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The enigma of *in vivo* oxidative stress assessment: isoprostanes as an emerging target

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Abstract

Oxidative stress is believed to be one of the major factors behind several acute and chronic diseases, and may also be associated with ageing. Excess formation of free radicals in miscellaneous body environment may originate from endogenous response to cell injury, but also from exposure to a number of exogenous toxins. When the antioxidant defence system is overwhelmed, this leads to cell damage. However, the measurement of free radicals or their endproducts is tricky, since these compounds are reactive and short lived, and have diverse characteristics. Specific evidence for the involvement of free radicals in pathological situations has been difficult to obtain, partly owing to shortcomings in earlier described methods for the measurement of oxidative stress. Isoprostanes, which are prostaglandin-like bioactive compounds synthesized *in vivo* from oxidation of arachidonic acid, independently of cyclooxygenases, are involved in many human diseases, and their measurement therefore offers a way to assess oxidative stress. Elevated levels of F_2-isoprostanes have also been seen in the normal human pregnancy, but their physiological role has not yet been defined. Large amounts of bioactive F_2-isoprostanes are excreted in the urine in normal basal situations, with a wide interindividual variation. Their exact role in the regulation of normal physiological functions, however, needs to be explored further. Current understanding suggests that measurement of F_2-isoprostanes in body fluids provides a reliable analytical tool to study oxidative stress-related diseases and experimental inflammatory conditions, and also in the evaluation of various dietary antioxidants, as well as drugs with radical-scavenging properties. However, assessment of isoprostanes in plasma or urine does not necessarily reflect any specific tissue damage, nor does it provide information on the oxidation of lipids other than arachidonic acid.

Keywords: *antioxidants*; *free radicals*; *human*; *inflammation*; *isoprostanes*; *oxidative strain*; *oxidative stress*

Introduction

One of the major common features of acute and chronic diseases, which cause high morbidity and mortality globally, is the surplus formation of various free radicals (reactive oxygen or nitrogen species). These can originate from reactions to cell injury in general, incorrect activation of phagocytic cells in chronic inflammatory situations, diminished levels of antioxidants, etc. Free radicals are formed endogenously within the cell during diverse metabolic processes *in vivo*, and can also be formed endogenously upon exposure of the body to a number of toxins, pesticides, pharmaceutical agents, environment pollutants, radiation injury, etc. However, the formation of free radicals is also an indispensable part of normal cell metabolism. Once biosynthesized, these molecules may serve as bioregulators of several functions of the body through signal transduction, thus involving diverse biochemical processes. This leads to further exclusive formation of new biomolecules that are necessary to maintain normal homoeostasis, signal transduction and protein function, and to ensure cell survival.

However, the measurement of free radicals or their endproducts is difficult, particularly since the free radicals are very reactive and spontaneously short lived. Therefore, scientists have primarily focused on the detection of endproducts or their by-products, which have quite diverse characteristics. In 1989, William A. Pryor, Co-Editor-in Chief of Free Radical Biology and Medicine, cited: "One of the greatest needs in this field now is the availability of non-invasive tests to probe oxidative stress status in humans". The lack of such tests has been a major obstacle not only in exploring the

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pathogenesis of various oxidative stress-related diseases, but also in evaluating the epidemiological findings regarding vitamins and diseases and in designing a rationale for therapeutic targets against the free radical-mediated diseases. Such data are needed to resolve the constant debate on the role of antioxidants in various diseases.

Isoprostanes are a newly discovered group of prostaglandin-like compounds, which are biosynthesized from esterified arachidonic acid through a non-enzymic free radical-catalysed mechanism and have very short half-lives (16 min in humans and about 4 min in rabbits) (1–3). Several of these compounds retain biological activities, as confirmed mainly by pulmonary and vasoconstrictive, and even inflammatory activities (2, 3). Both human and experimental studies reveal that isoprostanes are augmented in both acute and severe chronic inflammation, ischaemia–reperfusion, diabetes, atherosclerosis, obesity, lung and liver diseases, etc (see Table 1). Furthermore, existing evidences advocate that isoprostanes are authentic indicators of free radical-mediated lipid peroxidation and oxidative stress (2, 4). Having potent biological activities and also involvement in both normal physiology and pathophysiology, the measurement of these compounds has attracted huge attention, evident from an exponential increase in the number of publications in the field since mid-1990s. However, it is to be remembered that assessment of isoprostanes in plasma or urine does not necessarily provide information on any specific tissue damage unless it is performed in certain tissue or fluids collected from the tissue in question, and it does not reflect oxidation of lipids other than arachidonic acid.

Lipid peroxidation in relation to oxidative stress

In a wider designation, lipid peroxidation in the organic systems involves all oxidation of fatty acids that occurs *in vivo*. Lipid peroxidation can occur rapidly in the body through both enzymic and non-enzymic reactions, the latter being primarily focused in most studies. The enzymic pathway is well documented in the oxidation of arachidonic acid through cyclooxygenases and lipoxygenases with the formation of bioactive prostaglandins, leukotrienes or lipoxins, respectively (5–7). This review will primarily cover free radicals mediated by non-enzymic lipid oxidation products. One important reason for the key roles of these free radicals is the fact that free radical-catalysed oxidized lipids can instantly modify the cell membrane fluidity, which in turn can interact with the different membrane-adhering enzymes and membrane-bound receptors. In addition, many of these oxidized lipids may possess bioactivity as vasodilators or vasoconstrictors/bronchoconstrictors, or induce inflammation in neighbouring cells (see below).

It is well acknowledged that polyunsaturated fatty acids (PUFAs) with two or more double bonds are more prone to oxidation than the saturated and monounsaturated fatty acids (8). This is largely due to the instability (weak energy of attachment) of the hydrogen atom adjoining the double bond. This means that the hydrogen atom can be abstracted easily through a reactive radical attack (Fig. 1). Lipid peroxidation *in vivo* requires a PUFA and an oxidant inducer, which form a free radical intermediate. The free radical intermediate reacts with oxygen to form a peroxyl radical (LOO·). The unpaired electrons of the peroxyl radicals further abstract a hydrogen atom from another PUFA to form a lipid hydroperoxide, which may decay to form alkoxyl or peroxyl radicals. These radicals may also attract adjoining diverse membrane proteins. The reaction of the peroxyl radical with other fatty acids generates a carbon-centred radical which, in turn, will be able to react with oxygen to form another peroxyl radical. This radical continues its reaction to the PUFAs, and a propagation reaction initiates a chain reaction that is maintained until a termination

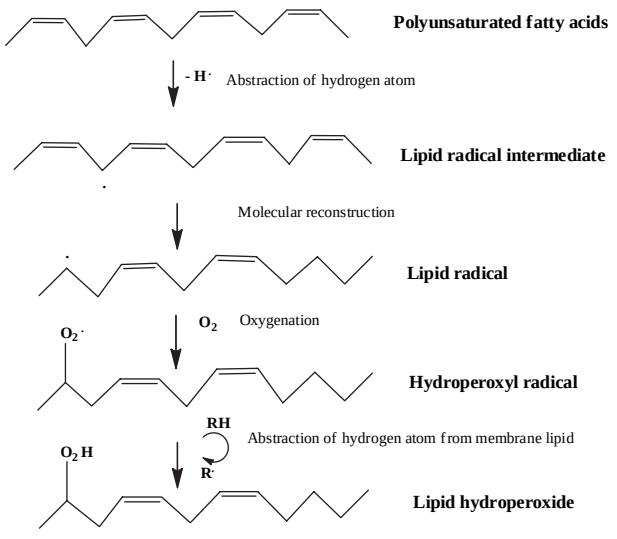

Fig. 1. Basic principle of non-enzymic lipid peroxidation involving polyunsaturated fatty acids.

reaction starts by one or several endogenous chain-breaking antioxidants or by exogenously applied antioxidants, dietary radical scavengers or drugs (9). However, excess endogenous formation or exogenous administration of these biocombating factors may also lead to pro-oxidative effects in certain conditions which, so far, have been less studied.

The oxidative reaction processes are often perceived as being merely destructive in nature. However, they are also crucial elements of normal human body functions, as essential actors in biochemical reactions involved in the control of physiological functions such as cell signalling, vascular tone, cell generation and degeneration, and defence against microorganisms (10–14). The physiologically essential reactive species seem to be necessary to maintain the normal basal oxidant–antioxidant balance and host defences in the mammalian system. However, when a surplus formation of these bioactive oxidation products exceeds the capacity of endogenous cellular antioxidant defence mechanisms, these products may intricate in various cell and organ damage by upsetting the normal physiology in such a fashion that they instigate and/or speed up the disease processes. Furthermore, overstressed or damaged cells in disease conditions will perhaps initiate the production of these active compounds *in vivo* and consequently inflate cellular or damage that potentiates the disease process.

Isoprostanes as biomarker of *in vivo* lipid peroxidation

Indications of non-enzymically auto-oxidized prostaglandin-resembling substances arising from unsaturated fatty acids were first demonstrated *in vitro* in late 1960s (15, 16), and also frequently seen in the laboratory after storage of arachidonic acid in the freezer. However, the biological significance of these prostaglandin isomers *in vivo* was not uncovered until 1990. The discovery of isoprostanes was the starting point of a new era in the detection and quantification of free radical-catalysed lipid peroxidation products (1). Isoprostanes are stable lipid peroxidation products after experimental oxidative injury (4, 17). Besides having selective bioactivity, esterified isoprostanes may also exert biological effects within the cells where they are formed through reaction with the adhering tissues (3).

The formation F_2-isoprostanes primarily requires arachidonic acid, molecular oxygen and free radicals. Unlike cyclooxygenase (COX)-derived primary prostaglandins, isoprostanes are formed *in situ* esterified with tissue phospholipids and subsequently released in free acid form following hydrolysis, presumably by phospholipases (18, 19). The enzymic cleavage step is also, at least theoretically, an important rate-limiting step for the formation and release of free isoprostanes in the circulation, which can be synchronized by variety of endogenous or exogenous factors (20). A simplified scheme of the formation of F_2-isoprostanes from arachidonic acid is shown in Fig. 2.

8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$; Fig. 2), a major F_2-isoprostane, and its related isomers are augmented significantly in a number of syndromes that are thought to be associated with oxidant injury, and the measurement of F_2-isoprostanes in a range of tissues and body fluids is generally regarded as a trustworthy biomarker for *in vivo* determination of lipid peroxidation by free radical pathways (1, 4, 9, 17, 20). 8-Iso-$PGF_{2\alpha}$ has about 10 times higher basal levels than enzymically produced $PGF_{2\alpha}$ and the free form of this compound is easily detectable in numerous body fluids by sensitive and specific analytical approaches. Measurement of the esterified and free isoprostanes is suitable in the tissues, and thus could be used as an approach to oxidative stress measurement for target tissue injury of interest (21, 22). Specific antibodies to the isoprostanes can also be applied

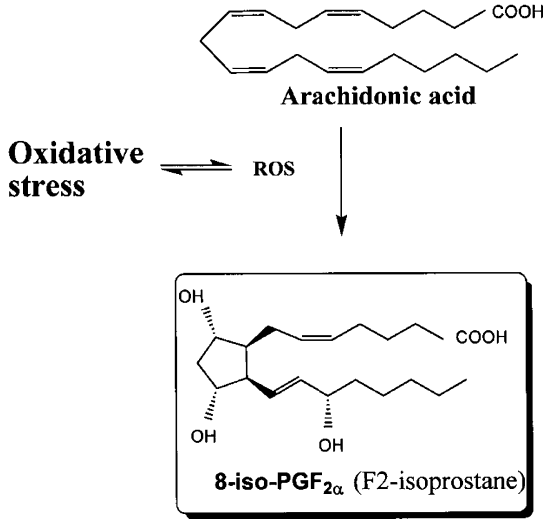

Fig. 2. Simplified scheme of 8-iso-$PGF_{2\alpha}$ (F_2-isoprostanes) formation from arachidonic acid by free radical catalysis. ROS: reactive oxygen species; PGF: prostaglandin F.

for *in situ* localization of the compound by immunostaining of the oxidative stress-injured tissues. A novel study of such identification of oxidative stress-induced cytoplasm of neurons in Alzheimer's pathology has recently been described (23). This localization of bioactive isoprostanes by immunostaining with specific antibody is promising for further detection of free radical-mediated tissue damage in different diseases, and also offers possibilities for the detection of therapeutic effects of various radical scavengers in disease-related injuries.

Measuring malondialdehyde (MDA), the previously most commonly used measure of lipid peroxidation, is much less sensitive measuring than the increase in esterified isoprostanes in the liver (80- versus 2.7-fold) (24). Other comparative *in vivo* studies did not show any correlation between the increase in isoprostanes and MDA levels (25, 26). Very few studies have shown a correlation between protein oxidation and that of isoprostanes, mainly owing to the different character of these oxidative processes (radicals involved, timing, duration, cellular components, etc.). Even though measuring isoprostanes merely reflects the oxidation of arachidonic acid rather than of the total lipid pool, it is thought that other lipids might have also been oxidized 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 (27). Nonetheless, it is vital to scrutinize when, what and which biological samples are preferential for measurement to avoid any risk of overlooking short- or long-lasting secretion of isoprostanes. Furthermore, incorrect collection, preservation and hazardous preparation (during extraction, purification and hydrolysis) of the samples before analysis are plausible factors in the artefactual formation of these compounds or impurity-affected analytical errors.

The methodology for isoprostane analysis

As already mentioned, measurements of MDA, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides, conjugated dienes, etc., have various methodological limitations (2, 28). Although gas chromatography and mass spectrometry (GC-MS) were originally required for the detection and quantification of isoprostanes, other assay methods such as liquid chromatography (LC)–mass spectrometry, GC-MS-MS, LC-MS-MS, radioimmunoassays and enzyme immunoassays (1, 29–31) are now also available. GC-MS-MS and LC-MS-MS methods require expensive and highly sensitive instruments to distinguish low levels of the compound *in vivo*, and well-trained technical staff, and sample preparation and characterization are complex. The more easily handled immunoassays are less specific and quantitative, unless antibodies are extensively confirmed for the cross-reactivities and other accuracy tests have been carried out. Nevertheless, immunoassays have been indispensable means for original innovation in the medical science since the early 1970s, which is also true for isoprostane analysis. A recent multilaboratory network study was organized by National Institute of Environmental Health Sciences (NIEHS), NIH, USA (BOSS-II and BOSS-III). Various oxidative stress biomarkers measured by different methods were used. The study showed that measurement of F_2-isoprostanes in body fluids by different methods is indeed a consistent biomarker of *in vivo* oxidative stress (32, 33).

Oxidative stress *in vivo*

Carbon tetrachloride (CCl_4)-induced oxidative stress is a conventional model in animals and has been used for decades (9). CCl_4 introduced orally to experimental animals swiftly transforms to the trichloromethyl radical ($\cdot CCl_3$) or other radicals in the liver that subsequently induce *in vivo* oxidative stress. Measuring F_2-isoprostanes using this model, also following administration of antioxidants such as vitamin E, has been a most important tool in the evaluation of this biomarker of oxidative stress, as evidenced by several such studies (21, 22, 25). A massive amount of esterified 8-iso-$PGF_{2\alpha}$ was found in the liver tissues 2 h after the oral administration of 2.5 ml kg^{-1} CCl_4 to rats, whereas the free 8-iso-$PGF_{2\alpha}$ levels were quite low (19). In an additional study, it was shown that free 8-iso-$PGF_{2\alpha}$ levels increased 17-fold in plasma and 53-fold in urine from the basal levels 4 h after CCl_4 (2 ml kg^{-1}) administration to rats (25). At 6 h, free 8-iso-$PGF_{2\alpha}$ levels in the plasma and urine had increased seven-fold in plasma and 87-fold in urine (Fig. 3). The levels of F_2-isoprostanes were still significantly elevated after 24 and 48 h compared with the baseline after the administration of CCl_4 (22). The inhibitory effect of vitamin E or other antioxidants supported the theory that oxidative stress was involved in increasing the F_2-isoprostanes upon CCl_4 administration (9, 21, 22).

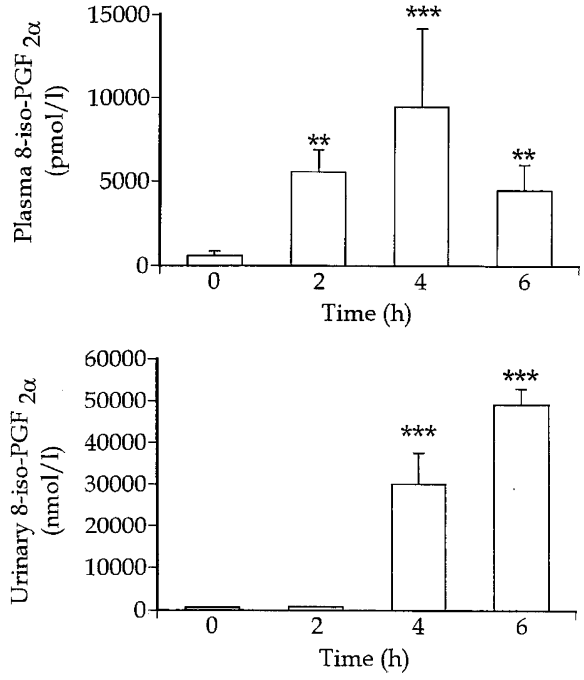

Fig. 3. Levels of free 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in plasma and urine at different times after oral administration of CCl_4 to rats (2 ml kg^{-1}). ***p <0.001, **p <0.01. [Reprinted with kind permission from Basu, 1999 (25)].

Ischaemia is the primary reason for tissue damage in, for example, cardiac infarction and stroke. It can be induced experimentally in laboratory animals (34). Reperfusion causes further local and remote organ damage that is frequently observed in intensive care units. Free radical formation at the location of injury is involved in this process, which subsequently induces oxidative stress in the whole body (35). In a well-established experimental porcine model, oxidative injury was assessed by the measurement of 8-iso-$PGF_{2\alpha}$ in plasma samples collected from both systemic circulation and the jugular bulb, which mainly drains the brain, 2 and 5 min after cardiac arrest (36). 8-Iso-$PGF_{2\alpha}$ increased rapidly in both in the systemic circulation (Fig. 4, upper panel) and jugular bulb plasma (Fig. 4, lower panel) at both time-points. In two additional studies with increased time of cardiac arrest (8 min of ventricular fibrillation) it was shown that 8-iso-$PGF_{2\alpha}$ levels increased in jugular bulb plasma concomitant with increases in the levels of a COX-mediated $PGF_{2\alpha}$ metabolite, hypoxanthine, and lactate (37, 38). A further increase in cardiac arrest time (up to 12 min of ventricular fibrillation) led to an additional increase in 8-iso-$PGF_{2\alpha}$ in the jugular bulb plasma (35). In addition, the levels of plasma 8-iso-$PGF_{2\alpha}$ correlated with the neurological score of the experimental animals at 24 h following cardiac arrest and resuscitation (37). Similarly, increased formation of F_2-isoprostanes following experimental cardiac arrest and resuscitation has recently been found in brain tissue (39). The rapid appearance of 8-iso-$PGF_{2\alpha}$ in the plasma and urine was also observed during ischaemia–reperfusion in experimental spinal cord ischaemia in pigs (40) and canine models (41).

Taken together, these studies support the concept that F_2-isoprostanes not only increase in models of ischaemia–reperfusion, but also play a bioactive role in the consequences of this injury. The increase in F_2-isoprostanes in the brain could be counteracted by the administration of various radical scavengers such as α-phenyl-*N*-tert-butyl nitrone (PBN), sulfonated α-phenyl-*N*-tert-butyl nitrone (S-PBN) and methylene blue, which further supports the theory that oxidative stress is involved in ischaemia–reperfusion injury (37, 38, 42).

Isoprostanes in other human diseases

Table 1 shows a comprehensive list of diseases where isoprostanes have been measured, and this list is growing progressively. Elevated levels in smokers were an early indication of the involvement of isoprostanes (68) and this has been corroborated in several other studies (69, 111). Increased, but less elevated levels of F_2-isoprostanes have also been demonstrated in former smokers (69).

Major controversies in this field remain over whether isoprostanes are implicated in Alzheimer's disease and type 1 diabetes. In Alzheimer's diseases, increased levels of F_2-isoprostanes have been found specifically in the cerebrospinal fluid, neurons and brain tissues (23, 89, 90, 112, 113). One study also demonstrated increased levels in the urine and plasma (91), which was not confirmed in other studies (93, 114). The reason for this inconsistency may be that biological samples were not taken at identical stages of the disease or that different drugs embroidered the outcome. The majority of the studies with Alzheimer's disease have revealed that increased formation of isoprostanes is seen mainly in neuronal tissues or in the cerebrospinal fluid.

Concerning type 1 diabetes, one report described an elevated level of 8-iso-$PGF_{2\alpha}$ in urine from patients with type 1 diabetes (43). However, a number of other reports showed no such differences in patients with type 1 diabetes compared with controls (Table 1) (44–48). Young Swedish patients

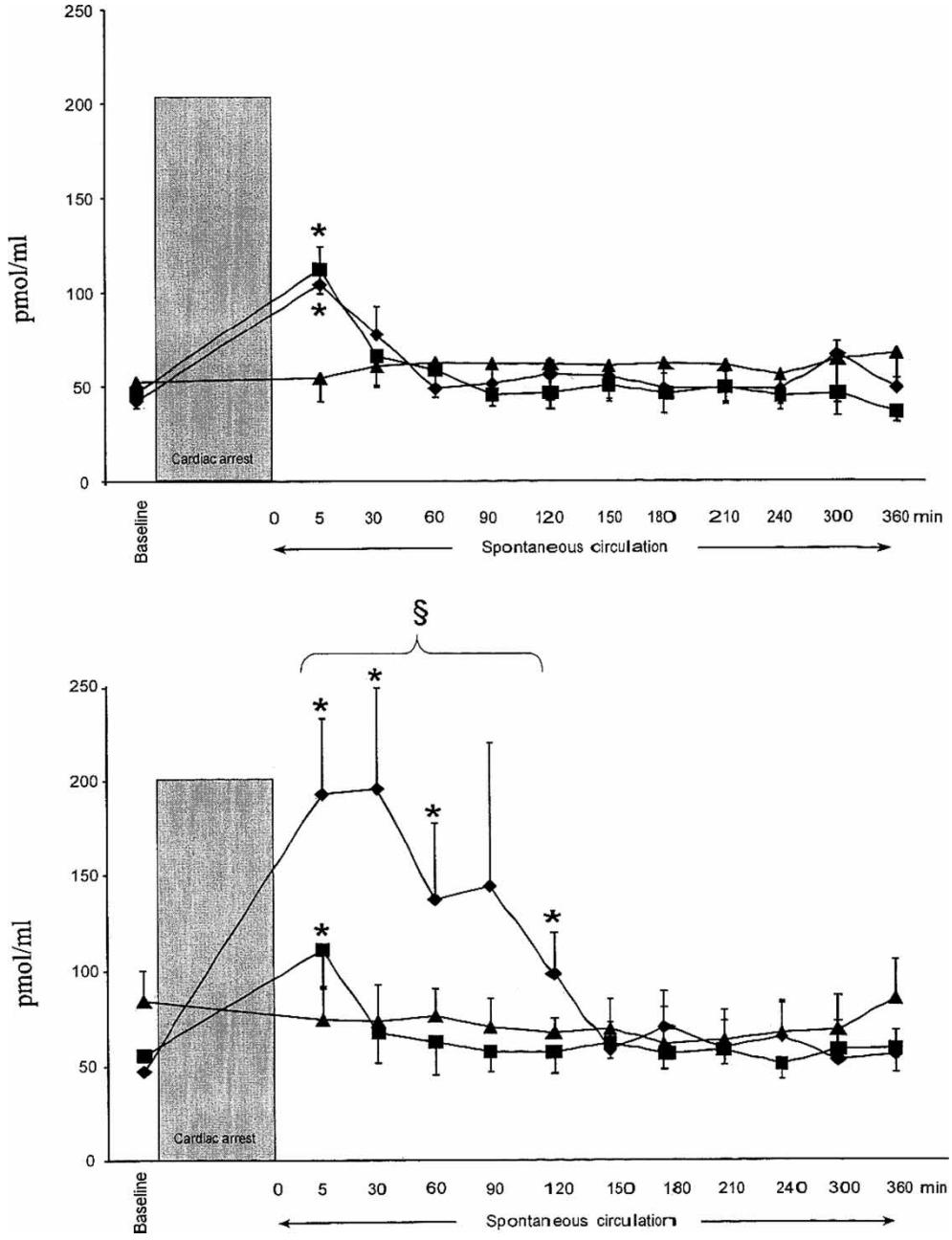

Fig. 4. Upper panel: Mixed venous plasma levels of 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) at baseline and after restoration of spontaneous circulation (ROSC). Lower panel: Jugular bulb plasma levels of 8-iso-$PGF_{2\alpha}$ at baseline and after ROSC. ◆: Group ventricular fibrillation (VF) 5; ■: group VF2; ▲: control group. *: Significant difference versus baseline; §: significant difference between group VF5 versus other groups. Values are expressed as means ± SEM. [Reprinted with permission from Basu et al., 2000 (36)].

with type 1 diabetes had no increase in 8-iso-$PGF_{2\alpha}$ concentrations compared with matched controls (46). Similarly, there was no increase in the urinary 2,3-dinor-5,6-dihydro metabolite of 8-iso-$PGF_{2\alpha}$ in type 1 diabetes (45). However, one study showed increased levels of 8-iso-$PGF_{2\alpha}$ in the early phase of diabetes onset and the level was stabilized after 1 year, in conjunction with control of the metabolic status (44). Thus, it is assumed that differences in the metabolic control in terms of HbA_{1C} and fasting glucose, hyperlipidaemia or degree of vascular damage consecutively affect the degree of lipid peroxidation and oxidative stress. The role of isoprostanes in other areas, e.g. osteoporosis, is unclear. However, it has been shown that urinary levels of 8-iso-$PGF_{2\alpha}$ are related to bone mineral density in humans, indicating that oxidative stress might be involved also in osteoporosis (115).

Table 1. F_2-Isoprostanes in human physiology and pathology

Pathological condition	Selected references	Observations
Diabetes		
Type 1 diabetes	Davi (43, 44)	Elevated
	O'Byrne (45)	Did not differ
	Vessby (46)	Did not differ
	Hoeldtke (47)	Did not differ
	Gleisner (48)	Did not differ
Type 2 diabetes	Gopaul (49, 50)	Elevated
	Davi (43)	Elevated
	Murai (51)	Elevated
	Helmersson (52) (elderly men >7 years' disease duration)	Elevated
Metabolic syndrome/lipid metabolism		
Hypercholesterolaemia	Davi (53)	Elevated
	Reilly (54)	Elevated
	Roberts (55)	Elevated
	Raal (56)	Did not differ
Type IIa hypercholesterolaemia	Cracowski (57)	Did not differ
Cardiovascular diseases		
Atherosclerosis	Pratico (58)	Elevated
	Gniwotta (59)	Elevated
	Mehrabi (60)	Elevated
Cardiopulmonary bypass	Ulus (61)	Elevated
	Delanty (41)	Elevated
Coronary reperfusion/angioplasty/ percutaneous coronary intervention	Reilly (62)	Elevated
	Berg (63)	Elevated
	Iuliano (64)	Elevated
Angiography	Berg (63)	Elevated
Coronary artery disease	Shishehbor (65)	Elevated
Heart failure	Cracowski (66)	Elevated
	Mallat (67)	Elevated
Cardiovascular risk factors		
Smoking	Morrow (68)	Elevated
	Helmersson (69)	Elevated
Passive smoking	Ahmadzadehfar (70)	Elevated
Former smokers	Helmersson (69)	Elevated
Pulmonary diseases		
Asthma	Montuschi (71)	Elevated
	Wood (72)	Elevated
	Baraldi (73)	Elevated
Acute respiratory distress syndrome	Carpenter (74)	Elevated
Cystic fibrosis	Collins (75)	Elevated
	Ciabattoni (76)	Elevated
	Montuschi (77)	Elevated
Pulmonary hypertension	Cracowski (78)	Elevated
Obstructive pulmonary diseases	Pratico (79)	Elevated
	Montuschi (80)	Elevated
Interstitial lung diseases	Montuschi (81)	Elevated
Reproductive diseases		
Pre-eclampsia	Morris (82)	Did not differ
	Ishihara (83)	Did not differ
	Regan (84)	Did not differ
	Walsh (85)	Elevated
	Barden (86, 87)	Elevated

Table 1 (*Continued*)

Pathological condition	Selected references	Observations
Pregnancy with Down's syndrome	Perrone (88)	Elevated
Neurodegenerative diseases		
Alzheimer's disease	Montine (89, 90)	Elevated
	Pratico (91)	Elevated
	Feillet-Coudray (92)	Did not differ
	Montine (93, 94)	Did not differ
	Casadesus (23)	Differed
Huntington's disease	Montine (94)	Did not differ
	Montine (95)	Elevated
Parkinson's diseases	Fessel (96)	Did not differ
Dementia with Lewy body disease	Fessel (96)	Did not differ
Migraine	Helmersson (97)	Did not differ
Spinal cord injury	Monga (98)	Elevated
Multiple sclerosis	Mattsson (99)	Elevated
Other diseases		
Prostate cancer	Camphausen (100)	Did not differ
Haemodialysis	Handelman (101)	Elevated
	Ikizler (102)	Elevated
Kidney transplantation	Cracowski (103)	Did not differ
	Basu (104)	Elevated
Dialysis patients	Lauzurica (105)	Elevated
Hypertension with renovascular disease	Minuz (106)	Elevated
Inflammatory diseases		
Rheumatoid arthritis	Basu (107)	Elevated
Psoriatic arthritis	Basu (107)	Elevated
Reactive arthritis	Basu (107)	Elevated
Osteoarthritis	Basu (107)	Elevated
Systemic sclerosis	Cracowski (108)	Elevated
	Stein (109)	Elevated
Periodontal disease	Wolfram (110)	Elevated
Physiological conditions		
Human pregnancy	Morris (82)	Elevated
	Ishihara (83)	Elevated

Foreword theory of oxidative strain compared with oxidative stress

In healthy aerobic organisms, the formation of reactive radical species of diverse activity appears to be finely tuned by the body's efficient antioxidant defence system. Nonetheless, once the production exceeds the regular physiological equilibrium, the mammalian body experiences potentially deleteriously distress, frequently referred to as oxidative stress. It is rarely described whether mild or low grade oxidative stress has any function in physiology, apart from being seen in chronic diseases such as diabetes, obesity and atherosclerosis, and also in smoking. Moderately higher levels of isoprostanes are seen in these conditions, compared with the more pronounced response seen in acute inflammatory situations.

It seems now that a mild increase in basal isoprostanes is also a component of certain basic physiological states such as normal pregnancy (82, 83). When an increased oxidative activity occurs as part of a normal physiological state, it may be considered as an "oxidative strain" rather than an oxidative stress. In other words, oxidative strain could be regarded as a condition in which increased free radical-mediated reactions are necessary to maintain specific functions. In such specific circumstances, the body requires a mild oxidative imbalance. This situation is different from the chronic low-grade inflammatory conditions in chronic diseases, where a low but sustained increase in F_2-isoprostanes occurs (52, 69, 116), or the high increase in F_2-isoprostanes seen in chronic severe inflammatory conditions, such as rheumatic

diseases, asthma or acute respiratory distress syndrome (ARDS) (71, 77, 81, 107).

Role of antioxidants on isoprostane formation

A central issue remaining to be elucidated is the role of exogenous antioxidants in different human diseases where oxidative stress is involved in the pathology. This issue can be addressed indirectly by studies of effects on reliable oxidative stress biomarkers such as isoprostanes. In rats with CCl_4-induced hepatotoxicity, a high dose of vitamin E ($20\ g\ kg^{-1}$ diet of all-rac-tocopheryl succinate for 3 weeks) affected non-enzymic lipid peroxidation (21). Administration of vitamin E before the CCl_4 treatment resulted in significantly lower levels of urinary and liver free 8-iso-$PGF_{2\alpha}$. 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 has also been shown that antioxidant therapy ameliorated the progression of atherosclerosis and isoprostane formation in animal studies (117).

Recent human studies, however, show that α-tocopherol in varying doses (200–2000 IU per day for 8 weeks or 200 IU per day for 2 weeks) did not affect the concentrations of F_2-isoprostanes (118, 119). Vitamin E supplementation also did not affect levels of F_2-isoprostanes in moderate cigarette smokers (120). In another study in which cigarette smokers consumed a diet high in polyunsaturated fat a pro-oxidant effect of supplementary vitamin E was even observed (121). However, when hypercholesterolaemic patients were treated with high doses (800–3200 IU per day), but not with lower doses (100–400 IU per day) of vitamin E, a significant decrease in the plasma F_2-isoprostane level was noted after 16 weeks (55), as seen in the animal studies (21). Other studies, however, have shown that vitamin E supplementation reduced the urinary concentration of F_2-isoprostanes in patients with type 2 diabetes (43), cystic fibrosis (76), hypercholesterolaemia (53) and homozygous homocystinuria (122), and also reduced urinary F_2-isoprostane levels in hepatic cirrhosis and alcoholic liver disease (123).

In a mechanistic study in which the formation of different isomers of CLA-induced F_2-isoprostanes was followed 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 per day) (119). Neither did 28 days' supplementation with various doses of α-tocopherol (15, 100, 200 and 400 IU per day) have any effect on the basal levels of F_2-isoprostanes in healthy human subjects (124). Together, these studies show that vitamin E supplementation has a varying antioxidative effect in studies related to different doses or patients or population groups or stage of pathogenesis of diseases, possibly depending on the basal lipid peroxidation process or even the pharmacogenomics in these individuals. Measurement of isoprostanes would be a relevant tool in the further study of the efficacy of various antioxidants.

Oxidative modification of low-density lipoproteins and the subsequent formation of foam cells are considered to affect the formation of atherosclerotic lesions, and both *in vitro* and various experimental studies show that oxidative stress is involved in all stages of coronary artery disease (125–128). However, recent large clinical trials have failed to demonstrate any clinical outcome of antioxidant supplementation (129). This is possibly due to the fact that the majority of these intervention studies neither had any reliable way of determining systemic oxidant stress, nor recruited the patients according to degree of oxidant stress. More studies are needed to prove or refute the role of antioxidant therapy in atherosclerosis. The most momentous question in this context is whether this disease is caused by oxidative stress or whether oxidative stress is merely a consequence of other disease processes.

Conclusions

Oxidative stress is believed to be one of the major processes underlying several acute or chronic diseases. Isoprostanes are biologically potent free radical-catalysed compounds, and current evidence suggests that they are reliable *in vivo* biomarkers of oxidative stress. Isoprostanes are involved in normal physiology such as human pregnancy, as well as in acute and chronic inflammation pathology. These non-enzymically, rapidly formed compounds not only are bioactive themselves, but also seem to be useful as novel early biomarkers of oxidative stress, and further could be applied to study the efficacy of antioxidants and other free radical scavengers.

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