ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 8, 2008

DOI: 10.1086/ars.2007.1956

Comprehensive Invited Review

F_2-Isoprostanes in Human Health and Diseases: From Molecular Mechanisms to Clinical Implications

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ABSTRACT

Oxidative stress is implicated as one of the major underlying mechanisms behind many acute and chronic diseases, and involved in normal aging. However, the measurement of free radicals or their end products is complicated. Thus, proof of association of free radicals in pathologic conditions has been absent. Isoprostanes are prostaglandin-like bioactive compounds that are biosynthesized *in vivo* independent of cyclooxygenases, principally through free-radical catalyzation of arachidonic acid. Isoprostanes are now considered to be reliable biomarkers of oxidative stress, as evidenced by an autonomous study organized recently by the National Institutes of Health (NIH) in the United States. A number of these compounds have potent biologic activities such as vasoconstrictive and certain inflammatory properties. Isoprostanes are involved in many human diseases. Additionally, elevated levels of F_2-isoprostanes have been seen in normal human pregnancy and after intake of some fatty acids, but their physiologic assignments have not yet been distinctive. This evidence indicates that measurement of bioactive F_2-isoprostanes in body fluids offers a unique noninvasive analytic utensil to study the role of free radicals in physiology, oxidative stress–related diseases, experimental acute or chronic inflammatory conditions, and also in the assessment of various antioxidants, radical scavengers, and drugs. *Antioxid. Redox Signal.* 10, 1405–1434.

I. INTRODUCTION

Oxidative stress is supposed to be one of the key underlying pathologies of several acute and chronic diseases, also associated with progressive aging and certain physiologic processes. However, the measurement of free radicals or their end products is complicated because free radicals are reactive, short-lived, and of diverse characteristics. Thus, support for the participation of free radicals in pathologic situations, particularly *in vivo,* has often been lacking or remains a subject of controversy. Isoprostanes, a group of prostaglandin-like compounds, are biosynthesized mainly from esterified arachidonic acid through a nonenzymatic free radical–catalyzed reaction *in vivo* that has opened a novel aspect of free-radical biology (16, 180). Some of these short-half-life compounds possess potent biologic activities, mainly through pulmonary and renal vasoconstriction through activating thromboxane receptors or other independent mechanisms, and thus they may contribute to vascular or tissue injury through pathophysiologic effects. Studies have shown that isoprostanes act as full or partial agonists through thromboxane receptors (262). After rapid hydrolysis and further metabolism, primary isoprostanes and their β-oxidized products are found in the plasma and later excrete efficiently into the urine. Both clinical and experimental studies have reported the association of isoprostanes with severe acute or chronic inflammatory conditions, such as asthma, atherosclerosis, chronic obstructive pulmonary diseases (COPD), diabetes, ischemia–reperfusion, rheumatic diseases, septic shock, and many more. A number of studies have shown that the F_2-isoprostanes are reliable biomarkers of lipid peroxidation and could, therefore, be used as potential *in vivo* indicators of oxidant stress in diverse conditions and in the evaluation of various antioxidants, diets, or drugs. Because bioactive F_2-isoprostanes (mainly 8-iso-$PGF_{2\alpha}$) are regularly formed in various tissues and rather small amounts of these potent compounds are found in unmetabolized form in plasma and higher levels in urine in normal basal condition, their role in the regulation of normal physiologic function might be of additional importance, yet to be disclosed. This review provides an overall up-to-date picture of isoprostanes as biomarkers of oxidative stress and their molecular mechanisms related to their formation, mechanism of action, cellular effects, metabolism, detection, and their further consequences in human health and diseases, in addition to their responses to various therapies such as antioxidants and drugs.

II. GENERAL ASPECTS OF LIPID PEROXIDATION, OXIDATIVE STRAIN, AND OXIDATIVE STRESS

It is well documented that polyunsaturated fatty acids with two or more double bonds are more subject to oxidation than the saturated and monounsaturated fatty acids (96). This is principally because of the instability (weak energy of attachment) on the hydrogen atom adjacent to the double bond that could simply be abstracted by a reactive radical species attack. Lipid peroxidation *in vivo*, through a free-radical pathway, is a reaction path that requires a polyunsaturated fatty acid (PUFA) and a reactant oxidant inducer that together form a free-radical intermediate. Figure 1 presents a general schematic principle of lipid oxidation from polyunsaturated fatty acids. The free-radical intermediate subsequently reacts with oxygen to generate a peroxyl radical ($LOO^{\cdot}$). The unpaired electrons of the peroxyl radicals additionally abstract a hydrogen atom from another PUFA to form a lipid hydroperoxide that may decompose to form alkoxyl or peroxyl radicals. These radicals may in addition attract different adjoining proteins attached to the cell membrane. This reaction of peroxyl radical to fatty acids forms a carbon-centered radical that consecutively may be capable of reacting with oxygen to form another peroxyl radical. This radical persists in its reaction to the PUFAs, and a propagation reaction starts and is sustained, awaiting a termination reaction initiated by a variety of endogenous inhibiting factors, for instance, chain-breaking antioxidants or exogenously administered antioxidants and drugs (13).

The issue arises whether an ongoing lipid peroxidation is detrimental to mammalian survival or has any positive consequences on health. It is commonly expressed in the literature

that all oxidative reaction processes *in vivo* are merely destructive in nature, generally designated as a state of oxidative stress. Conversely, it could be simply argued that they are also critical elements of human body and our daily existence. Abundant evidence supports that reactive free-radical species are physiologically very important to exert a variety of vital biochemical reactions, including lipid oxidation that systematizes many significant physiologic functions, including cell signaling, vascular tone, cell generation and degeneration, control of cellular homeostasis, defense against microorganisms, and human pregnancy (16, 19, 45, 84, 85, 190, 191), and most likely several other yet-unidentified essential functions of the body. Therefore, a mildly increased essential lipid oxidation *in vivo* to execute a definite physiologic task can be considered an "oxidative strain" in nature rather than designated as so-called more detrimental "oxidative stress" (14). In other words, "oxidative strain" can be defined as "a mild disturbance in the prooxidant/antioxidant balance in support of the preceding to perform a specific physiologic function that is beneficial to health," as evidenced in normal human pregnancy. This is essentially unlike the oxidative stress that is normally unfavorable to health.

The physiologically essential reactive species in a controlled behavior appears to be obliged to uphold the normal basal oxidant–antioxidant balance and host defences in the mammalian system. Nonetheless, although these reactions devastate a distinct physiologic threshold level through pathologic contribution or environmental pollutants, an assortment of primary reaction products, including several intermediates, are produced that either are biologically active compounds of dissimilar nature or are inactive in the biologic systems. When the excess formation of these bioactive oxidation products overwhelms the capacity of endogenous cellular antioxidant defense mechanisms, these products may affect various cell and organ damage by distressing the normal physiology in such a manner that they activate or accelerate (or both) the disease processes. Additionally, overstressed or injured cells in diseased condition possibly may also initiate the production of these active compounds *in vivo*, and consequently increase cellular or muscle damage that leads the mammalian body to experience a further severity of the pathologic state and consequent disease flare-up. Hence, the determination of lipid peroxidation regardless of its consequences through initiating oxidative strain or oxidative stress remains a scientific challenge in understanding human physiology and pathophysiology. Concepts have been lately urbanized on the free radical–mediated lipid peroxidation, and their further health consequences through initiating oxidative strain or oxidative stress after the establishment of isoprostanes are reliable biomarkers of free radical–mediated reactions *in vivo*.

III. MOLECULAR MECHANISMS OF ISOPROSTANE FORMATION

Autooxidation of lipids is well known. Early confirmation of formation and mechanism of formation of nonenzymatically autooxidized prostaglandin-like compounds from fatty acids was established *in vitro* by Nugteren and others (195, 204, 219). It has long been recognized that careless storage of unsaturated fatty acids leads to the formation of a number of degraded prostaglandin-like compounds *in vitro*. Nonetheless, the significance of nonenzymatic formation of these prostaglandin derivatives *in vivo* was not elucidated until 1990. The detection of isoprostanes, a family of prostaglandin-like compounds generated *in vivo* by nonenzymatic free radical–catalyzed peroxidation of arachidonic acid, initiated a new epoch on the significance and detection of nonenzymatic lipid peroxidation products (16, 176, 180, 183). However, a few early studies also described the formation of isoprostanes *via* a cyclooxygenase (COX) pathway from human platelets and monocytes (124, 211,

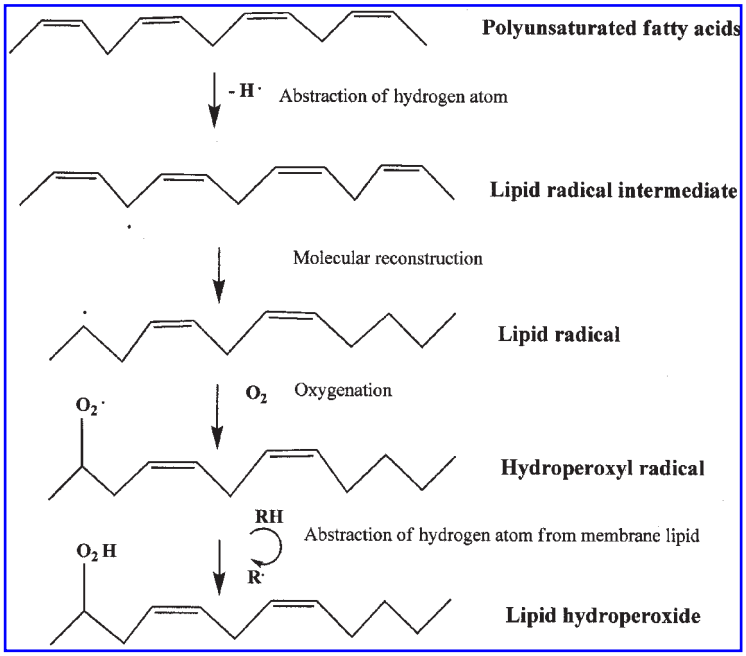

FIG. 1. Basic theory of free radical–mediated lipid peroxidation involving polyunsaturated fatty acids.

215). It is now evident that formation of isoprostanes *in vivo* through the COX pathway is minimal. Unlike primary prostaglandins, isoprostanes do not demand cyclooxygenases for their bioformation. Further, structural characteristic distinction between isoprostanes and COX-mediated prostaglandins is that the former predominate with *cis* side chains to the cyclopentane ring compared with the latter in *trans* orientation (197).

Two nomenclature systems of F_2-isoprostanes were described earlier (234, 261). The Eicosanoid Nomenclature Committee has approved a nomenclature system for F_2-isoprostanes in which carboxyl carbon is designated as C-1, and different regioisomer classes are designated by carbon number of the side chain where the –OH group is located (261). Alternative isoprostane classes are designated based on ω-carbon (234). Over time, several denominations of F_2-isoprostanes have been used in the literature. These are 8-iso-$PGF_{2\alpha}$, 8-epi-$PGF_{2\alpha}$, F_2-isoPs, 15-F_2-IsoP and 15-F_{2t}-IsoP, iPF2alpha-III, and iPF2alpha-VI. 8-Iso-$PGF_{2\alpha}$ and 15-F_{2t}-IsoP are the two most commonly expressed F_2-isoprostanes in the publications.

The mechanism of formation of isoprostanes from an arachidonic acid precursor is shown in Fig. 2. An abstraction of a bisallylic labile hydrogen atom occurs first (Step 1), and after addition of an oxygen molecule to arachidonic acid, four positional peroxyl radicals form (Step 2). Further, endocyclization occurs (Step 3), and an additional oxygen molecule is eventually added to form four unstable PGG_2-like bicyclic endoperoxide intermediates (Step 4). These bicyclic endoperoxide intermediates are then reduced by the presence of glutathione to result in the parent isoprostanes of various series (Step 5). Depending on the mechanism of formation, four F-ring isoprostane regioisomers are formed, which are designated as either 5-, 8-, 12-, or 15-series regioisomers on the basis of carbon atom to which the side chain hydroxyl-group is positioned (175). Because F-ring compounds are isomeric to the COX-derived primary $PGF_{2\alpha}$, these compounds are collectively called F_2-isoprostanes (181). Other isoprostanes of series D_2 and E_2, thromboxane A_2, and cyclopentanone -A_2 and -J_2 are also formed *in vivo* by rearrangement of PGH_2-like isoprostane intermediate (53). Unlike COX-derived primary prostaglandins, isoprostanes are shown to be formed *in situ* in esterified form to tissue phospholipids and subsequently released in free acid form after hydrolysis of the ester moiety, presumably by phospholipases (176).

A range of other isoprostane-like compounds, so-called F_3-isoprostanes formed *in vitro* from the common fish oil [eicosapentanoic acid (EPA, 20:5, n3)] have been identified (89, 193). Their basal levels are too low to detect *in vivo,* but they are detectable in experimental animals after supplementation of EPA (89). Numerous other isoprostane-like compounds, collectively called neuroprostanes and highly reactive acyclic-ketoaldehydes, isoketal-like compounds designated neuroketals, are biosynthesized through peroxidation of docosahexaenoic acid

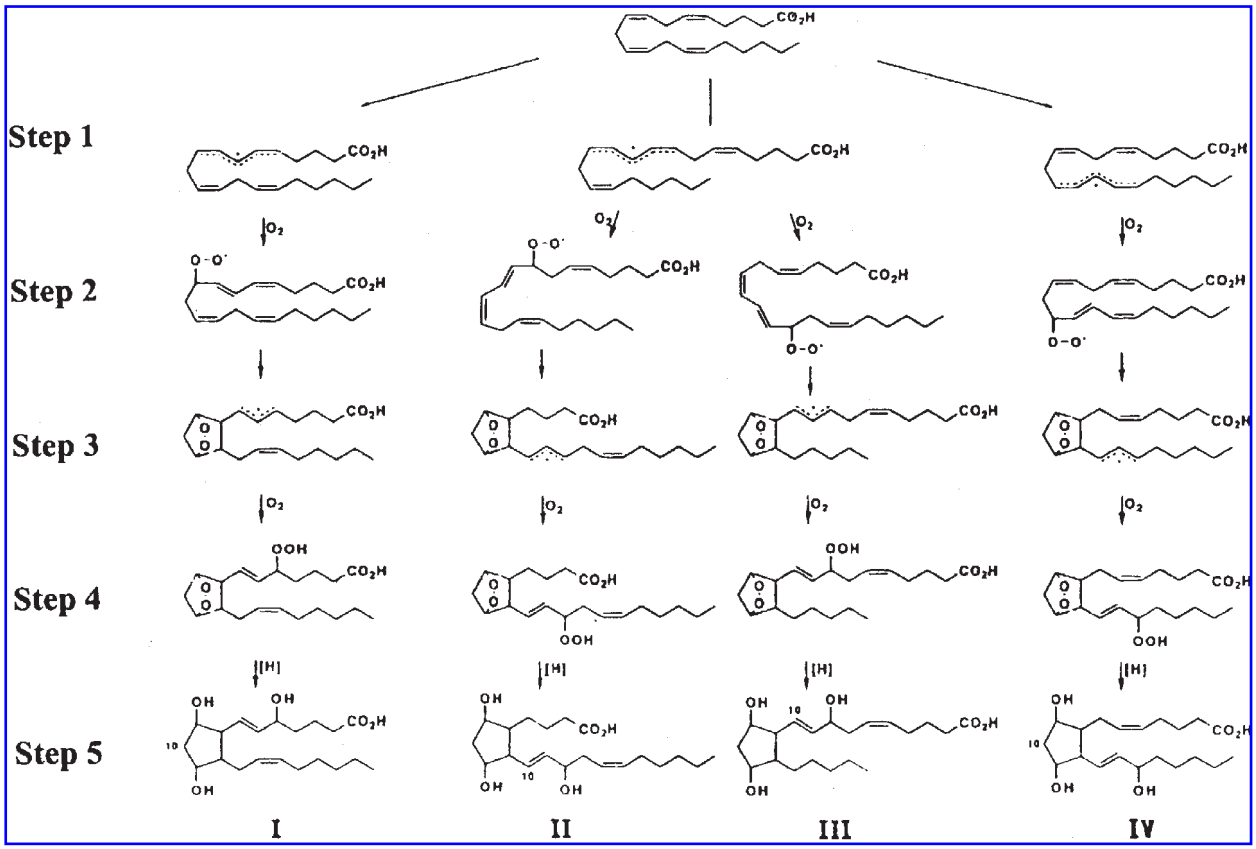

FIG. 2. Mechanism of formation of isoprostanes by free radical–mediated catalysis of arachidonic acid. (Adapted from ref. 173, with permission.)

(DHA, 22:6, n3), which is principally found in the brain and proposed to be involved in the neuronal injury (194, 230). However, 8-iso-$PGF_{2\alpha}$ has also been identified *in situ* in neuronal injury (see Alzheimer's disease section later). Other new products of lipid peroxidation, isofurans that are formed through a substituted tetrahydrofuran ring, were recently described (228). These compounds mainly form endogenously when oxygen concentrations increase in the cells by disfavouring isoprostanes formation.

A. F_2-Isoprostane–induced prostaglandin $F_{2\alpha}$ formation

Numerous studies have reported that both F_2-isoprostanes and cyclooxygenase-catalyzed $PGF_{2\alpha}$ are simultaneously formed in experimental models of oxidative stress by CCl_4 (20, 248) and septic shock induced by LPS (21, 23, 24) or induced ischemia–reperfusion (32), and also are involved in several human pathologies such as type 2 diabetes (92, 105), smoking (103), obesity (37), rheumatic disease (39), liver transplantation (29), cardiopulmonary bypass surgery (267), elective percutaneous coronary interventions, and coronary angiography (40). Prostaglandins might be one of the major conceivable links between oxidative stress–induced inflammatory responses involving two distinct biochemical pathways: free radicals and cyclooxygenases. Recently, a study was designed to investigate to what extent exogenous 8-iso-$PGF_{2\alpha}$, a major F_2-isoprostane, can modify cyclooxygenase-catalyzed endogenous $PGF_{2\alpha}$ formation, given that prostaglandins are inflammatory and potent vasoactive compounds that are involved in various essential physiologic functions, and also form instantly during acute and chronic inflammation (15, 19). An immediate appearance and disappearance of free 8-iso-$PGF_{2\alpha}$ was seen in both plasma and urine within a short interval after IV administration of 8-iso-$PGF_{2\alpha}$ to the rabbits. A succeeding but differential kinetics of formation of $PGF_{2\alpha}$ than 8-iso-$PGF_{2\alpha}$ was observed with a rapid and pulsatile elevation of plasma 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of primary COX-catalyzed $PGF_{2\alpha}$. Within a short while, this compound was excreted efficiently as an intact compound into the urine throughout the 3-h experiment after intervention (Fig. 3). An eightfold augmentation of $PGF_{2\alpha}$ metabolite in plasma at 10 min and a 12-fold increment in the urine at 30–60 min after the IV administration of 8-iso-$PGF_{2\alpha}$ was recorded, and the levels were sustained throughout the 3-h experiment. This observation indicates that pharmacologically administered or endogenously generated 8-iso-$PGF_{2\alpha}$ during an oxidant stress induces prostaglandin formation. This happens presumably *via* the conventional cyclooxygenase-mediated arachidonic acid oxidation that may well be inflammatory and vasoconstrictive itself to the cells, and causes additional gross harmful cellular effects. COX-mediated $PGF_{2\alpha}$ formation may be a primary but key biologic upshot. It may occur either after 8-iso-$PGF_{2\alpha}$ administration *in vivo* or surplus endogenous formation of F_2-isoprostanes during oxidant stress in a variety of diseases because of the potent biologic role of primary prostaglandins to amend diverse physiologic and pathophysiologic statuses in the body by affecting vasoconstrictive and inflammatory mechanisms (see references earlier). Additionally, F_2-isoprostanes may also activate $PGF_{2\alpha}$ receptor (FP receptor) and other prostaglandin receptors (118, 125, 126). Thus, a signaling message might relate to oxidative stress–induced isoprostane formation and the subsequent manifestation of the inflammatory response due to prostaglandin $F_{2\alpha}$ formation through the cyclooxygenases activation.

IV. MOLECULAR MECHANISMS OF ACTION AND BIOLOGIC EFFECTS

Isoprostanes, mainly 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2, possess potent biologic effects in a number of biologic systems, and thus they may also serve as pathologic mediators of oxidant stress through their vasoconstrictive and inflammatory properties. A simplified sketch of the molecular mechanism of action and biologic effects is shown in Fig. 4. Specifically, the biologic action of 8-iso-$PGF_{2\alpha}$ has been shown to be mediated partially by interaction with the vascular TXA_2/PGH_2 receptors (262), and perhaps also through some unknown mechanisms such as inducing diverse product formation through activating other biochemical pathways (see later). Biologic relevance could be achieved locally in the tissues if the free 8-iso-$PGF_{2\alpha}$ levels reach at a microgram concentration or higher, as known at present. Nonetheless, currently very little is known of the biologic effects of other isoprostanes. This is because synthetic isoprostanes are not currently available commercially. It has hitherto not been established whether isoprostanes have any distinct receptors for their biologic effects, as earlier suggested (136). However, a recent review by Morrow (174) highlighted this important issue with current data on the existence of a specific isoprostane receptor for their bioaction. After formation, esterified isoprostanes to phospholipids are not as active as those in their free-acid form. With the existence of efficient hydrolyzing enzymes in diverse tissues in the mammalian body, the esterified isoprostanes rapidly metabolize to their bioactive free-acid form. When such compounds are available as free acid at a high concentration, which usually happens after a certain physiologic (see later) or pathologic situation, they affect first the integrity and fluidity of the membranes, and consequently, the adjacent tissues, causing a state of so-called *in vivo* oxidative strain or oxidant stress. Isoprostanes, particularly 8-iso-$PGF_{2\alpha}$, have well-known vasoconstrictive effects in a variety of organs [*e.g.*, aortic ring segments (271), brain (109), cerebral arterioles (111), kidney (180, 262), the lung (7, 41), pulmonary artery (180), and retinal vessels (127)]. The vasocontractile response to 8-iso-$PGF_{2\alpha}$ is reported to be dependent on extracellular Ca^{2+} by both L- and T-type Ca^{2+} channels, and perhaps also on protein kinase C (88, 220). Further, an inverse correlation has been reported between plasma levels of 8-iso-$PGF_{2\alpha}$ and both endothelium-dependent and endothelium-independent vasodilation in healthy women subsequent to infusion of methacholine or sodium nitroprusside (240). This evidences a possible interaction between circulatory 8-iso-$PGF_{2\alpha}$ and an impaired vasodilation in women. Nonetheless, no definite data have yet been published on the relation of endothelial function and isoprostanes.

The character of isoprostanes as mediators of oxidant stress and signaling molecules of other biochemical cascades *in vivo* is still at its infancy. Nevertheless, Morrow (174) recently reviewed this important issue. It was shown recently in rabbits that intravenous administration of 8-iso-$PGF_{2\alpha}$ induced COX-mediated $PGF_{2\alpha}$ formation (15) (see earlier). As discussed, for-

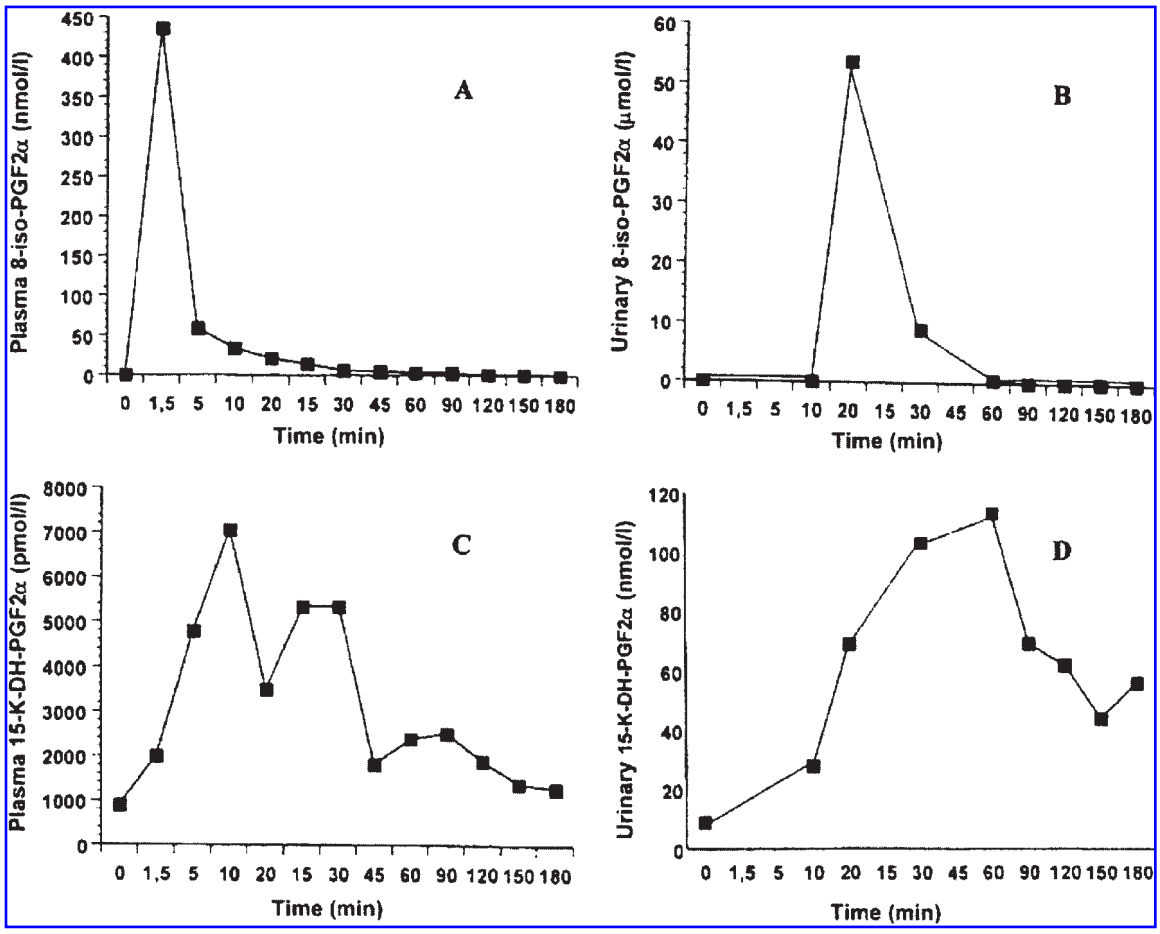

FIG. 3. (A) Time-course elimination of intravenously administered 8-iso-$PGF_{2\alpha}$ in plasma. The kinetics of plasma levels of 8-iso-$PGF_{2\alpha}$ after IV administration of unlabeled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$). Time zero is the time of administration of 8-iso-$PGF_{2\alpha}$. (**B**) Time-course elimination of intravenously administered 8-iso-$PGF_{2\alpha}$ in urine. The kinetics of urinary levels of 8-iso-$PGF_{2\alpha}$ after IV administration of unlabeled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$). (**C**) Time-course formation of COX-catalyzed product $PGF_{2\alpha}$ in plasma. The kinetics of plasma levels of 15-keto-dihydro-$PGF_{2\alpha}$ as a result of primary $PGF_{2\alpha}$ formation after IV administration of unlabeled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$). (**D**) Time-course elimination of $PGF_{2\alpha}$ metabolite in urine. The kinetics of urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ as a result of primary $PGF_{2\alpha}$ formation after IV administration of unlabeled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$). (Adapted from ref. 14, with permission.)

mation of COX-mediated $PGF_{2\alpha}$ has also been seen subsequent to CCl_4-induced F_2-isoprostane production in rats, showing that these two structurally intimately related but biosynthetically dissimilar compounds have deviant kinetics of biosynthesis and release, and circuitously supporting the view of activation of cyclooxygenases (20, 248). 8-Iso-$PGF_{2\alpha}$ causes vasoconstriction through inducing thromboxane formation in the endothelium (127), and earlier observed airway obstruction pharmacologically by 8-iso-$PGF_{2\alpha}$ may partly be due to endogenous TXA_2 formation (199).

V. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Although it is crucial, the pharmacokinetics of F_2-isoprostanes, mainly 8-iso-$PGF_{2\alpha}$, has been seldom studied. It is always critical to understand the pharmacokinetic properties of a particular compound or its end products for qualitative and quantitative assays and also to assess bioactivity. A similar judgment can also be made for F_2-isoprostanes. Metabolism studies showed that the bioactive isoprostanes that are produced *in situ,* preferentially in their esterified form in the tissues, metabolized to their free-acid form (17, 18). After biosynthesis within the tissues, these compounds are readily absorbed and randomly distributed in both the esterified and free-acid form (176, 250). A range of hydrolytic enzymes are ubiquitous in the body, and they are largely accountable for the formation of free isoprostanes because of their esterified moiety in the tissues. This includes preferentially phospholipase A_2 and also platelet-activating factor (PAF) acetylhydrolyses (176, 251). This key reaction process in the cells is the first step of enzymatic bioconversion of esterified isoprostanes to free isoprostanes in the tissues. After formation as free compounds, they are efficiently released into the peripheral circulation. This quick de-esterifi-

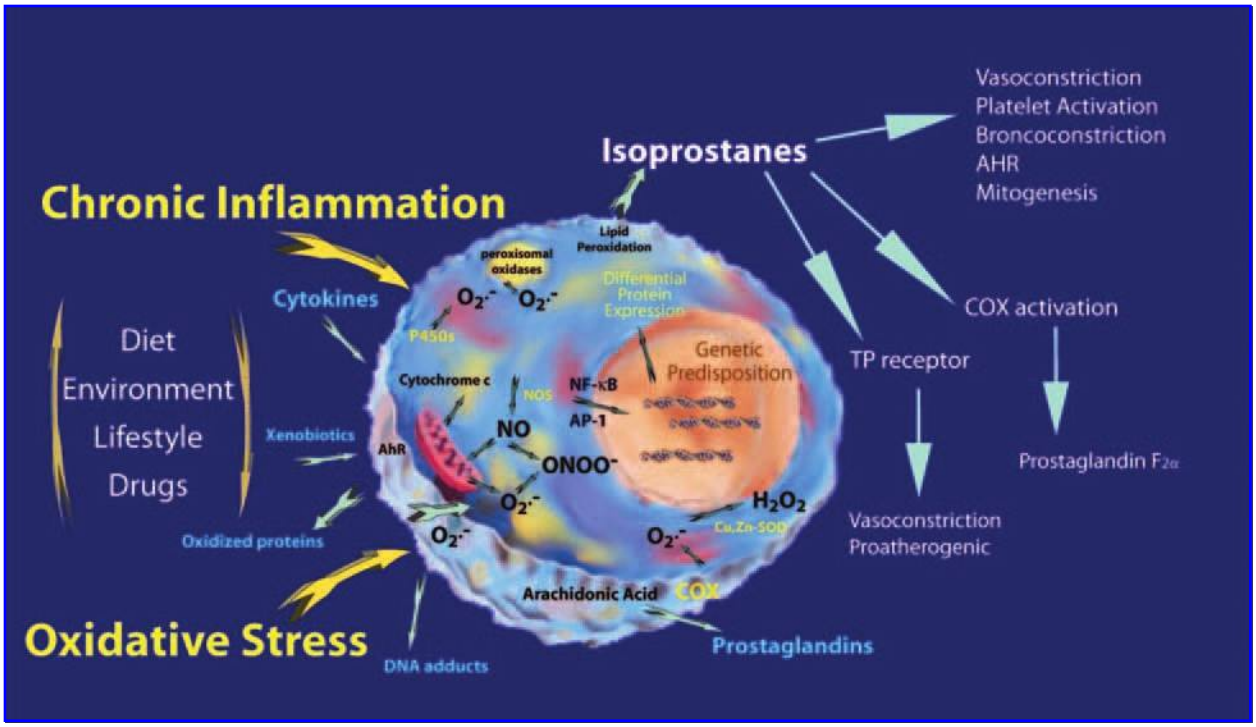

FIG. 4. A simplified sketch of the molecular mechanism of action and biologic effects of F_2-isoprostanes *in vivo*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

cation phase might perhaps be viewed as one of the rate-limiting steps for the release of free isoprostanes in the tissues and their additional availability in the peripheral circulation. This important action contrasts to the events when the primary prostaglandins are formed from arachidonic acid by cyclooxygenase catalyzation, and usually biosynthesize instantly in free-acid form in the tissue phospholipids. Several other endogenous or exogenous factors are also involved in the rate-limiting steps of isoprostane formation and release into the circulation [see (25)].

In a pharmacokinetic and metabolic study, in which tritium-labeled 8-iso-$PGF_{2\alpha}$ was administered over 1 h into a male subject, 75% of the infused compound was excreted into the urine during the following 4.5 h (229). Further characterization of the 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ has been identified as a major metabolite in urine, which represented for 29% of the total radioactivity infused. This major urinary metabolite of 8-iso-$PGF_{2\alpha}$ in humans usually was degraded through one step of β-oxidation of the primary fatty acid 8-iso-$PGF_{2\alpha}$ (229). An additional major metabolite of 8-iso-$PGF_{2\alpha}$ in humans was found to be 2,3-dinor-8-iso-$PGF_{2\alpha}$ (229). The half-life of 8-iso-$PGF_{2\alpha}$ has been found to be ~16 min in humans.

Similarly, when tritium-labeled 8-iso-$PGF_{2\alpha}$ was administered intravenously in rabbits, the total infused radioactivity emerged instantaneously in the blood, and vanished rapidly from the circulation (18). A total of 80% of the total radioactive 8-iso-$PGF_{2\alpha}$ was recovered in the urine within 4 h after the start of the experiment. The plasma half-life of 8-iso-$PGF_{2\alpha}$ in the rabbit was shown to be 1 min in the distribution phase. The half-life in the terminal elimination phase was ~4 min, which is somewhat shorter than that in humans. Numerous polar β-oxidized metabolites became visible in the plasma within 2 min, and finally they were efficiently excreted into the urine. α-Tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ was identified as a major urinary metabolite in the rabbits, together with several other β-oxidized products. The metabolism of 8-iso-$PGF_{2\alpha}$ to α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ and other β-oxidized products occurs in several steps in the rabbit (18), a metabolism course that resembles the COX-catalysed primary $PGF_{2\alpha}$ degradation in this species (18, 54). The levels of β-oxidized metabolites of 8-iso-$PGF_{2\alpha}$ in the plasma were increased within 5 min after administration of radiolabeled 8-iso-$PGF_{2\alpha}$ (18). A simplified metabolic pathway and the enzymes and reaction processes involved in the degradation process of 8-iso-$PGF_{2\alpha}$ in the rabbit are shown in Fig. 5.

Metabolism of isoprostanes has been shown to occur essentially through like pathways as enzymatically formed primary prostaglandins. Studies *in vitro* with various tissues (17) and experiments *in vivo* demonstrated that oxidation of the 15-hydroxy group at C-15 by 15-prostaglandin dehydrogenase (15-PGDH) is the initial step of 8-iso-$PGF_{2\alpha}$ degradation (18). A reduction of C-13,14 double bond by Δ^{13}-reductase and formation of 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ occurred in the subsequent step of metabolism. Therefore, 15-PGDH and Δ^{13}-reductase are the central enzymes involved in the degradation of 8-iso-$PGF_{2\alpha}$, and perhaps these enzymes are also responsible for the metabolism of other isoprostanes. Although various hydrolytic, oxidative, and reductive enzymes, including phospholipases, 15-PGDH, and Δ^{13}–reductase, respectively, are found in all parts of the body (4, 34, 38), possible formation of

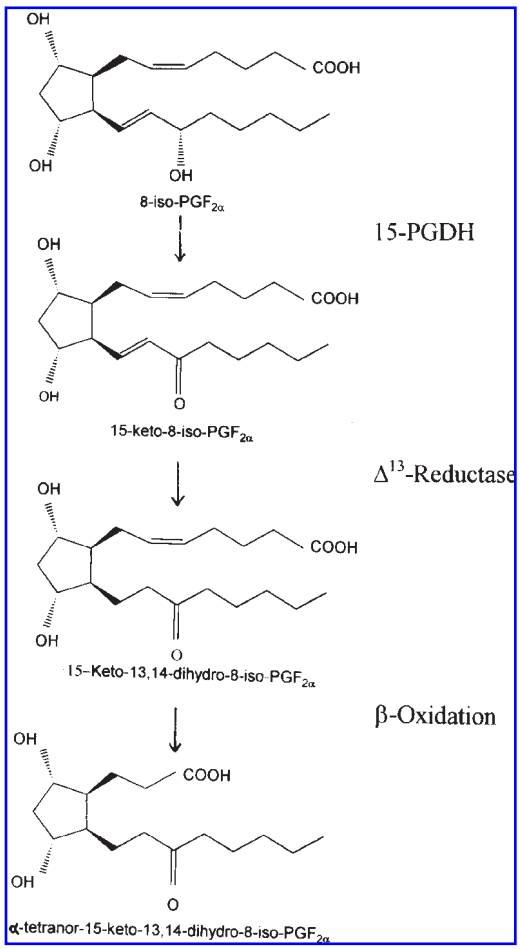

FIG. 5. Metabolism of 8-iso-PGF$_{2\alpha}$ to 15-keto-dihydro-8-iso-PGF$_{2\alpha}$ and subsequently to more polar β-shorter C-16 metabolites. 15 PGDH, 15-prostaglandin dehydrogenase. (Adapted from ref. 17, with permission.)

F_2-isoprostanes in any tissue at basal status and after an induction of oxidant stress would be followed by a rapid degradation and further release into the circulation unless a deficit of the *de novo* metabolizing enzyme systems was present. Although 15-PGDH and Δ^{13}-reductase are rich in lung, liver, and kidney, these key organs are the main metabolism sites for isoprostanes. However, metabolism of isoprostanes may also occur in all parts of the body, resembling prostaglandin metabolism (4, 35).

Both β- and ω-oxidation are very common reactions in later steps of prostaglandin metabolism. It has been revealed in rabbits that 15-keto-13,14-dihydro-8-iso-PGF$_{2\alpha}$ swiftly metabolizes through two steps of β-oxidation largely to α-tetranor-15-keto-13,14-dihydro-8-iso-PGF$_{2\alpha}$ and also to numerous other β-oxidized metabolites (18). Together, the human and rabbit studies described earlier confirm that β-oxidation is the common degradation pathway in the later step of metabolism of 15-keto-13,14-dihydro-8-iso-PGF$_{2\alpha}$. Therefore, this step of metabolism of F_2-isoprostanes is species specific, at least in regard to the formation of dissimilar structural end products. Both the dinor and tetranor metabolites of 8-iso-PGF$_{2\alpha}$ are detectable in plasma and urine in more-elevated concentrations than are their initial parent compounds (18). In rat hepatocytes, metabolism of 8-iso-PGF$_{2\alpha}$ yields a tetranor compound, designated 2,3,4,5-tetranor-8-epi-PGF$_{2\alpha}$ (54).

VI. ISOPROSTANES AS BIOMARKERS OF LIPID PEROXIDATION AND OXIDATIVE STRESS

A simplified schematic formation of 8-Iso-PGF$_{2\alpha}$, a major F_2-isoprostane from arachidonic acid, is shown in Fig. 6. This compound or its related isomers or both are increased significantly in a number of diseases that are believed to be associated with oxidant injury, and the measurement of F_2-isoprostanes in a range of tissues and body fluids is usually regarded as a reliable biomarker of *in vivo* determination of lipid peroxidation by a free-radical pathway (13, 16, 25, 162, 163, 175, 180). 8-Iso-PGF$_{2\alpha}$ has ~10 times higher basal levels than the enzymatically produced PGF$_{2\alpha}$ in plasma, and the free form of this compound is easily detectable in a number of body fluids by sensitive and specific analytic approaches. Measurement of the esterified and free isoprostanes is suitable in the tissues, and thus it could be used as an approach to oxidative stress measurement for tissue injury of interest (177, 248). Specific antibodies to the isoprostanes can also be applied in the *in situ* localization of the compound by immunochemistry of the oxidative stress–injured tissues (described later in the detection section).

Measuring commonly applied MDA (malondialdehyde) as a measure of lipid peroxidation is much less sensitive than the increase (80- *vs*. 2.7-fold) in esterified isoprostanes in the liver

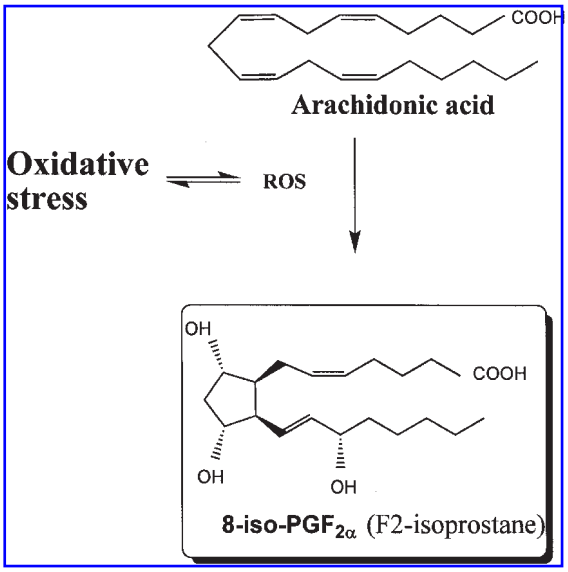

FIG. 6. A simplified scheme of biosynthesis of 8-iso-PGF$_{2\alpha}$ (F_2-isoprostane) from arachidonic acid. ROS, Reactive oxygen species; PGF, prostaglandin F. (Adapted from ref. 13, with permission).

(137). Other comparative *in vivo* studies did not show any correlation in the increase of isoprostanes with MDA levels (20, 99). Even though measuring isoprostanes merely reflects the oxidation of arachidonic acid rather than the total lipids pools, it is thought that other lipids also might have been oxidized to a certain extent in the process of lipid peroxidation. Additional advantages of measuring isoprostanes are that the levels of these compounds are not exaggerated by the lipid content of the diet (93, 225) or by preservation when the biologic samples are stored frozen in proper conditions (16). Nonetheless, it is very vital to scrutinize when, what, and which biologic samples are preferential for measurement of isoprostanes to avoid any risk of overlooking a brief or long-lasting secretion of isoprostanes. Furthermore, inappropriate collection, hazardous preparation (during extraction, purification, and hydrolization) and preservation of the samples before analysis are plausible factors of artefactual formation of these compounds or impurity-affected analytic errors in isoprostane analysis.

VII. ISOPROSTANE DETECTION AND ASSAYS

A major scientific question and challenge has been the determination of free radicals and oxidative stress *in vivo*. Recent studies elucidate that lipid peroxidation is a faster reaction process in pathologic situations than oxidation of other biomolecules, such as proteins, DNA, or carbohydrates. When considering quantitation of lipid peroxidation *in vitro* and *in vivo*, measurements of F_2-isoprostanes have a clear advantage over the other commonly available assay methods such as TBARS (thiobarbituric acid–reactive substances), MDA, lipid hydroperoxide, exhaled alkanes, such as ethane and pentane, or conjugated dienes. These latter methods are usually fraught with various methodologic boundaries in application, more in the *in vivo* than *in vitro* studies (62, 96, 232). These include specificity and sensitivity of the methods, unspecific product formation through alternative biochemical pathways, and the levels of the formed products were largely influenced by the lipid contents of the diet or other artifacts. A typical and most commonly used method of lipid peroxidation is MDA analysis through a TBARS reaction assay. The determination of MDA is an unspecific method of lipid peroxidation that represents a by-product formation during TXA_2 formation through the cyclooxygenase pathway. With this understanding, a recent independent multilaboratory network study on oxidative stress using the CCl_4-induced hepatotoxicity model in rats and analysis of several oxidative stress biomarkers (both protein and lipid oxidation) by different methods was organized independently by the National Institute of Environmental Health Sciences, NIH, U.S.A. (BOSS-II and BOSS-III studies). This confirms that measurement of F_2-isoprostanes in plasma and urine by different analytic methods (GC-MS, LC/MS/MS, radioimmunoassay, and enzyme immunoassays), MDA (measured by GC-MS), and 8-OHdG in urine represent potential candidates for general biomarkers of *in vivo* oxidative stress (119, 120).

Gas chromatography with mass spectrometry (GC-MS) was the first assay technique used in the early discovery and quantitation of isoprostanes. Other assay methods such as liquid chromatography (LC)-mass spectrometry, GC-MS-MS, LC-MS-MS, radioimmunoassays, and enzyme immunoassays have now been developed both by individual research groups and commercially. These rapidly exploited the impact of novel isoprostanes in human health and diseases (22, 179, 207, 274). Table 1 shows a list of such methods that are commonly used for the determination of F_2-isoprostanes in body fluids and tissues. The mass spectrometry–based methods, although specific, theoretically have the drawback of being expensive and relatively laborious to execute because of the formation of numerous closely related structural isomers, which need extensive chromatographic purification steps before analysis and to dis-

TABLE 1. F_2-ISOPROSTANE DETERMINATIONS BY DIVERSE METHODS

Analytic methods	*Specificity/sensitivity*	*Selected references*	*Sample purification*
GC-MS	NR	179	Extraction, TLC
	9.4–15.1%; 10 pg/ml	192	Solid-phase extraction
	χ = 1,009–1,076; SD, 5–29	2	Solid-phase extraction, TLC
	4%; 20 pg	184	Solid-phase extraction
	6.7%, 3.7%, and 8%, 5.6%	169	Reversed-phase extraction, HPLC
	NR	218	Solid-phase extraction, HPLC
	50 pg/ml	273	HPLC
	3.4% and 4.5%	49	Solid-phase extraction, TLC
	8.8–8.9%	200	Extraction, TLC
	4–10% and 2–5%; 25 pg/ml	42	Solid-phase extraction
	24–32%; 40 pg/ml	263	Liquid-phase extraction, HPLC
	~6%; ~5 pg	151	Solid-phase extraction, TLC
LC-MS-MS/LC-MS	0.03–0.06%; 0.02–0.05%	131	Solid-phase extraction, HPLC
	<12% and <9%; 9 pg	132	Solid-phase extraction, LC
EIA	2%, 2.9%, and 3.7%, 11%	274	Solid-phase extraction, TLC
	100 pg/ml	241	Not known
RIA	NR	274	Solid-phase extraction, HPLC
	14.5%, 12.5%; 8 pg/ml	22	No sample preparation

LC-MS, liquid chromatography-mass spectrometry; EIA, enzyme immunoassay; RIA, radioimmunoassay; NR, not reported.

tinguish their mass characteristics. Further, mass spectrometric methods often require appropriate derivatization procedures and have a comparatively lower sample-analyzing ability, and are therefore difficult to use in large clinical, experimental, and epidemiologic studies. This has always been challenging in the determination of prostaglandin-like compounds *in vivo*. Unlike sample extraction/purification procedures applied in a number of the previously mentioned assays, including the enzyme immunoassays, these have often been shown to be a basis for errors rather than a benefit in the performance of isoprostane assays. Even though mass spectrometry–based methods are relatively more specific than many others, as claimed by potential users, they necessitate a skilled technical staff and a high sensitivity-assay technique to detect low levels of these compounds *in vivo*. On the contrary, immunoassays, although they are less specific or quantitative than GC-MS methods except the antibodies, are extensively verified for their cross-reactivities and other accuracy tests and have been found to be essential tools for new discoveries in medical and pharmaceutical sciences for decades. Immunoassays have a huge sample-analyzing capacity at fairly low expense, if the requirements are fully satisfied, and if the assays are validated and the results are confirmed through the experimental animal models of oxidant injury, establishing that an augmentation of the measured compound is warranted with the specific experimental design. A model of such experimentation is CCl_4-induced oxidant stress and to study isoprostane formation, where tissues, plasma and urine ought to be analyzed and the results carefully related with their kinetics of formation (22, 179, 229, 248). Although immunoassays have been often considered semiquantitative because of accuracy problems, a well-validated technique could be a significant tool for evaluating free radicals–mediated reactions in clinical research, where a large numbers of samples must be analyzed at an affordable cost, as well as in mechanistic studies of oxidative stress in basic research.

A radioimmunoassay has been developed and validated by raising a specific antibody with which free 8-iso-$PGF_{2\alpha}$ can be measured reasonably reliably in most of the body fluids devoid of any extraction or hydrolyzation procedures (revealed to be the major problem in the accuracy of various isoprostane assays) (22). This method can also be used to measure total levels (esterified and free) of this compound in target sites of interest (*e.g.*, tissues or biologic fluid collected from certain key organs) (248, 250). The accuracy is lower in the tissue measurements than in assays in body fluids because of the need for extraction and hydrolyzation of the tissue samples, as discussed earlier. A number of enzyme immunoassays that require extensive sample hydrolyzation, extraction, and purification steps are now commercially accessible with variable outcomes.

When considering assessment of isoprostanes in the tissue measurement of the esterified and free isoprostanes is appropriate, it could be used as an approach to oxidative stress measurement for tissue injury (177, 248). Specific antibodies to the isoprostanes can also be used for *in situ* localization of the compound by immunostaining of the oxidative stress–injured tissues. A novel study of such recognition of oxidative stress–induced cytoplasm of neurons in Alzheimer pathology has lately been described (52). This localization of bioactive isoprostanes by immunostaining with specific antibody is a new way of detection of free radical–mediated tissue damage and also opens possibilities for therapeutic application of various radical scavengers in disease-related damage.

Most of the assays of isoprostanes to date have primarily focused on assessment of 8-iso-$PGF_{2\alpha}$ in body fluids that are major products of the arachidonic acid oxidation process *in vivo*. Numerous other F_2-isoprostanes of the $ipF_{2\alpha}$-IV series also originate at high concentrations in the urine and are considered to be consistent parameters of oxidant stress (129). While measuring the urinary metabolites of 8-iso-$PGF_{2\alpha}$, the selection of a suitable end product is of significance, because the metabolic profile and the *in vivo* manifestation of the different metabolites of this compound at a later stage of the metabolism are unlike among species, as described earlier (34, 38, 229). Tetranor metabolites are the key urinary products of 8-iso-$PGF_{2\alpha}$ in rabbits, whereas the dinor metabolite of 8-iso-$PGF_{2\alpha}$ is the central product in humans. Because the levels of isoprostanes differ between laboratories as a result of differences in the assay methods applied, and also because some assays measure total compound (esterified plus free), and others, only free compound, each laboratory should at least have its own basal levels calculated from various species. Thus, it is of importance to observe the following considerations when evaluating isoprostanes in diverse experimental or clinical situations.

1. It is essential to check which isoprostanes are to be measured: free isoprostanes or a total compound (esterified plus free isoprostane) after hydrolysis of the matrix.
2. Variation in the basal levels also is dependent on the previously mentioned factor.
3. When considering isoprostane levels in acute experimental oxidative stress or an inflammatory scenario, a higher difference ought to be obtained from basal levels because of inclusion of fewer subjects or experimental animals in the study.
4. A 10% difference in the levels of isoprostanes is sufficient to detect a chronic pathologic augmentation compared with basal levels (103, 105) when a sufficient number of patients are included in the study, because of large variation in the basal isoprostane levels, as discussed later.

VIII. BASAL VARIATION OF F_2-ISOPROSTANES IN HUMANS

It is of vital importance to know the basal levels of F_2-isoprostanes in humans to evaluate both experimental and clinical-study settings. It is equally imperative to know how levels of this parameter fluctuate during a 24-h period and between days in healthy humans. Recalling these questions are unexpectedly exceptional compared with the number of published reports on F_2-isoprostanes as a biomarker of oxidative stress. This section briefly summarizes reports related to the intraday and daily variation of 8-iso-$PGF_{2\alpha}$ excretion in humans.

A. Variation within the day

The basal plasma level of free 8-iso-$PGF_{2\alpha}$ is shown to be ~28 pg/ml (range, 10–80 pg/ml) in healthy subjects, with a

wide variation among individuals (22). A part of the variability in the levels could be due to different analytic methods used by various investigators, but it is equally true that subject variability is observed in free 8-iso-$PGF_{2\alpha}$ levels with use of same analytic method (25). No studies have been performed systematically in plasma; rather they have been performed with urinary samples in healthy human subjects (see later). Because the metabolism of 8-iso-$PGF_{2\alpha}$ is fast and a large amount of this compound is excreted intact into the urine, measurements of 8-iso-$PGF_{2\alpha}$ in urine samples to study basal variation is hypothesized to be appropriate.

When a 24-h urine sample, a spot urine sample in the morning, and spot urine samples at varying hours during the day was collected from 10 healthy subjects of varying ages for the measurement of 8-iso-$PGF_{2\alpha}$, it was found that a variation in isoprostane levels occurs within the day (Fig. 7). Nonetheless, no significant difference was found at the cluster level comparing mean values of 8-iso-$PGF_{2\alpha}$ in urine collected at different times during the day, or collected in the morning, compared with mean levels of 8-iso-$PGF_{2\alpha}$ in the 24-h urine sample (101). This was also established in a larger study with healthy individuals, in which the mean level of 8-iso-$PGF_{2\alpha}$ in the morning urine in the whole group did not differ from the mean level of 8-iso-$PGF_{2\alpha}$ in the 24-h collection (33). In addition, no diurnal variation of 8-iso-$PGF_{2\alpha}$ at the group level could be observed when mean values from five different urinary collections were compared (225). No statistical circadian variation was observed at the group level between mean values of three 8-h collections during a 24-h period in 10 subjects (274). Good correlations have been observed between levels of 8-iso-$PGF_{2\alpha}$ in the morning urine and levels of 8-iso-$PGF_{2\alpha}$ in the 24-h urinary collection, and in the spot urine collected at different times during the day and the 24-h urine, respectively (Fig. 8) (101). In another study, a good correlation between the levels of urinary 8-iso-$PGF_{2\alpha}$ collected in the morning or collected during 24 h is reported (225). Collectively, it could be assumed that clearly a diurnal variation in levels of urinary 8-iso-$PGF_{2\alpha}$ exists during the day in individual subjects rather than group levels. Conversely, when 8-iso-$PGF_{2\alpha}$ is studied on a group level, as performed in most clinical studies, the collective data are in agreement with a nonexisting circadian variation. Additionally, F_2-isoprostanes measured in the urine samples collected in the morning, or in several spot urine samples, satisfactorily correspond to the daily F_2-isoprostane excretion. Dinor metabolites of F_2-isoprostanes could also be analyzed in the urine to depict the levels of F_2-isoprostane excretion in humans. The expected levels are much higher than the parent F_2-isoprostane.

Measurement of major urinary metabolites of 8-iso-$PGF_{2\alpha}$ (2,3-dinor-5,6-dihydro-$PGF_{2\alpha}$ in human urine or α-tetranor-15-keto-13,14-dihydro-$PGF_{2\alpha}$ in urine from other species such as rabbits or rats), in addition to parent 8-iso-$PGF_{2\alpha}$, provide useful parameters to predict the 8-iso-$PGF_{2\alpha}$ formation *in vivo* (18, 54, 229). These metabolites are the major metabolites in humans, rats, and rabbits, as earlier discussed. The advantage of measuring F_2-isoprostanes or their metabolites in urine is recommended when considering a noninvasive sample collection over a selected period.

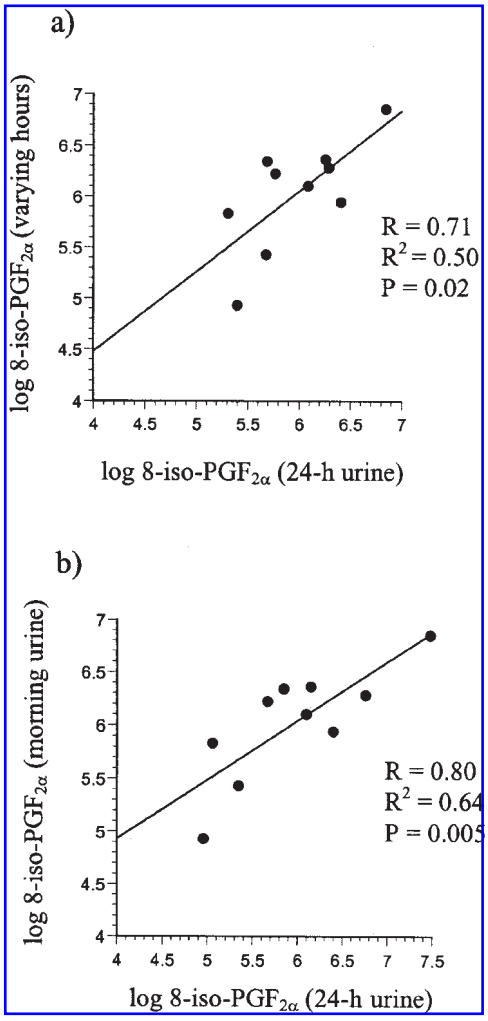

FIG. 8. Linear correlations of 8-iso-$PGF_{2\alpha}$ levels in (a) spot urine samples during a day (mean) and 24-h urine samples, and (b) morning urine samples, and 24-h urine samples, in 10 healthy subjects. (Adapted from ref. 24, with permission.)

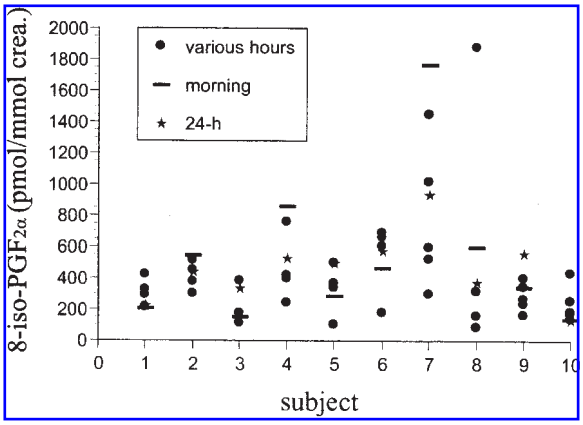

FIG. 7. 8-Iso-$PGF_{2\alpha}$ levels in urinary samples collected at various hours, in the morning, and over a 24-h period in 10 healthy subjects. (Adapted from ref. 24, with permission.)

B. Variation between days

The intrasubject coefficient of variation in 8-iso-$PGF_{2\alpha}$ in morning urine during 10 consecutive days varied from 18 to 104% in healthy subjects, with a coefficient of variation of 42% for all 13 subjects during 10 consecutive days (102). This deviation includes both a biologic variation and an intraassay variation of the assayed samples. The variation due to the analysis technique used in the mentioned study has a coefficient of variation of ~12–15%, leaving the residual observed variation (26–30%) to individual biologic variation. The intrasubject variation for the duration of 3 days varied from 2.1 to 10.5% in nonsmokers and 4.5 to 24.3% in smokers (6).

Together, daily and individual variations between days are the features to take into account when setting up a clinical study. However, the variation may differ depending on the study settings and the subjects included in the study.

IX. OXIDATIVE STRESS MODEL AND ISOPROSTANES FORMATION

A. CCL_4-induced oxidative stress

Carbon tetrachloride (CCl_4) is a well-known toxic compound that induces cirrhosis and oxidative injury in the liver. This model has been extensively used in experimental models of oxidative stress and lipid peroxidation in animals for decades (13). Carbon tetrachloride swiftly transforms to trichloromethyl radical ($\cdot CCl_3$) or other radicals in the liver, and subsequently induces *in vivo* oxidative stress when administered orally to the experimental animals. Measuring F_2-isoprostanes by using this model and also after administration of antioxidants, specifically vitamin E, has been revealed to be a unique tool in the establishment of F_2-isoprostanes as biomarkers of oxidative stress, as evidenced by several such studies (20, 119, 120, 177, 248). A substantial amount of esterified 8-iso-$PGF_{2\alpha}$ was seen in the liver tissues at 2 h after the oral administration of (2.5 ml/kg) CCl_4 to the rats, whereas the free 8-iso-$PGF_{2\alpha}$ levels of this compound in the liver tissue were rather low (250). In a further study, it was found that free 8-iso-$PGF_{2\alpha}$ levels increased 17-fold in plasma and 53-fold in urine from the basal levels at 4 h after CCl_4 (2 ml/kg) administration to the rats (20). At 6 h, free 8-iso-$PGF_{2\alpha}$ levels in the plasma and urine increased sevenfold in plasma and 87-fold in urine, respectively, when the animals were killed (Fig. 9). The levels of F_2-isoprostanes were still significantly higher after 24 and 48 h as compared with the baseline after the administration of CCl_4 (177). Thus, data indicate that CCl_4-induced formation of F_2-isoprostanes is linked to lipid peroxidation rather than to hepatic necrosis. A key proportion of the esterified isoprostanes that are formed in the tissue is consequently released into the peripheral circulation in free acid form, given that the hydrolysis of the esterified compounds is instantaneous.

To confirm whether oxidative stress is involved in CCl_4 intervention in the experimental animals, the increase of F_2-isoprostanes in the body fluids has been counteracted by the administration of vitamin E or other antioxidants (13, 177, 248) (see later). In this context, in a mechanistic study, a high dose of vitamin E (20-g/kg diet of all-rac-tocopheryl succinate for 3 weeks) was supplemented to affect nonenzymatic lipid peroxidation in rats with CCl_4-induced hepatotoxicity (2.5 ml/kg) (248). Rats supplemented with vitamin E before CCl_4 treatment had significantly lower levels of urinary and liver free 8-iso-$PGF_{2\alpha}$, together with lower hepatic toxicity, than had rats treated with CCl_4 alone. Thus, lipid peroxidation during experimental hepatic oxidative injury and inflammatory response could be reduced by daily dietary supplementation of high doses of vitamin E. It also showed that antioxidant therapy ameliorated the progression of atherosclerosis and isoprostane formation (216). Thus, supplementation of high doses of vitamin E in animal models may affect free radical–induced oxidative injury and advocates for a role of these antioxidants in oxidative stress–related pathologies. However, the role of vitamin E in regulating isoprostane formation in human studies is not yet completely convincing, except supplementation with higher doses with longer duration (231) (see later).

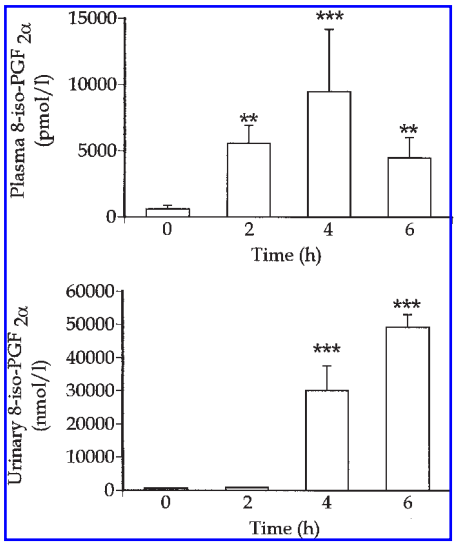

FIG. 9. The levels of free 8-iso-$PGF_{2\alpha}$ in peripheral plasma and urine at different times after oral administration of CCl_4 to rats (2 ml/kg). (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (Adapted from ref. 19, with permission.)

B. Other chemical-induced oxidative stress

In early experimental studies, administration of either diquat to selenium-deficient rats to normal rats resulted in a dramatic increase in F_2-isoprostanes levels, which correlated with the tissue injury caused by the free radical–mediated oxidative stress (179). Plasma levels of F_2-isoprostanes have been reported to correlate with the severity of alcohol-induced liver injury in different dietary models in rats (189). However, isoprostane formation through other experimental oxidative-stress models is not so common as in the CCl_4-induced oxidative stress model.

X. ISOPROSTANES IN ANIMAL MODELS

A. Acute inflammation and oxidative stress

It is well known that gram-negative bacteria encompass a cellular fence covered with endotoxin, a lipopolysaccharide,

that is released during bacterial lysis. This substance is toxic and is formed by bacteria during infection, inducing acute and chronic inflammation by the release of a broad range of biochemical mediators in the host (258). Human gram-negative shock, frequently observed in intensive care units, could be simulated by the administration of endotoxins (*LPS/E. coli*) in pigs, which is a conventional model of experimental septic shock and induction of acute inflammation (153). Sepsis and endotoxemia *via* inflammatory stimuli induce production of thrombin that converts fibrinogen to fibrin, with aggregation of platelets, and serves as an inflammatory mediator affecting endothelial cells and smooth muscle and myocardial cells. Natural responses to endotoxemia include cardiovascular and metabolic deterioration, activation of the coagulation system, complement, and cytokine cascades, and generation of potent products of arachidonic acid cascades that in concert lead to insufficient cardiac performance (244).

Several experimental acute-inflammation studies revealed that both plasma and urinary levels of 8-iso-$PGF_{2\alpha}$ increased significantly in a well-established porcine model of septic shock subsequent to intravenous administration of *LPS/E. coli,* and in addition, with an augment of arterial $Paco_2$ (23, 24, 31) (Fig. 10). However, free radical–mediated F_2-isoprostane formation was increased with a different kinetics, magnitude, and duration of formation than cyclooxygenase-catalyzed $PGF_{2\alpha}$, a marker for inflammatory response during endotoxemia. Nevertheless, the survival of pigs after induced endotoxemia was reliant not on the kinetics of formation and the levels of $PGF_{2\alpha}$ metabolite in the circulation, but somewhat on the extent of oxidative stress, as indicated by the stepwise and higher extent of F_2-isoprostane formation (24). In a similar porcine experimental model, it was also shown that endotoxemia caused quick $PGF_{2\alpha}$ formation in the myocardium, and thus, cyclooxygenase-mediated acute inflammation in the anesthetized pigs. This could readily be detected biochemically in myocardial microdialysate by nearly threefold augmentation of plasma $PGF_{2\alpha}$ metabolite (187). This myocardial inflammatory progression paralleled a deteriorated left ventricular performance and might, therefore, be a mediator of myocardial dysfunction in septic shock. Therefore, induction of acute inflammation by endotoxemic challenge is linked to increased primary $PGF_{2\alpha}$ formation and also to time-related increase of free radicals–mediated F_2-isoprostane formation, a cellular metabolism outcome of an inflammatory response. Thus, these experiments support the notion that F_2-isoprostanes are involved in acute inflammation, and $PGF_{2\alpha}$ might be one of the vital biochemical links to acute inflammation-mediated free radical formation and initiation of oxidative stress.

Among several drugs used against septic shock, only propofol, a commonly used anesthetic drug in surgery and intensive care units, has been shown effectively to counteract levels of F_2-isoprostanes. It affected less a major clinical outcome, arterial oxygen tension (Pao_2) in endotoxemic pigs after LPS intervention, and thus, restored survival of the experimental pigs (31). Together, it could be assumed that decreasing F_2-isoprostanes in acute inflammatory conditions improves the critical clinical outcome in endotoxemia.

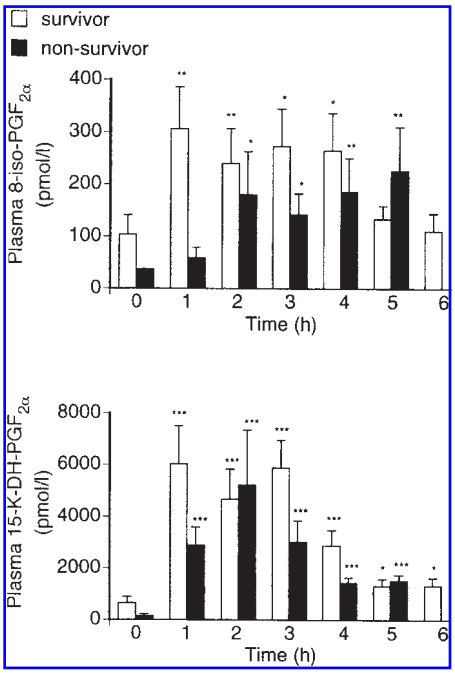

FIG. 10. The levels of $PGF_{2\alpha}$ in plasma as assessed by 8-iso-$PGF_{2\alpha}$ in pigs from both the survivors and the nonsurvivors after induction of septic shock by intravenously administered LPS. Time after i.v. infusion of LPS is denoted on the x-axis (Adapted from ref.23, with permission.)

B. Ischemia–reperfusion and acute oxidative stress

Ischemia–reperfusion injury is the key reason for tissue injury after cardiac infarction and stroke. In experimental animal studies, cardiac arrest and resuscitation were induced in pigs. This induced ischemia–reperfusion condition may further lead to subsequent cerebral injury in the experimental animals (280). This model is a well-established experimental study protocol for ischemia–reperfusion and subsequent brain injury. Reperfusion causes local and remote organ damage that is a severe and commonly observed as a clinical manifestation in intensive care units. This state is a characteristic sign of association of free radical formation at the site of tissue damage and ensuing induction of oxidative stress in the whole body after a reduced blood flow in the brain (28). In this porcine model of cardiac arrest and cardiopulmonary resuscitation, oxidative injury was determined by the measurement of 8-iso-$PGF_{2\alpha}$ in plasma samples taken frequently from both systemic circulation and the jugular bulb that primarily drains the brain after 2 and 5 min of cardiac arrest (32). 8-Iso-$PGF_{2\alpha}$ was elevated quickly both in the systemic circulation (Fig. 11, upper panel) and in jugular bulb plasma (Fig. 11, lower panel) at both interventions after cardiac arrest and after resuscitation. In two supplementary studies with amplified time of cardiac arrest (8 min of ventricular fibrillation), 8-iso-$PGF_{2\alpha}$ levels increased in jugular bulb plasma simultaneous with increases in the levels of hypoxanthine, lactate, and a COX-mediated $PGF_{2\alpha}$ metabolite (134, 135). A further increase of cardiac arrest time (up to 12 min of ventricular fibrillation) led to an additional increase of 8-iso-$PGF_{2\alpha}$ in the jugular bulb plasma (28). Increased levels of plasma 8-iso-$PGF_{2\alpha}$ were found to be linked to neurologic

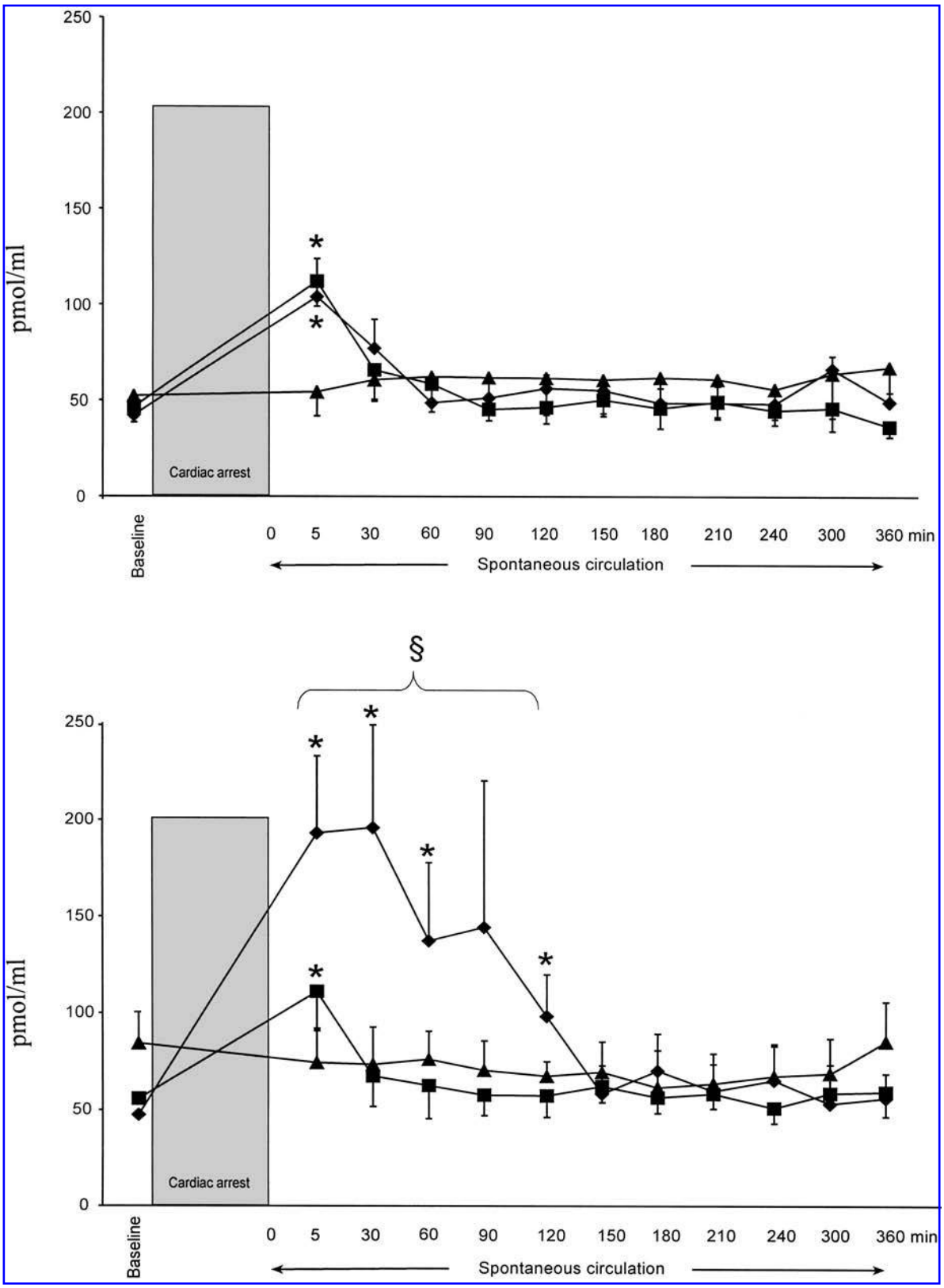

FIG. 11. The levels of 8-iso-$PGF_{2\alpha}$ in plasma in pigs after induction of cardiac arrest and cardiopulmonary resuscitation. Mixed venous plasma levels of 8-iso-$PGF_{2\alpha}$ at baseline and after ROSC (*top*). Jugular-bulb plasma levels of 8-iso-$PGF_{2\alpha}$ at baseline and after ROSC (*bottom*). ◆, group VF5; ■, group VF2; and ▲, control group. *Significant difference *versus* baseline; significant difference between intervention groups and control group. Values are expressed as mean ± SEM. ROSC, Restoration of spontaneous circulation. (Adapted from ref. 31, with permission.)

deficits of the experimental animals at 24 h after cardiac arrest and resuscitation (134). A comparable finding of the appearance of 8-iso-$PGF_{2\alpha}$ in arterial and venous blood and brain tissue were recently reported after cardiac arrest and resuscitation in the pigs, which corroborates these findings (113). A fast appearance of 8-iso-$PGF_{2\alpha}$ in the plasma and urine was also observed during ischemia–reperfusion in experimental spinal cord ischemia in pigs (12). Increased production of 8-iso-$PGF_{2\alpha}$ also was seen in acute coronary thrombolysis/reperfusion in an experimental canine model (75). Overall, these studies support the notion that F_2-isoprostanes not only increase in experimental models of ischemia–reperfusion as an indicator of oxidative injury but that they may also play a significant role in the pathophysiologic sequel of the tissue damage. The increase of F_2-isoprostanes in the brain after experimental cardiac arrest and cardiopulmonary resuscitation has been counteracted in different degrees by the administration of various free radical scavengers such as PBN, S-PBN, and methylene blue, which suggests that oxidative stress is concerned in ischemia–reperfusion-related brain injury (134, 135, 149, 150). The clinical outcome, such as cerebral blood flow, was normalized by S-PBN (sulfophenyl-*N-tert*-butyl-nitrone), and an improved 24-h neurologic outcome was seen after administration of PBN (α-phenyl-*N-tert*-butyl-nitrone) in this experimental model of cardiac arrest and resuscitation. This supports the notion that by counteracting increased levels of peripheral F_2-isoprostanes, clinical improvements could be achieved that are favorable in cardiac arrest–induced brain injury.

XI. ISOPROSTANES IN PHYSIOLOGY

The evidence of involvement of isoprostanes in other aspects of physiologic status is basically still unexplored. Because isoprostanes are constantly formed *in vivo* in humans and other species, and the levels vary within the day and among days in humans, it could be presumed that isoprostanes are conceivably compulsory in the body to accomplish diverse unreported customary functions resembling other cyclooxygenase-catalyzed prostaglandins. In a recent study, elevated levels of plasma and urinary 8-iso-$PGF_{2\alpha}$ were found in normal pregnant women compared with nonpregnant women with a regular menstruation cycle using no contraceptives, NSAIDs, or vitamins (115). In another study, it was established that postmenopausal women have higher urinary 8-iso-$PGF_{2\alpha}$ levels compared with premenopausal women (104). In addition, the urinary levels of 8-iso-$PGF_{2\alpha}$ were higher in young men than in premenopausal women (112). Therefore, in studies with young men, postmenopausal women, and pregnant women, it was evident that isoprostane levels are regulated in normal physiologic conditions. Healthy women with uncomplicated singleton pregnancies of 24–28 weeks had increased F_2-isoprostanes and MDA levels compared with nonpregnant women (242). This indicates that isoprostanes and thereby increased free radical reactions in mild form are necessary in the body during normal human pregnancy. Further proof of such increase in longitudinal studies with human pregnancy is yet to be reported.

XII. ISOPROSTANES IN CHRONIC DISEASES

Since the early confirmation that F_2-isoprostanes are reliable biomarkers of oxidative stress *in vivo*, the involvement of F_2-isoprostanes and thereby oxidative stress has been described in a large number of human diseases. Table 2 shows a comprehensive list of diseases in which isoprostanes have been shown to be involved, and the number of the diseases in which isoprostanes are found is growing progressively. Nevertheless, several of the findings of involvement of isoprostanes in certain pathologic states are new and unparalleled observations.

A. Atherosclerosis

Atherosclerosis is an intricate, multifactorial vascular disease associated with narrowing of the carotid, coronary, and femoral arteries, among others, by the formation of stable and unstable plaques, depending on the grade of lipid accumulation and inflammation. It is also classified as a chronic inflammatory disease associated with several common critical health-risk factors such as diabetes, hypertension, obesity, dyslipidemias, and smoking. These different pathologic conditions eventually lead to ischemic heart disease with clinical syndromes evidenced by considerably reduced blood flow to the myocardium. Although the primary mechanism(s) causing carotid atherosclerotic plaque to develop into symptomatic disease are still uncertain, data suggest that mediators of inflammation and oxidative stress are not only the leading cause of formation of plaque but also may be involved in rapid progression of atheromatous lesions, plaque rupture, and intraluminal thrombosis (74). Several of risk factors for atherosclerosis, such as diabetes, obesity, smoking, and thickening of the intima–media of the carotid artery, are associated with increased low-grade inflammation, as evidenced by a moderate but significant increase of isoprostanes together with cytokines (IL-6) and acute-phase proteins (hsCRPs) in the body fluids (26, 37, 103, 105, 245, 282). Carotid intima–media thickening is an early signal of development of atherosclerosis. However, no correlation between urinary 8-iso-$PGF_{2\alpha}$ and intima–media thickening has been observed in a recently studied Swedish cohort in elderly men of age 77 (282).

1. Smoking. Cigarette smoking, which involves accumulation of polycyclic aromatic hydrocarbons in respiratory tissues, may lead to miscellaneous pathologies including accelerating atherosclerosis and respiratory disease and future development of cardiovascular diseases (198). Early confirmation of the involvement of isoprostanes has been shown among the smokers (178). This has further been corroborated in several other studies (3, 103, 165). Increased levels of F_2-isoprostanes are found among former smokers, but the levels are less than those among current smokers (103).

2. Diabetes. Compared with a healthy population, persons with type 1 diabetes are coupled to an increased risk of microvascular complications and premature atherosclerosis. Atherosclerosis is considered to be partially a consequence of chronic low-grade inflammation and oxidative stress. A major

TABLE 2 F_2-ISOPROSTANES IN HUMAN HEALTH AND COMMON DISEASES THAT RELATE TO OXIDATIVE STRESS

Disease status	*Selected references*	*Observations*
Cardiovascular diseases		
Atherosclerosis	214	Elevated
	91	Elevated
	148	Elevated
Cardiopulmonary bypass	268	Elevated
	75	Elevated
Coronary reperfusion/ angioplasty/PCI	223	Elevated
	40	Elevated
	116	Elevated
Angiography	40	Elevated
Coronary artery disease	243	Elevated
	276	Elevated
Heart failure	67	Elevated
	140	Elevated
Hypertension with RVD	152	Elevated
Cardiovascular risk factors		
Smoking	178	Elevated
	103	Elevated
Passive smoking	3	Elevated
Former smokers	103	Elevated
Obesity	122	Higher
	245	Higher
	270	Higher
Hypercholesterolemia	69	Elevated
	233	Elevated
	220	No difference
	224	Elevated
Type 2a hypercholesterolemia	65	No difference
Type 1 diabetes	71 70	Elevated
	196	No difference
	278	No difference
	108	No difference
	90	No difference
Type 2 diabetes	92, 94	Elevated
	71	Elevated
	185	Elevated
	105, Elderly men, >7-yr disease duration	Elevated
Pulmonary diseases		
		Higher
Asthma	166	Elevated
	285	Elevated
	8	Elevated
	79	Elevated
	168	Elevated
	154	Elevated
ARDS	51	Elevated
Cystic fibrosis	57	Elevated
	55	Elevated
	167	Elevated
Pulmonary hypertension	61	Elevated
Chronic obstructive pulmonary disease	208	Elevated
	165	Elevated
Interstitial lung disease	164	Elevated
Hepatic diseases		
Hepatorenal syndrome	182	Elevated
Liver transplantation	48	Elevated
Alcoholic liver disease	146	Elevated
	213	Elevated
Biliary cirrhosis	1	Elevated
Renal diseases		
Dialysis patients	128	Elevated
	114	Elevated
Renal injury	110	Elevated
Neurodegenerative diseases		
Alzheimer's disease	156, 158	Elevated
	210	Elevated
	81	No difference
	159, 160	No difference
	52	Difference
Huntington's disease	160	No difference
	157	Elevated
Parkinson's disease	83	No difference
Dementia with Lewy body disease	83	No difference
Migraine	104	No difference
Spinal cord injury	155	Elevated
Multiple sclerosis	143	Elevated
Reproductive diseases		
Preeclampsia	173	No difference
	115	No difference
	221	No difference
	272	Elevated
	9, 10	Elevated
Pregnancy with Down's syndrome	203	Elevated
Inflammatory diseases		
Rheumatoid arthritis	39	Elevated
Psoriatic arthritis	39	Elevated
Reactive arthritis	39	Elevated
Osteoarthritis	39	Elevated
Systemic sclerosis	64	Elevated
	253	Elevated
Periodontal disease	283	Elevated
Other diseases		
Prostate cancer	50	No difference
Hemodialysis	97	Elevated
	114	Elevated
Chronic fatigue syndrome	123	Elevated
Kidney transplantation	66	No difference
	29	Elevated
Crohn's disease	59	Elevated
Osteoporosis	30	Higher
Down's syndrome	212	Elevated
Healthy physiologic conditions		
Human pregnancy	173	Elevated
	115	Elevated

TABLE 3. EFFECTS OF ANTIOXIDANTS ON F_2-ISOPROSTANE FORMATION

Antioxidants (dose supplemented)	*Subjects/conditions*	*Observation*	*Outcome*	*Reference*	*Comments*
d-α-Tocopherol acetate (200 mg/day)	20/healthy	2 wk	→	246	placebo-controlled
α-Tocopherol (500 mg/day)	55/type 2 diabetes	6 wk	↓ plasma → urine	286	
Mixed tocopherols	55/type 2 diabetes	6 wk	↓ plasma → urine	286	
α-Tocopherol (800 IU/day)	38/athletes	2 mo	↑	145	
α-Tocopherol (16–400 mg/day)	38/healthy	21 day	→	27	
d-α-Tocopherol (200–2,000 IU/day)	25/healthy	8 wk	→	147	placebo-controlled
d-α-Tocopherol (800 IU/d or higher)	35/high cholesterol	16 wk	↓	233	placebo-controlled
α-Tocopherol (500–1,000 mg/day)	33/systemic sclerosis	3 wk	→	63	
α-Tocopherol (400 IU/day)	11/end-stage renal disease	1 and 2 mo	↑	247	
dl-α-Tocopherol acetate (600 mg/day)	10/type 2 diabetes	2 wk	↓	71	controls missing
dl-α-Tocopherol acetate (100 or 600 mg/day)	22/high cholesterol	2 wk	↓	69	controls missing
dl-α-Tocopherol acetate (600 mg/day)	7/homocysteinemia	2 wk	↓	72	controls missing
d-α-Tocopherol (182 mg/day)	23/high cholesterol	12/36 mo	↓	121, 238	ASAP study, only men
Vitamin C (1,000 mg/day), vitamin E (300 mg/day)	22/ultramarathon runners	3 wk	↓	142	
dl-α-Tocopherol acetate (300/600/1,200 mg/day)	11–12/smokers	3 wk	→	201	placebo-controlled
Vitamin E* (100 or 800 U/day)	7/smokers	5 day	→	222	controls missing
Vitamin E (800–1,200 ID/day)	80/overweight-obese	6 mo	↓	260	
Tocotrienols (200 mg/day)	17/high cholesterol	4 wk	→	186	mixed _-and α-tocotrienol
Vitamin C (30–2,500 mg/day)	7/healthy		→	130	young women only
Vitamin C (500 mg/day)	25/high cholesterol	12/36 mo	→	121	ASAP study
Vitamin E (130 mg/day), C (1,500 mg/day), β-carotene (9 mg/day), zinc (45 mg/day), Se (76 μg/day), garlic (150 mg/day)	54/allergic adults	4 wk	→	78	
Vitamin C (1,500 mg/day)	18/type 2 diabetes	3 wk	→	68	placebo-controlled
Vitamin C	67/smokers	2 mo	↓	76	
Vitamin C (2,000 mg/day)	5/heavy smokers	5 day	↓	222	controls missing
Vitamin C (500 mg/day)	42/overweight smokers	60 day	↓	77	placebo-controlled
α-TE (43 mg) + carotenoids (0.45 mg)	33/healthy	11 wk	→	269	supplemented in spreads
α-TE (111 mg) + carotenoids (1.24 mg)	33/healthy	11 wk	↓	269	supplemented in spreads
dl-α-Tocopherol (31 mg/day) + vitamin C (272 mg/day)	17/healthy 18/smokers	90 day	→	117	placebo-controlled
d-α-Tocopherol (182 mg/day) + vitamin C (500 mg/day)	28/high cholesterol	12/36 mo	→	121, 238	ASAP study
Vitamin E* (500 mg/day) + vitamin C (200 mg)	69/mild dementia	12 wk	→	56	placebo-controlled
Vitamin E (400 IU/day), vitamin C (500 mg/day)	15/children with FH* or FCD*	6 wk	→	80	
Vitamin E* (800 U/day) + vitamin C (200 mg/day)	4/heavy smokers	5 day	↓	222	controls missing
β-Carotene (50 mg/day)	55/smokers	4 yr	→	144	
Coenzyme Q_{10} (200 mg/day)	19/type 2 diabetes	12 wk	→	107	placebo-controlled

→, F_2-isoprostane formation unaltered compared with controls (or baseline); ↓, F_2-isoprostane formation reduced compared with controls (or baseline).
*Type and preparation of vitamin E not specified.
α-TE, α-tocopherol equivalents; FH, familial hypercholesemia; FCH, phenotype of the familial combined hypercholesemia.

controversy still exists about whether isoprostane levels are higher in type 1 diabetes patients. Concerning type 1 diabetes, one report described elevated levels of 8-iso-$PGF_{2\alpha}$ in urine from these patients (71). Nonetheless, a number of other reports showed no such discrepancies in isoprostane levels in patients with type 1 diabetes compared with controls (Table 2) (70, 90, 108, 196, 278). Metabolically well-controlled young Swedish type 1 diabetes patients had no increase in 8-iso-$PGF_{2\alpha}$ concentrations compared with those of matched controls (278). Likewise, no increase in the urinary 2,3-dinor-5,6-dihydro metabolite of 8-iso-$PGF_{2\alpha}$ was established in type 1 diabetes (196). Increased levels of 8-iso-$PGF_{2\alpha}$ were found in the early phase of diabetes onset and stabilized after 1 year in conjunction with other metabolic status (70). Therefore, it could be assumed that differences in the metabolic status (such as Hb_{A1C} and fasting glucose), hyperlipidemia, or degree of vascular damage in these populations consecutively affected the degree of lipid peroxidation and oxidative stress.

Type 2 diabetes consists of progressive hyperglycemia, insulin resistance, and pancreatic β-cell failure that relate to the development of atherosclerosis. Elevated levels of 8-iso-$PGF_{2\alpha}$ have been found in plasma or urinary samples from type 2 diabetes patients, compared with the nondiabetic controls (71, 92, 94, 185). In a large cross-sectional study in elderly men (77 years), it was shown that the 24-h urinary level of 8-iso-$PGF_{2\alpha}$ was significantly higher in men with type 2 diabetes ($n = 101$) than in the control men ($n = 585$) of the same age (105). A subgroup of these patients with disease duration of >7 years since disease diagnosis did not show any difference between the type 2 diabetes patients and control subjects. Consequently, type 2 diabetes seems to be associated with a higher isoprostane level only in patients with disease duration >7 years, at least in this population. However, this study only includes elderly patients of age 77. Induced hyperglycemia in patients with type 2 diabetes undergoing a glucose tolerance test has also been reported to cause an acute increase in isoprostane levels (239). A positive relation has also been observed between glucose and F_2-isoprostanes in some studies (72, 94), but not in the others (92, 105). Therefore, current research indicates that, like type 1 diabetes, type 2 diabetes seems to be related to various degree of oxidative stress, depending on the glycemic status of the subjects.

3. Hypertension. Hypertension is a serious clinical risk factor for the development atherosclerosis. F_2-isoprostanes levels in mild-to-moderate essential hypertension were similar to those in normotensive patients in some studies (58, 138, 152). In another study, it was shown that hypertension is not directly associated with 24-h urinary F_2-isoprostanes (275). However, plasma and urinary F_2-isoprostanes were significantly lower in treated compared with untreated hypertensive men, but not women. Urinary F_2-isoprostanes iPF(2α)-III in pulmonary hypertension patients were 2.3 times higher than those in the controls (60). Thus, it is assumed that oxidative stress has a differential effect on the severity of hypertension.

4. Obesity. Obesity is strongly related to the metabolic syndrome, which is associated with low-grade inflammation and oxidative stress (Table 2). Inflammatory factors originating from obesity-induced visceral fat may initiate oxidative stress that has been associated with insulin resistance and cardiovascular risk factors. Obese men had significantly higher plasma concentrations of 8-epi-$PGF_{2\alpha}$ than did nonobese men, and plasma levels of 8-epi-$PGF_{2\alpha}$ were significantly correlated with body mass index (BMI) (270). In a recent cross-sectional study from the Framingham Heart Study, abdominal visceral and subcutaneous adipose tissue volumes were related to F_2-isoprostanes (206). Women with BMI of >28 were shown to have higher formation of 8-iso-$PGF_{2\alpha}$ despite the distribution of fat (73). Patients with cardiovascular risk factors except obesity are linked with enhanced oxidative stress. Further, loss of weight has been associated with lower 8-iso-$PGF_{2\alpha}$ levels (73). However, conflicting reports have been presented in epidemiologic studies. A positive correlation between BMI and 8-iso-$PGF_{2\alpha}$ levels is claimed in some studies (44, 77, 122) but not corroborated in a recent study of elderly men (105).

In a current report with adolescents in Minnesota, it was shown that urinary 8-iso-$PGF_{2\alpha}$ levels are significantly related to BMI, and a significant interaction was found between BMI and insulin resistance. F_2-isoprostanes levels were higher in heavily insulin-resistant adolescents (those with high BMI and low M_{LBM}) in which M_{LBM} (*i.e.*, glucose use/kg lean body mass, LBM) adolescents compared with groups with (a) thin, insulin sensitive (those with low BMI and high M_{LBM}); (b) thin, insulin resistant (those with low BMI and low M_{LBM}); and (c) heavy, insulin-resistant adolescents (high BMI and high M_{LBM}) (245). By recruiting adolescents in a cohort of both thin and heavy subjects with a broad range of insulin resistance, it not only identified the risk related to fatness but also identified an interaction between fatness, insulin resistance, and oxidative stress. Collectively, these studies indicate that obesity is closely related to oxidative stress and subsequent higher isoprostane formation.

B. Neurologic diseases

The major controversies in this field still remain whether isoprostanes are implicated in Alzheimer's disease (Table 2). In Alzheimer's disease, increased production of F_2-isoprostanes has been found specifically in the cerebrospinal fluid, neurons, and brain tissues (52, 156, 158, 161, 217), and in one study, in the urine and plasma (210). A recent novel study suggested that increased 8-iso-$PGF_{2\alpha}$ is located in hippocampal pyramidal neurons in postmortem tissues from aged-matched Alzheimer's disease tissues compared with control tissues (Fig. 12). However, no such change was found in the plasma or urine by the others (82, 159). The rationale for this inconsistency is perhaps that the biologic samples are not taken at an identical stage of the disease, or different biologic samples were assayed, or different drugs that the patients normally take might have affected the study outcome. A majority of the studies with Alzheimer's disease revealed that increased formation of isoprostanes localized mainly in the neurons, tissues, or in the CSF, rather than in the plasma or urine. Nevertheless, none of the studies has shown any prospective data on the increased levels of isoprostanes in Alzheimer's disease. Isoprostanes have been shown to be implicated in several other neurologic diseases, such as multiple sclerosis (95), Huntington's disease (157), Creutzfeld–Jacob's disease (157), and aneurysmal subarachnoid hemorrhage (133).

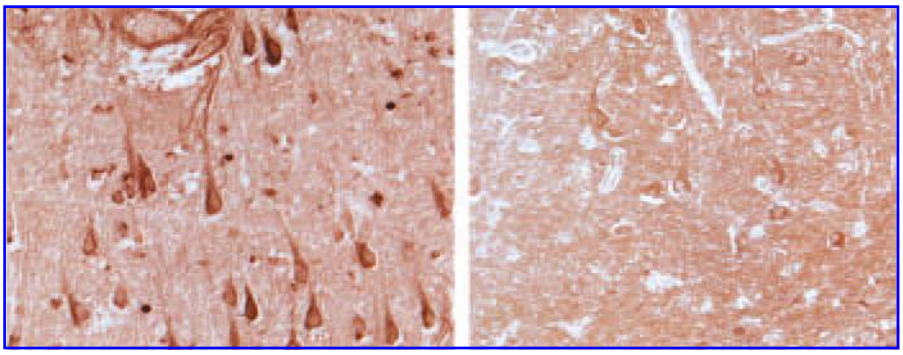

FIG. 12. Immunohistochemical localization of isoprostanes in Alzheimer's disease (AD) patients and control brain. Neurons in hippocampus of AD patients stain intensively in a fixed section treated with anti-8-iso-$PGF_{2\alpha}$ antibody (*left*). Similar staining in neurons in the hippocampus in age-matched control (*right*). (Adapted from ref. 51, with permission.)

Neuroprostanes are formed from docosahexaenoic acid (C22:6; n-3, DHA) and are considered to be specific for neuronal oxidative stress. Recent studies have shown that neuroprostanes are superior biomarkers of oxidative stress to F_2-isoprostanes when considering neurologic diseases (5, 162). DHA oxidizes both *in vitro* and *in vivo* to form F(2)-isoprostane-like compounds termed as F(4)-neuroprostanes. DHA is specifically enriched in neuronal membranes, making the F(4)-neuroprostanes more-sensitive and specific markers of neuronal oxidative damage, and thus it could specifically be used as biomarker of oxidative stress in neurologic diseases and their relation to Alzheimer's and Parkinson's diseases.

C. Lung diseases

F_2-isoprostanes have been shown to be increased in various body fluids or exhaled breath condensate in several pulmonary diseases, such as asthma (8, 79, 154, 166, 168, 184, 202, 209, 285), interstitial lung disease (164), cystic fibrosis (55, 57, 165), pulmonary hypertension (61), chronic obstructive pulmonary disease (COPD) (43, 165), and ARDS (51). These clearly show that isoprostanes, and thereby oxidative stress, are intimately involved in most of the pulmonary diseases (Table 2).

D. Reproductive diseases

Preeclampsia is a multifactorial disease that is primarily symbolized by pregnancy-induced hypertension and onset of proteinuria, usually taking place in middle to late pregnancy. About 2–3% of all pregnant women in the world develop preeclampsia, which is the foremost cause of maternal and fetal morbidity and mortality. Abnormal placentation and endothelial dysfunction are considered to trigger its clinical manifestations of the pathophysiologic changes, perhaps leading to decreased placental perfusion and in a later state, to maternal syndrome. Increasing evidence suggests that preeclampsia is associated with both increased oxidative stress and reduced antioxidant defenses, which has led to the theory that oxidative stress may play an important task in the pathogenesis of preeclampsia, and thus hypothetically could be counteracted by supplementation with various antioxidants. Nonetheless, whether oxidative stress is the underlying pathology in the disease outburst remains unclear. The preeclamptic patients were shown not to have a clear indication of increased oxidative stress (9, 115), and supplementation of either vitamin E or vitamin C did not resolve the pathogenesis of preeclampsia in large population studies (205, 235). This could be due to inadequate doses or duration of different antioxidant therapy or both, as shown for vitamin E supplementation in participants with polygenic hypercholesterolemia (231). In addition, it could also be because no clear indication exists of involvement of oxidative stress in preeclampsia (see later).

Conflicting results have been obtained regarding F_2-isoprostanes in preeclamptic patients (Table 2). Several studies have shown increased levels of plasma, urinary, or placental isoprostanes (9, 10, 98, 272), but in other recent studies, no such differences have been found (173, 221). In a recent study, it was shown that the levels of plasma and urinary 8-iso-$PGF_{2\alpha}$ in severe preeclampic patients did not differ from those in pregnant controls at the same pregnancy stage (115). However, the γ-tocopherol levels were significantly lower in the preeclamptic patients than in normal pregnant and nonpregnant controls. The difference in the levels of isoprostanes in various studies might possibly be due to differences in the sampling regimen, or isoprostanes are measured in various body fluids collected from unlike stages and body compartments. These perhaps added complexity in determining the role of oxidative stress in the pathogenesis of preeclampsia.

E. Inflammatory diseases

Rheumatoid arthritis is a systemic autoimmune chronic inflammatory disease characterized by inflammation in synovial joints. Earlier it was shown that cyclooxygenase-catalyzed products of arachidonic acid, specifically, PGE_2 and PGI_2 levels, were increased in the synovial fluid collected from knee joints of arthritic patients (39, 46, 47). In a recent study, local (knee joints) and systemic measurements of inflammatory response index in conjunction with oxidative stress were determined in the synovial fluid and plasma, respectively. High levels of systemic 8-iso-$PGF_{2\alpha}$ were detected among the patients with rheumatoid arthritis, psoriatic arthritis, reactive arthritis, and osteoarthritis (39). In addition, high levels of 8-iso-$PGF_{2\alpha}$ were found in the synovial fluid collected from these patient groups. Urinary concentrations of a tetranor-dicarboxylic acid metabolite of F_2-isoprostanes concentrations were significantly higher in patients with scleroderma than in healthy controls (254). Urinary levels of 8-iso-$PGF_{2\alpha}$ in systemic sclerosis patients were higher compared with those in sex-matched healthy controls (284). Similarly, urinary levels of iPF2α-III were approximately twice as high in patients as in control subjects (64).

F. Other diseases and conditions

Isoprostanes are involved in a number of other diseases (Table 2), and the number is constantly increasing with the diseases that earlier were not reported.

A number of studies with various end points, such as exercise or ultramarathon runs, have been shown to increase lipid peroxidation and isoprostanes systemically (141, 142, 236, 252). 8-Iso-$PGF_{2\alpha}$ levels were higher in healthy subjects after 3 h of knee-extensor exercise (86, 87). Muscle damage resulting from eccentric exercise leads to higher F_2-isoprostanes concentrations at 72-h after exercise (237). It seems that extensive exercise may lead to free radical formation through muscle damage and after isoprostane formation.

XIII. REGULATION OF ISOPROSTANE FORMATION BY ANTIOXIDANTS AND FATTY ACIDS

A. Human studies

Even though many diseases have been proposed to be related to oxidative stress, a central issue remains to be elucidated on what role oxidative stress actually plays in a particular human disease pathology without therapeutically following up a certain drug, antioxidant, or radical scavenger of their antioxidative properties, and prevent the disease out-burst. Although very complex, this can be indirectly answered if we can regulate several reliable *in vivo* oxidative stress indices such as isoprostanes production, and further by following disease initiation and progression with effective antioxidative therapy.

Oxidative modification of low-density lipoprotein and subsequent formation of foam cells are considered to have an impact on the pathogenesis of atherosclerosis. *In vitro* and various experimental *in vivo* studies have suggested that oxidative stress is involved in all segments of coronary artery disorders (139, 255–257). This has prompted the study of antioxidant vitamins, such as α-tocopherol or β-carotene or both, in the prevention of the initiation and progression of cardiovascular diseases. However, a recent meta-analysis from different large randomized clinical trials (total patients, 81,788) failed to demonstrate the beneficial effects of vitamin E for the prevention of cardiovascular diseases and does not support the practice of routine use of vitamin E in future primary and secondary prevention trials in patients at high risk of coronary artery disease (281). This lack of efficacy of vitamin E may possibly be because the majority of these intervention studies in humans had no reliable determination of systemic oxidant stress, nor were the patients recruited with higher basal levels of oxidant stress. In other words, do these populations need antioxidant supplementation without knowing their oxidative stress status to judge the efficacy of α-tocopherol, or is it the right population or right antioxidant to test the antioxidant hypothesis? The proper therapeutic doses or the duration of antioxidant supplementation necessary to counteract the oxidative stress is not known, nor is it clear at which stage of disease pathogenesis antioxidant therapy is mandatory and should be given to the patients to achieve maximal protection from oxidative damage of tissues and the subsequent disease consequences. It is equally uncertain which chemical or prodrug form of antioxidant should be supplemented, so that the accurate bioavailability of the active molecule at the site of radical formation is present for combating an *in vivo* oxidative stress. The most momentous query is whether this disease caused by oxidative stress is merely a complication and consequence of other action? Many of these and other related questions must be scientifically evaluated and should be taken into consideration by choosing a reliable *in vivo* biomarker of oxidative stress, such as F_2-isoprostanes or a group of trustworthy biomarkers of oxidative stress.

Table 3 shows the effect of different antioxidants on F2-isoprostane formation. Scientists have been eager to appraise one of most widespread vitamins in this regard, vitamin E. A recent human study showed that α-tocopherol given in increasing doses (200–2,000 IU/day for 8 weeks) did not affect the concentrations of F_2-isoprostanes (147). Neither did vitamin E (200 IU/day for 2 weeks) have any effect on the basal F_2-isoprostane level in another study in healthy human subjects (246). In a mechanistic study in which the formation of different isomers of conjugated linoleic acid (CLA)-induced F_2-isoprostanes was followed up in healthy subjects, no decrease in the urinary 8-iso-$PGF_{2\alpha}$ level was seen during 4 weeks of supplementation with vitamin E (200 IU/day) (246). Neither did 28 days of supplementation with various doses of α-tocopherol (16, 100, 200, and 400 IU/day) have any effect on the basal levels of F_2-isoprostanes in healthy human subjects (27). Vitamin E supplementation did not affect the levels of F_2-isoprostanes in moderate cigarette smokers (201). In a study in which cigarette smokers consumed a diet high in polyunsaturated fat, a prooxidant effect of supplementary vitamin E was observed (277). However, when hypercholesterolemic patients were treated with doses (800–3,200 IU/day) of vitamin E for 20 weeks, a significant decrease in the F_2-isoprostane level was noted only after 16–20 weeks of supplementation (Fig. 13) (233), as seen earlier in the animal studies with higher doses (248). Nonetheless, no such decrease was seen in patients given lower doses of vitamin E (100–400 IU/day) for 20 weeks in the same study (Fig. 14) (233). Other studies have shown that vitamin E supplementation reduced the concentration of F_2-isoprostanes in patients with type 2 diabetes (71), cystic fibrosis (55), hypercholesterolemia (69), and homozygous homocystinuria (72). Vitamin E has been

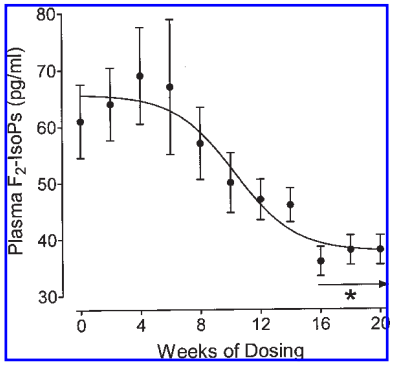

FIG. 13. Time course of suppression of plasma levels of F_2-isoprostanes in polygenic hypercholesterolemia subjects supplemented with 3,200 IU/day of vitamin E. *$p < 0.005$ compared with time 0. (Adapted from ref. 231, with permission.)

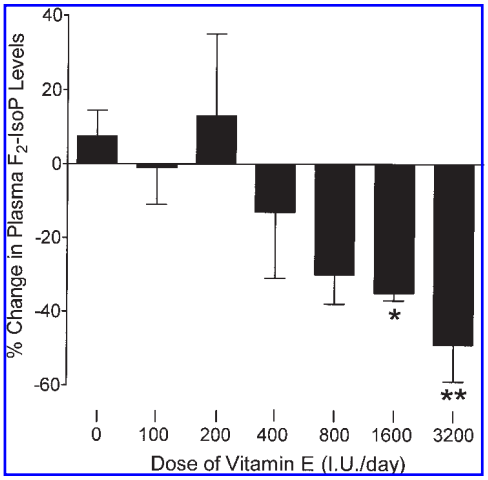

FIG. 14. Relation between the daily doses of vitamin E supplemented for 16 weeks and reduction of plasma levels of F_2-isoprostanes in polygenic hypercholesterolemia subjects. *$p < 0.03$ compared with placebo; *$p < 0.005$ compared with placebo. (Adapted from ref. 231, with permission.)

shown to suppress urinary F_2-isoprostane levels in hepatic cirrhosis and alcoholic liver disease (146). Collectively, these studies illustrate that vitamin E supplementation has a varying antioxidative effect in studies related to different doses, patients, population groups, or stage of pathogenesis of disease, possibly depending on the basal lipid peroxidation process or even the pharmacogenomics in these individuals. Thus, measurement of isoprostanes would be a relevant tool in the study of efficacy of antioxidants of various natures in the coming decades. This might inspire renewed interest in vitamin E or other antioxidant supplementation in human subjects.

B. Animal studies

Several studies report that administration of antioxidants improved diverse disease progression in animal models of human diseases in which isoprostane formation is greater than that in the controls. Vitamin E suppresses F_2-isoprostane generation in urine, plasma, and vascular tissue of ApoE-deficient mice (216). Aortic lesion areas and isoprostane levels in the arterial wall were also decreased without reducing cholesterol levels by vitamin E supplementation. This study shows that oxidative stress is increased in ApoE-deficient mice, a characteristic indication of atherosclerosis development, and this can be reduced by the oral administration of vitamin E. However, no such effect has been observed in human studies. In a recent study in ApoE- and Tpa-deficient mice, dietary α-tocopherol supplementation restored circulating aortic levels of α-tocopherol and reduced atherosclerosis (259). Nevertheless, similar dietary supplements did not reduce disease in ApoE-deficient mice. Dietary supplementation with a synthetic vitamin E analogue (BO-653), either alone or in combination with α-tocopherol, decreased atherosclerosis in ApoE- and in Apo- and Tpa-deficient mice. However, differences in atherosclerosis were not related to F_2-isoprostanes. Obesity and hyperlipidemia caused increased urinary isoprostanes in mice deficient in both leptin and low-density lipoprotein receptor (ob/ob; $LDLR^{-/-}$) (100). After supplementation with vitamin E (2,000 IU/kg) in lean and obese $LDLR^{-/-}$ mice, no reduction of urinary isoprostanes was observed. Thus, any reduction of systemic oxidative stress has been observed by vitamin E supplementation. A report showed that early supplementation with vitamin E has a beneficial effect on diabetes-induced endothelial dysfunction in resistant arteries without any effect on isoprostane formation (279). Together, these studies indicate that vitamin E has differential effects in different animal models with various end points.

XIV. REGULATION OF ISOPROSTANE FORMATION BY FATTY ACIDS

Consumption of certain dietary fats may lead to a risk of initiating cardiovascular diseases in humans, possibly through induction of oxidative stress and subsequently directed at low-grade inflammation. A large number of clinical studies have been performed to elucidate whether a certain polyunsaturated fatty acid (PUFA) is beneficial or detrimental to cardiac health, where isoprostanes as biomarkers of oxidative stress have been evaluated. When a diet consisting of saturated fat was substituted by a diet with high amounts of linoleic acid (18:2, n-6), levels of urinary F_2-isoprostanes increased in healthy subjects (265). Linoleic acid is a precursor of arachidonic acid that possibly is responsible in the increased formation of *in vivo* F_2-isoprostanes. Altering fat quality from saturated fat to a rapeseed oil–based diet rich in α-linolenic acid (18:3, 2-3) did not change the formation and excretion of urinary F_2-isoprostanes (249). In addition, vaccenic acid (18:2, *trans*-11), a *trans*-fatty acid, also elevated urinary F_2-isoprostanes (266) through metabolic conversion to conjugated linoleic acid (CLA). The differences in the formation of urinary F_2-isoprostanes in these studies could possibly be linked to the number and position of the double bonds in the fatty acid side chain. A number of studies with supplementation of CLA in healthy subjects and subjects with metabolic syndrome showed an increase in both plasma and urinary F_2-isoprostanes (33, 36). Levels of urinary F_2-isoprostanes return to normal 2 weeks after cessation of intake of CLA, as depicted in Fig. 15. In examination of which of the common CLA isomers are specific inducer of isoprostane formation *in vivo*, CLA isomer (18:2, *trans*-10, *cis*-12) was shown to be more specific than either *cis*-9, *trans*-11 CLA or a mixture of two isomers of CLA: *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA (226, 227, 246).

Supplementation of fish PUFAs [eicosapentanoic acid (EPA) or docosahexanoic acid (DHA)] to healthy subjects, type 2 diabetic patients, and overweight hyperlipemic or postmenopausal women reduced the levels of isoprostanes compared with those in control subjects or baseline values (106, 170–172, 188). Maternal fish oil supplementation in pregnancy lowers plasma and urinary isoprostanes in neonates at high risk of atopy (11). However, a solid dietary fat containing fish oil redistributed lipoprotein subclasses in LDL and HDL without affecting isoprostane formation (264). Thus, these studies mean that different fatty acids have different effects, depending on the chemical structure of the compound. They can either induce or reduce *in vivo* isoprostane formation in humans.

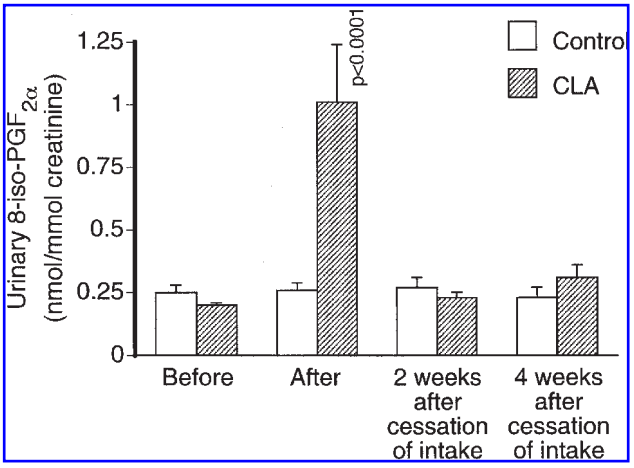

FIG. 15. Morning urinary levels of 8-iso-$PGF_{2\alpha}$ in control subjects and subjects treated with CLA for 1 month, and 2 and 4 weeks after the cessation of treatment. The *p* value indicates the significance of a difference between the changes in the two groups. (Adapted from ref. 32, with permission.)

XV. COALESCE THEORY OF OXIDATIVE STRESS AND ISOPROSTANES

Why are isoprostanes necessary in the establishment of the oxidative-stress theory? Appropriate biomarkers to study free radicals or their end-products formation is an indispensable requirement to conclude the presence of oxidative stress in a certain disease or mechanistic study of disease initiation, and its potential counteraction by diet, antioxidants, and drugs. Before isoprostanes were found to be consistent biomarkers of oxidative stress *in vivo*, a majority of the earlier accessible biomarkers of oxidative stress severely suffered from methodologic limitation of a diverse nature. This made it difficult to judge the authenticity of the involvement of free radicals in certain diseases or therapeutic challenges against free radical formation. Isoprostanes are bioactive compounds that are involved in both the physiologic and pathophysiologic status of the mammalian body, and, in addition, measuring these compounds also reflects the characteristic signs of endogenous reactions of free radicals that evidence biologic consequences of oxidative stress. These emerging roles of isoprostanes in oxidative stress and inflammation open a new frontier of oxidative-stress research both preclinically and clinically in the coming decades. These might introduce novel concepts in future understanding of oxidative stress *in vivo* and potential therapies against a vast number of oxidative stress–related and inflammation-related diseases.

XVI. CONCLUSIONS

Oxidative stress is believed to be the major underlying pathogenic origin of several acute or chronic inflammatory diseases and also is thought to be associated with the normal aging process. Despite the earlier availability of several biomarkers of oxidative stress, it is now evident that the majority of the assay methods were unreliable when assessing oxidative stress *in vivo*, which directed us to erroneous interpretation of the role of oxidative stress in various diseases. Current evidence proposes that isoprostanes are biologically potent free radical–catalyzed compounds with vasconstrictive and certain inflammatory properties and are consistent *in vivo* biomarker of oxidative stress. It also suggests that isoprostanes, being bioactive compounds, are involved in normal physiology, such as in human pregnancy, and also are involved in acute and chronic inflammation pathology. The importance of isoprostanes seems to be of great significance in medical science, because these nonenzymatic, rapidly formed compounds from fatty acids in the body not only are potent to ascertain some biologic effects but also could be used as novel *in vivo* biomarkers of oxidative stress. Isoprostanes could further be used to study the efficacy of antioxidants, diet, drugs, and other radical scavengers and in crucial mechanistic studies of oxidative stress. These provide a unique prospect to understand more on oxidative strain–related physiologic performance and oxidative stress–related diseases, and possibly also in future prediction, diagnosis, and treatment of diseases with different therapies.

ABBREVIATIONS

AHR, Airway hyperresponsiveness; ARDS, acute respiratory distress syndrome; BMI, body mass index; $\cdot CCl_3$, trichloromethyl radical; CCl_4, carbon tetrachloride; CLA, conjugated linoleic acid; COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; CSF, cerebrospinal fluid; DHA, docosahexanoic acid; EIA, enzyme immunoassay; EPA, eicosapentanoic acid; FP, FP receptor ($PGF_{2\alpha}$ receptor); GC-MS, gas chromatography–mass spectrometry; GC-MS-MS, gas chromatography–mass spectrometry–mass spectrometry; Hb_{A1C}, hemoglobin A1C; hsCRP, high specific C-reactive protein; $iPF_{2\alpha}$, IV, F_2-isoprostane 4 series; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin-$F_{2\alpha}$; IU, international unit; LC-MS-MS, liquid chromatography–mass spectrometry–mass spectrometry; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; MDA, malondialdehyde; M_{LBM}, glucose use per kilogram lean body mass; NSAIDs, nonsteroidal antiinflammatory drugs; 8-OhdG, 8-hydroxy-2'-deoxyguanosine; $Paco_2$, arterial partial pressure of carbon dioxide; Pao_2, arterial oxygen tension; PAF, platelet-activating factor; PBN, α-phenyl-*N*-*tert*-butyl-nitrone; PCI, percutaneous coronary intervention; PG, prostaglandin; 15-PGDH, 15-prostaglandin dehydrogenase; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PUFA, polyunsaturated fatty acid; RIA, radioimmunoassay; S-PBN, sodium-2-sulfophenyl-*N*-*tert*-butyl-nitrone; TBARS, thiobarbituric acid–reactive substances; TXA_2, thromboxane.

ACKNOWLEDGMENTS

I am indebted to all colleagues and students collaborating in many of the studies cited herein. Professor Denis Cellewaert is acknowledged for kind assistance in preparing Fig. 4.

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Date of first submission to ARS Central, October 17, 2007; date of final revised submission, March 10, 2008; date of acceptance, March 10, 2008.

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