of metabolic abnormalities associated with oxidant stress.

Thus, 

F₂ isoprostanes may have practical implications for the use of vitamin E supplements for cardiovascular prevention in diabetic patients, and may help to resolve some of the uncertainty about the optimal dose of vitamin E supplementation. Moreover, present evidences suggest the need to re-evaluate the response of potential target populations based on non-invasive measurements of F₂ isoprostane formation as a rationale basis for new large-scale interventional trial design.

9. Conclusion and summary

Isoprostanes are emerging as a new class of biologically active products of arachidonic acid metabolism of potential relevance to diabetes and cardiovascular diseases. Their formation seems to reflect primarily, if not exclusively, a nonenzymatic process of lipid peroxidation in vivo. Enhanced urinary excretion of 8-iso-PGF₂α has been repeatedly characterized in association with diabetes mellitus. Besides providing a reliable, noninvasive index of lipid peroxidation in this setting, measurements of specific F₂ isoprostanes, such 8-iso-PGF₂α, in urine may provide sensitive biochemical end-points for dose-findings studies of natural and synthetic inhibitors of lipid peroxidation. Although the biological effects of 8-iso-PGF₂α in vitro suggest that it and other isoeicosanoids [120,121] may modulate the functional consequences of lipid peroxidation in diabetes, evidences that this is likely in vivo are growing but are not yet conclusive at this time.

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