Clinical disease resulting from atherosclerosis continues to cause extensive mortality and morbidity and uses an exorbitant amount of the financial resources available for health care. Atherosclerosis is a complex, multifactorial, and chronic disease influenced by a wide variety of genetic, environmental, and behavioral activities. However, it is at least widely recognized that among the known risk factors, hypercholesterolemia is one of the most important. Recent clinical trials indicate that for most individuals, and certainly for those at high risk, the lower the plasma cholesterol level, the lower the risk for subsequent development of clinical events. The mechanisms by which elevated levels of lipoproteins, chiefly the apoB containing lipoproteins such as LDL, cause an acceleration of atherogenesis are only incompletely understood. Yet there seems to be an emerging body of evidence to support the contention that the atherogenicity of LDL derives in large part from the fact that it becomes modified in one or more ways. In particular, it is widely held that the oxidative modification of LDL is intimately involved in the atherogenic process.Indeed, there is now considerable evidence to support the so-called oxidation hypothesis of atherosclerosis. This hypothesis suggests that oxidation of lipids and lipoproteins, such as LDL, is important, if not obligatory for the atherogenic process. This formulation has not only been of great heuristic value in guiding research into the pathophysiology of lesion development, but if correct, the corollary is that inhibition of such lipid peroxidation should ameliorate or prevent atherosclerosis and its clinical sequelae. Although there is preliminary data to support a connection between enhanced lipid peroxidation and hypercholesterolemia, the lack of techniques sufficiently sensitive to reliably provide an index of lipid peroxidation in vivo has hampered efforts to study this relationship, particularly in humans.

The evidence that oxidation of LDL occurs in vivo is substantial and has been reviewed elsewhere in detail. In brief, the evidence includes (1) antibodies to oxidation-specific epitopes found on oxidized LDL (OxLDL) immunostain atherosclerotic lesions of animal models and humans, (2) LDL extracted from lesions has all of the physical, immunological, and biological properties of LDL oxidized in vitro, (3) although heavily oxidized LDL is not found in plasma, a small fraction of circulating LDL displays the chemical and immunological characteristics of minimally oxidized LDL, (4) oxidized lipids, including oxidized sterols, isoprostanes (see below), and products of the lipoxygenase pathway can be found in atherosclerotic lesions and in some cases in plasma, (5) OxLDL is immunogenic and autoantibodies to epitopes of OxLDL are found in plasma and lesions (an elevated titer of such autoantibodies is frequently associated with increased vascular disease), and (6) antioxidant treatment of various hypercholesterolemic animal models, with both natural and synthetic agents, reduces lesion progression independent of hypolipidemic activity (reviewed in Steinberg and Witztum). Of particular relevance is the recently reported study by Pratico et al., demonstrating that vitamin E treatment can reduce atherosclerosis in apoE deficient mice.

Data to support the oxidation hypothesis in humans is more limited. Although epidemiological data support a role for antioxidants in the prevention of clinical events, intervention trials thus far have given mixed results. This may be due, in part, to the fact that until now we have not had techniques to adequately provide an index of in vivo lipid peroxidation, which could be used to design and monitor effective antioxidant intervention trials to adequately test the oxidation hypothesis. At present, we lack measures to identify high-risk groups that would theoretically benefit most from antioxidant interventions, and we lack any reliable measure to determine the in vivo effectiveness of such interventions. In the absence of such information, current (and future) clinical trials testing natural (or synthetic) antioxidants, such as vitamin E, which utilize clinical endpoints, may give incorrect conclusions regarding the role of antioxidants. This is a possibility because of the inclusion of populations that would not be expected to benefit from antioxidant supplementation, and/or because the dose or agent yielded insufficient antioxidant protection. In contrast are the clinical trials in which the effectiveness of hypocholesterolemic therapy was firmly established; the most effective hypolipidemic agents were used to treat subjects at high-risk by virtue of high cholesterol levels.

Free radicals, principally derived from oxygen, have been implicated in many human diseases, including atherosclerosis. However, despite a vast interest in this field, the ability to measure this process in vivo has remained elusive. In particular, the oxidative modification of LDL in the arterial wall (or elsewhere) presumably begins with an oxidative attack on the surface polyunsaturated fatty acids (PUFA), which includes arachidonic acid. Once initiated (regardless of the initial event), non-enzymatic free radical–catalyzed mechanisms can then lead to modifications of all the components of LDL and initiate modifications of surrounding cells and structures as well. In the past, attempts to provide a measure of lipid peroxidation in vivo have included plasma measurement of thiobarbituric acid reacting substances and measurement of exhaled alkanes, but the accuracy of these techniques has been questioned. In one popular assay, LDL is isolated and the susceptibility of its PUFAs to undergo lipid peroxidation in response to an oxidant challenge is measured by the lag time preceding the formation of conjugated dienes.
Although useful as an index of the antioxidant protection conferred on LDL by a particular agent, its relationship to in vivo protection in tissues and, more important, to the atherogenic process itself, remains poorly defined.

It had been known for some time that prostaglandin (PG)-like compounds could be formed from nonenzymatic free radical–catalyzed peroxidation of arachidonic acid. In 1990, Morrow and colleagues demonstrated that a novel family of PG-like isomers, termed F2 isoprostanes, were formed as the result of free radical attack of cell membrane phospholipids. In contrast with enzymatically generated PG, which utilizes free arachidonic acid as substrate, the F2 isoprostanes are formed by oxidation of the arachidonic acid esterified to phospholipids in cellular membranes. They can also form when LDL undergoes either cell- or copper-mediated oxidation. They are released by a phospholipase activity and circulate in the plasma in free form or as phospholipid esters and can be excreted in urine, in part as distinct metabolites. Isoprostanes are mostly formed by nonenzymatic oxidation, yielding 4 different peroxyl radical isomers which can undergo further reactions theoretically yielding up to 64 different F2 isoprostane compounds or rearrangements to D2E2 of isothromboxanes. Although in theory, all of the isoprostanes and their various isomers may be produced randomly, different isoprostanes may be produced preferentially under conditions of oxidant stress. In vitro, some isoprostanes alter platelet and vasomotor activity, but the in vivo relevance of these observations is unknown.

Highly sensitive stable-isotope dilution assays using gas chromatography/mass spectrometry (GC/MS) have been developed to measure F2 isoprostanes. Morrow and Roberts have developed GC/MS-based methods to quantify levels of total free and esterified F2 isoprostanes in blood. Specific GC/MS assays for iP-F2α-III (formerly called 8-iso-PGF2α) and for iP-F2α-VI (formerly called iP-F2α-I) have also been developed by Patrono and colleagues. A discussion of the advantages of each method can be found in excellent reviews. Because iP-F2α-III has been shown to be a product of the cyclooxygenase (COX) pathway and a result of nonenzymatic lipid peroxidation, interventions affecting this pathway could theoretically confound measurements of total F2 isoprostanes. In practice, however, the COX pathway appears to contribute little to urinary excretion. In contrast, iP-F2α-VI does not appear to be generated by COX. In general, measurements of both F2 isoprostanes in urine have shown a high correlation. An additional benefit to urinary measurements is that the level of arachidonic acid precursor is very low and thus the potential for artificial generation of F2 isoprostanes ex vivo is minimal. A major disadvantage of this method is that measurements are confined to specialized laboratories, and even though the F2 isoprostanes are stable in frozen urine for several months, the ability to use this method is likely to be restricted. More promising for clinical use would be the development of immunoassays. Currently, an assay for iP-F2α-I is available, but validation of cross-reactivity with the many different isomers and/or metabolites has yet to be accomplished (in large part because these isomers have not yet been available in pure form). It is important to reemphasize that measurements of isoprostanes, whether in blood or urine, presumably reflect the summation of products of lipid peroxidation occurring throughout the body. They do not necessarily indicate enhanced lipid peroxidation in a specified organ, such as the artery, nor do such measurements necessarily relate directly to LDL oxidation.

Elevated levels of F2 isoprostanes have been observed in plasma and urine in animals and humans under a wide variety of conditions of enhanced oxidative stress (see references 8 and 9). For example, rats deficient in Vitamin E and selenium have elevated levels, as do humans undergoing reperfusion after thrombolytic therapy and following myocardial reperfusion after CABG. F2 isoprostane excretion is increased in chronic smokers and falls after cessation of smoking. Elevated plasma or urinary levels of F2 isoprostanes have also been reported in association with other cardiovascular risk factors, including both type 1 and type 2 diabetes and hypercholesterolemia (discussed below). Levels of isoprostanes are elevated in atherosclerotic lesions and by immunocytochemistry they localize to foam cells and the extracellular matrix. To date, there is relatively little data on the impact of antioxidant interventions on isoprostane levels in humans. In one study, 2 weeks of vitamin E (100 to 600 mg/day) decreased elevated immunoactive urinary iP-F2α-III excretion in a dose-dependent manner in hypercholesterolemic subjects, and in another study, vitamin C plus vitamin E reduced urinary levels in smokers. The recent elegant study by Pratico et al demonstrated a rise in iP-F2α-VI levels in plasma, urine, and arterial tissue in hypercholesterolemic apoE deficient mice, parallel to the ongoing progression of atherosclerotic lesions. Vitamin E administration led to a reduction in isoprostane excretion and inhibition of lesion formation without affecting plasma cholesterol levels. Plasma vitamin E levels correlated inversely with plasma, urinary, and lesion isoprostane levels, and with aortic lesion area. These data provide definitive evidence for a direct relationship between atherogenesis and in vivo lipid peroxidation.

Previous data have suggested that hypercholesterolemia was associated with enhanced oxidant production and increased lipid peroxidation, through use of an immunoassay for iP-F2α-III, demonstrated significantly higher urinary levels in hypercholesterolemic subjects than in controls. However, as noted above, some concerns remain about the specificity of the immunoassay methodology. In this issue, Reilly et al provide important definitive data that strongly link hypercholesterolemia and increased lipid peroxidation in patients with homozygous familial hypercholesterolemia (HFH) and in subjects with moderate hypercholesterolemia. This was accomplished using GC/MS techniques to measure the urinary excretion of both iP-F2α-III and iP-F2α-VI, demonstrating that the levels were significantly elevated in the patient groups as compared with age- and sex-matched controls. Furthermore, the levels of isoprostanes were elevated in isolated LDL fractions of the HFH patients, and the urinary isoprostane levels correlated with both serum cholesterol levels and LDL-isoprostane levels. Of interest, the content of iP-F2α-III was increased in each LDL particle, even after adjustment for cholesterol or arachidonate content. It is unclear if the iP-F2α-III esterified in LDL is the result of esterification of modified fatty acids formed elsewhere or reflects enhanced LDL peroxidation that has occurred in vivo.

The data of Reilly et al confirm and extend the earlier findings of Davi et al that isoprostane generation is increased.
in hypercholesterolemia. However, these studies do not address the fundamentally important issue of why hypercholesterolemia is associated with enhanced lipid peroxidation. Does the fact that even normcholesterolemic subjects excrete a certain level of \( F_2 \) isoprostanes imply an ongoing rate of lipid peroxidation in everyone, consistent with our aerobic environment, and that this process is simply exaggerated as plasma cholesterol levels are raised? This would be compatible with the correlations noted between urinary \( F_2 \) isoprostanes and total and LDL cholesterol levels.\(^{13,18}\) Thus, in relevant tissues, increased LDL levels might simply provide enhanced PUFA “substrate,” accelerating non-enzymatic lipid peroxidation. In the artery, the increased quantity of products derived from oxidation of LDL, such as lyso PC, oxidized phospholipids, hydroxy fatty acids, and isoprostanes, could all modulate the activity of cells in the artery. For example, localized high concentrations of lyso PC and iPF\(_2\)-\( \alpha \) (8-iso-PGF\(_2\)-\( \alpha \)) may be sufficient to alter vasomotor tone and/or affect platelet function. Products of early and late stages of OxLDL can activate endothelial cells, including, presumably, an increased ability to generate reactive oxygen species which, in turn, would serve to further amplify rates of lipid peroxidation.

However, the enhanced isoprostane excretion does not tell us where the increased lipid peroxidation is occurring or whether it is necessarily related to atherosclerotic lesion formation. Indeed, the work of Liao et al.\(^{19}\) demonstrates that the enhanced rates of lipid peroxidation associated with hypercholesterolemia occurs in organs other than the artery; thus, many organs, including the kidney, may be responsible for the enhanced urinary excretion of the \( F_2 \) isoprostanes. It is possible that once a chronic inflammatory lesion is established, as is believed to be the case with established atherosclerotic lesions,\(^{20}\) ongoing enhanced lipid peroxidation may occur independent of hypercholesterolemia. In the study of apoE deficient mice noted above, vitamin E therapy reduced atherosclerosis and isoprostane excretion without altering cholesterol levels.\(^{4}\) Important future experiments will be needed to determine whether hypolipidemic therapy in both young and older patients will also decrease the elevated urinary \( F_2 \) isoprostane levels, providing evidence for a causal relationship. Indeed, it is possible that the rather dramatic and rapid improvement in clinical outcomes seen in multiple hypolipidemic trials, and in at least one vitamin E trial (CHAOS), are due, in part, to a reduction in rates of lipid peroxidation within the artery wall.

As discussed above, there is preliminary data in animals and humans that measures of urinary isoprostanes reflect in vivo rates of lipid peroxidation and that antioxidant therapies can decrease isoprostane excretions. Further studies to confirm and extend these data and identify the variables (eg, age, gender, diet, drugs, presence of renal disease) that may alter urinary isoprostane measurements and the impact of antioxidants are needed. Correlation with other available measurements of lipid peroxidation and with measures of atherosclerosis are also needed. Despite these and other questions, the measurement of \( F_2 \) isoprostanes shows great promise as a useful technique for identifying populations that may have enhanced rates of lipid peroxidation and thus may benefit most from the benefits of antioxidant intervention. It should be noted that even among the HPHI patients, there was a 4- to 6-fold variation in isoprostane excretion and some even had isoprostane levels similar to controls. Isoprostane measurements should also prove useful in designing optimal antioxidant regimens and in monitoring their effectiveness in a clinical trial. We are frequently asked whether the medical establishment should recommend to the population as a whole the widespread use of antioxidant supplementation, such as vitamin E. As in other aspects of medical therapy, we firmly believe that this should not be done until the clinical efficacy of this intervention has been demonstrated. The accumulated data suggest that measurements of \( F_2 \) isoprostanes may enable us to more rationally design appropriate clinical trials and thus obtain an answer to the question: “To E or not to E.”

**References**


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