Eicosanoids and Isoeicosanoids: Indices of Cellular Function and Oxidant Stress

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ABSTRACT Arachidonic acid (AA) is an unsaturated fatty acid constituent of the phospholipid domain of cell membranes. It is subject to release via mobilization of phospholipases, particularly a cytoplasmic phospholipase A2. Thereafter, it may be metabolized by at least two cyclooxygenase (COX) isoforms to prostaglandins and related compounds, via lipoxigenases to leukotrienes and via p450-catalyzed metabolism to epoxyeicosatrienoic acids. Collectively, these bioactive lipids are termed eicosanoids. All of these lipids express potent bioactivity in vitro. Clinical studies have already demonstrated the importance of COX and lipoxigenase (LOX) products in human disease. The generation of models of COX, LOX and prostaglandin receptor gene inactivation is likely to broaden our insight into the importance of these compounds in vivo. Crystallization of the biosynthetic enzymes is likely to facilitate the development of highly specific inhibitors, as is the case already for COX-2. AA possesses intrinsic biological properties. It is also subject to free radical attack, generating isomeric eicosanoid species, the isoeicosanoids. These compounds may also express biological activity in vitro, although their importance in vivo is unclear. Development of specific assays for these compounds in urine suggests their utility as noninvasive indices of oxidant stress in vivo. J. Nutr. 128: 434S–438S, 1998.

KEY WORDS: • eicosanoids • isoeicosanoids • oxidant stress • isoprostanes • cyclooxygenase (COX)

In 1934, von Euler identified a lipid-soluble substance from semen that would stimulate uterine smooth muscle contraction and named it prostaglandin (von Euler 1934). Bergström and Sjovall (1960) isolated prostaglandin E from sheep prostate glands and showed that it was a 20-carbon fatty acid with blood pressure-lowering activity. Bergström et al. (1964) demonstrated the enzymatic conversion of arachidonic acid to prostaglandin E2. Because arachidonic acid is synthesized from linoleic acid in humans, these discoveries established prostaglandins as a product of the metabolism of essential fatty acids.

EICOSANOIDs: FORMATION AND BIOLOGICAL ACTIVITY

It is now known that arachidonic acid (AA)¹ is subject to metabolism by a wide array of bioactive lipid mediators. Two isoforms of the prostaglandin (PG) G/H synthase, colloquially known as cyclooxygenases (COX), catalyze the formation of PG and related compounds. Vane (1971) first demonstrated that COX was the target for aspirin inhibition of PG formation. Inhibition of COX-1 in platelets, with consequent suppression of formation of thromboxane (Tx) A2, underlies the efficacy of aspirin in the treatment of platelet-dependent vascular occlusion (Patrono 1994).

Although the expression of COX-1 may be regulated, it is usually expressed constitutively. Similarly, although COX-2 expression may be constitutive, particularly in the cells of the reproductive tract and in the nervous system (Yamagata et al. 1993), its expression is usually tightly regulated, particularly by cytokines, growth factors and tumor-promoting agents (Fu et al. 1990). These observations have implicated COX-2 in PG generation in inflammation and, perhaps, cancer (Tsujii et al. 1997). COX-1, by contrast, is expressed constitutively in normal gastric epithelium. Inhibition of COX-1 is thought to underlie the gastrointestinal side effects of commonly available nonsteroidal anti-inflammatory drugs (NSAID), all of which are quite nonselective between the two COX isoforms (Smith et al. 1996). Thus, the development of highly selective COX-2 inhibitors (Seibert et al. 1994) may promise compounds that are better tolerated and more efficacious than conventional NSAIDs. A potential caveat is that an evoked inflammatory response is impaired in mice deficient in COX-1, but not COX-2. The latter mice were also able to mount an inflammatory response to incidental infection (Langenbach 1995; Morham 1995). Both COX isoforms have now been crystallized (Browner 1996, Loll et al. 1995).

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4 Abbreviations used: AA, arachidonic acid; cox, cyclooxygenase; EET, epoxyeicosatrienoic acid; HFH, homoyzgenous familial hypercholesterolemia; IPF, F2 isoprostanes; NSAID, nonsteroidal anti-inflammatory drugs; PG, prostaglandin; PGD, prostaglandin D; PTCA, percutaneous transluminal coronary angioplasty; TP, Txa2 receptor; Tx, thromboxane.

Although G protein–coupled receptors for all of the PG have been cloned (Narumiya 1994), only the human pharmaco-
logy of specific antagonists of the TxX receptor (TP) has been characterized to date. It is likely that the recent genera-
tion of mice deficient in each of these receptors will further elucidate their in vivo biology. Similarly, there have been 
suggestions that eicosanoids might activate nuclear receptors (Forman et al. 1997). However, issues of specificity and con-
centration-response relationships with compounds actually formed in vivo must be resolved.

Lipoxygenases generate leukotrienes and related com-
pounds. These lipids express biological properties of likely rele-
cance to inflammatory responses in vivo. This is consistent 
with the phenotype expressed by mice deficient in the 5-lipox-
genase enzyme (Chen et al. 1994). Specific antagonists of 
sulfidopeptide leukotrienes have found efficacy in human 
asthma, as have specific inhibitors of the 5-lipooxygenase 
(O’Byrne 1994). Leukotrienes and related compounds have 
also been implicated in neuronal function, atherogenesis, cell-
ular proliferation and the regulation of vascular tone in vitro.
Again, the recent availability of mice deficient in specific li-
poxygenases may elucidate the in vivo relevance of these ob-
servations.

Much less is known of the biological importance of the epxynase catalyzed formation of epoxieicosatrienoic acids 
(EET) and related products, probably by p450 isozymes with high affinity for arachidonic acid as a substrate (McGiff 1991). 
These compounds are potent regulators of epithelial ion transport and vascular tone in vitro (Oyekan et al. 1994). However,
specific receptors for EET have yet to be cloned, and animals deficient in the AA-specific p450 isoforms (Wu et al. 1996)
have yet to be generated. Data suggestive of their importance in hypertension have been obtained with nonspecific inhibit-
ors (Makita et al. 1994). However, given the absence of a 
specific means of pharmacological inhibition of their synthesis 
or action, their role in pathophysiology is speculative at present.

In addition to these observations, cells may interact to gen-
erate novel transcellular products of AA (Marcus 1990). Simi-
larly, AA itself may directly modify cellular function. Thus, 
arachidonoylation of cellular proteins such as G proteins (Hal-
lak et al. 1994) or miniglucagon (Sauvadet et al. 1997) may modify their effects. It may directly regulate ion channels (Dx-
men et al. 1993) or influence gene expression (Barry et al. 1997).
Differential allosteric regulation of the two COX iso-
forms by AA can cause dramatic differences in isoform selec-
tivity for inhibitors, as a function of AA concentration (Swin-
ney et al. 1997).

A recent area of interest has been the potential importance of oxidized lipids in the modification of cellular function (Lehr et al. 1997). The focus of this review will be on candidate members of this species, a family of free radical–catalyzed products of arachidonic acid, the isoeicosanoids.

THE ISOEICOSANOIDS

Isoeicosanoids, isomers of enzymatically derived eicos-
aoids, are free radical–catalyzed products of arachidonic acid (Nugteren and Christ-Hazelhof 1980, O’Connor et al. 1984).
The existence of the F2 isoprostanes, isomers of PGF2α, in human plasma and urine was first described by Morrow et al. (1990). More recently, the free radical–dependent formation of E2 and D2 isoprostanes, isothromboxanes and isoleukotrienes has been reported.

Four classes of F2 isoprostanes (IPF) have been described

![FIGURE 1 The F2-isoprostane family of isomers can be divided into four classes; 8-epi PGF2α belongs to class IV and IPF2α-1 to class I. Although 8-epi PGF2α is a minor by-product of the cyclooxygenase (COX) pathways in vitro, there is no evidence that IPF2α-1 can be generated in this manner.](https://academic.oup.com/jn/article-abstract/128/2/434S/4724043/132403)
dental pathway might contribute to the formation of 8-epi PGF<sub>2α</sub> in settings of inflammation and cellular proliferation, in which COX-2 induction might be expected.

Despite the apparent lack of relevance of the enzymatic formation of 8-epi PGF<sub>2α</sub> to the use of its urinary excretion as an index of oxidant stress, it seemed prudent to develop methods to measure another F<sub>2</sub>-isoprostane that was not susceptible to enzymatic formation. IPF<sub>2α</sub>-I is a member of a distinct class of F<sub>2</sub>-isoprostanes (Waugh and Murphy 1996). It is not formed by COX and its excretion in volunteers is not suppressed by aspirin (Pratico et al. 1998). Urinary levels of IPF<sub>2α</sub>-I and 8-epi PGF<sub>2α</sub> are closely correlated (r = 0.57, P < 0.0001) in patients with hypercholesterolemia (Fig. 2). This is a setting of moderate COX-1 activation. These observations are consistent with the hypothesis that excretion of both compounds in urine reflects formation by a common mechanism—free radical-catalyzed generation of prostaglandin isomers. Thus it would appear that enzymatic formation of 8-epi PGF<sub>2α</sub> by COX is a trivial contributor to overall 8-epi PGF<sub>2α</sub> biosynthesis in vivo and should not detract from its usefulness as an index of oxidant stress.

**ISOPROSTANES AND OXIDANT STRESS**

Oxidative stress is thought to play an important pathophysiologic role in a variety of human diseases, including atherosclerosis, cancer and neurodegenerative disorders. However, difficulty in assessing radical generation in vivo has proven to be the major limitation to our understanding of this mechanism of human diseases. Traditional in vitro assays, directed against malondialdehyde or lipid hydroperoxides, are thought fallible when applied to clinical investigation, because of such factors as ex vivo generation of products and both the instability and nonspecificity of the analytes involved. Furthermore, it is unclear how ex vivo estimates of free radical generation, such as lipoprotein oxidizability or the formation of adducts detected by spin trapping, relate to oxidant stress in vivo.

The measurement of F<sub>2</sub> isoprostanes may represent an important development in the assessment of free radical generation and oxidant stress in vivo. They are remarkably stable compounds. Coordinate elevation of plasma and urinary isoprostanes in syndromes of extrarenal oxidant stress (Morrow et al. 1995, Pratico and FitzGerald 1996) implies that little is likely to be gained by measurement of metabolites, rather than the parent products, in urine. However, estimates of 8-epi PGF<sub>2α</sub> in plasma, where there is an abundance of lipid, may be confounded by its autooxidation ex vivo. Additionally, COX-1–dependent formation by platelets activated ex vivo might also undermine plasma-based measurements as indices of actual formation of 8-epi PGF<sub>2α</sub> in vivo. Thus measurement of a metabolite of 8-epi PGF<sub>2α</sub> (Roberts et al. 1996) might circumvent this problem if it is not formed in the cells of circulating blood. We have utilized gas chromatography/mass spectrometry to validate immunoassays of the parent compound. More recently, we have adopted an integrated approach that uses the coordinate measurement of urinary 8-epi PGF<sub>2α</sub> and IPF<sub>2α</sub>-I in the evaluation of oxidant stress in specific clinical settings.

**F<sub>2</sub> ISOPROSTANES IN SPECIFIC CLINICAL SYNDROMES**

There is a dose-dependent increase in urinary 8-epi PGF<sub>2α</sub> excretion in apparently healthy chronic cigarette smokers, which was not suppressed by aspirin. Both cessation of smoking, with supplementation of nicotine patches and short-term therapy with vitamin C (2000 mg/d), an endogenous antioxidant, attenuated the elevation of urinary 8-epi PGF<sub>2α</sub> excretion. Deficiency of vitamin C (Heitzer 1996) may render smokers particularly susceptible to the antioxidant effects of exogenous vitamin supplementation.

Oxidative stress is thought to play a critical role in atherogenesis. This hypothesis is based largely on indirect evidence. We have recently immunolocalized 8-epi PGF<sub>2α</sub> to monocyte/macrophages and vascular smooth muscle cells in human atherosclerotic plaque and demonstrated increased levels of this compound in atherosclerotic vessels, compared with normal arterial segments (Pratico et al. 1997). Furthermore, we have demonstrated along with others that F<sub>2</sub> isoprostanes are formed in LDL when it is oxidized in vitro. Therefore we designed specific studies to assess the biosynthesis of both 8-epi PGF<sub>2α</sub> and IPF<sub>2α</sub>-I in patients with homozygous familial hypercholesterolemia (HHF) and also in more moderate hypercholesterolemia. Urinary excretion of these isoprostanes was increased in both groups of hypercholesterolemic patients compared with their respective controls. Furthermore, the concentration of 8-epi PGF<sub>2α</sub> excreted in LDL was elevated and correlated with urinary excretion of this compound in a subset of these patients (Reilly et al. 1996b). Given the potentially distinct mechanisms that might result in free radical generation in smokers, one might anticipate an even greater increment in dyslipidemic individuals who smoke. Interestingly, the precise role of dietary lipids in isoprostane biosynthesis in normal individuals or in the setting of increased biosynthesis remains to be addressed definitively.

Oxidant stress has been implicated in vascular reperfusion after a period of ischemia. Examples include the regional myocardial stunning seen in animal models of coronary occlusion/reperfusion and in some patients after thrombolytic therapy, as well as in the global myocardial dysfunction seen after coronary
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