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REVIEW ARTICLE

Using Isoprostanes as Biomarkers of Oxidative Stress: Some Rarely Considered Issues

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Abstract

The measurement of F_2-isoprostanes by methods utilizing mass spectrometry is widely regarded as the best currently available biomarker of lipid peroxidation. F_2-isoprostanes and their metabolites can be measured accurately in plasma, urine, and other body fluids using mass spectrometric techniques, and detailed protocols have been published in several papers. However, many clinical studies and intervention studies with diets or supplements, have employed single "spot" measurements of F_2-isoprostanes on either plasma/serum or urine to estimate "oxidative stress." This review examines the validity of the common assumption that plasma and urinary F_2-isoprostane measurements are equivalent. It identifies scenarios where they may not be and where "spot" measurements can be misleading, with examples from the literature. We also discuss the controversial issue of whether and how F_2-isoprostane levels in plasma should be standardized against lipids, and, if so, which lipids to use. *Antioxid. Redox Signal.* 13, 145–156.

Introduction

The discovery of F_2-isoprostanes (F_2-IsoPs) by Morrow *et al.* (71) almost 2 decades ago has brought a new dimension in free radical lipid peroxidation research by creating a powerful tool to measure oxidative damage. F_2-IsoPs measurement by mass spectrometry is a robust chemical measurement relatively free of potential artifacts. Biological samples are subjected to various clean-up techniques and the F_2-IsoPs then measured by gas chromatography/mass spectrometry (GC/MS), gas chromatography tandem mass spectrometry (GC/MS/MS), or liquid chromatography tandem mass spectrometry (LC/MS/MS) (32, 38, 53, 71, 75, 85, 93, 95, 106). Detailed protocols have been published (49, 53, 71, 75, 98, 106), as well as several excellent reviews on this topic (9, 19, 28, 63, 64, 68, 73, 95). F_2-IsoPs are a group of compounds produced via nonenzymatic, free radical attack on arachidonic acid (38, 53, 68, 71, 75, 89), although additional sources have not been ruled out (discussed in Refs. 28 and 112). Other oxidation products of arachidonic acid such as A_2-, E_2-, D_2-, J_2-IsoPs, isothromboxanes, and isoketals have been studied (68, 89, 93), as have A_4-, D_4-, E_4-, and F_4-neuroprostanes from docosahexaenoic acid (77, 87), and also F_3-IsoPs and A_3/J_3-IsoPs from eicosapentaenoic acid (13, 26, 76), and hydroxyoctadecadienoic acid products from linoleic acid (73, 107). However, since F_2-IsoPs are the most widely studied "biomarker of oxidative stress" (and are often referred to as the "gold standard" to measure lipid peroxidation), they are the focus of this article. In this commentary, we do not aim to repeat what has already been covered extensively in the literature, but instead to highlight some points which may substantially affect interpretation of IsoPs measurement that have rarely been explicitly considered in the literature to date.

One point worth mentioning first is that there is incomplete agreement over what exactly is measured by the different MS techniques in use in different laboratories. Measurement of F_2-IsoPs is often equated to measurement of the compound, 8-isoprostaglandin $F_{2\alpha}$ but there are many other isomers and different MS-based methods measure different mixtures of these (49, 53, 68, 75, 89, 95, 96, 106). Roberts *et al.* (88) showed that 8-isoprostaglandin $F_{2\alpha}$ can be further metabolized via β-oxidation and subsequent reduction to give 2,3-dinor-8-isoprostaglandin $F_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-prostaglandin $F_{2\alpha}$ respectively (Fig. 1), which are present in significant amounts in human urine (18, 35, 75, 88). F_2-IsoPs can thus be measured (with rigorous attention to methodology) in plasma, serum, urine, and, less often, in other body fluids such as bronchoalveolar lavage fluid, exhaled breath condensate, and saliva (37, 58, 65–67) as "biomarkers" of oxidative stress. It is essential that samples containing lipids (all body fluids except urine) are analyzed at once upon collection, or immediately flash-frozen in liquid nitrogen and stored at minus 80°C in the presence of antioxidants such as BHT (butylated hydroxytoluene) to prevent artefactual oxidation of lipids on storage (53, 71). Hemolysis causes rapid lipid

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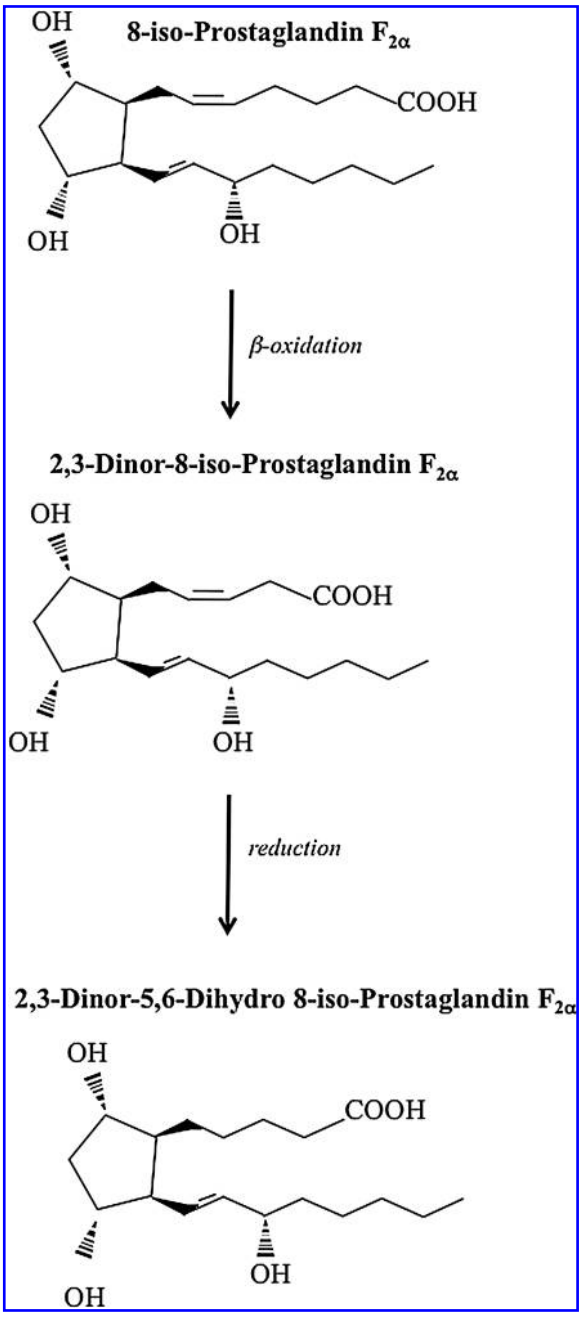

FIG. 1. Metabolism of 8-isoprostaglandin $F_{2\alpha}$.

oxidation and blood samples showing significant hemolysis should be avoided (24, 30).

However, several issues affecting the interpretation of F_2-IsoPs measurements have been given scant attention in the literature. First, many papers are now appearing in which single measurements of F_2-IsoPs levels are made on either plasma or urine samples, often in patients suffering various diseases who are compared with "normal controls." Other published papers report the effects of dietary changes or administration of antioxidant supplements in humans, measuring F_2-IsoPs levels in either plasma or urine before and after the intervention. It seems to be assumed by many authors of such papers that measurements either in plasma or in urine are equivalent, and only one needs to be examined, and often only at a single time point. The first question we wish to address in this review is whether these assumptions will always be true.

Hydrolysis, Excretion, and Metabolism

Most arachidonic acid (the precursor of F_2-IsoPs) in animals is not "free," but present in esterified forms as phospholipids in membranes and lipoproteins. F_2-IsoPs appear to be initially formed esterified on phospholipids and are then hydrolyzed into free forms by the actions of phospholipase A_2 (PLA_2) and platelet activating factor acetylhydrolase (PAF-AH) enzymes (19, 68, 69, 71, 89, 103, 104). In human plasma, the latter enzyme seems more important since plasma samples from patients lacking PAF-AH did not release F_2-IsoPs from esterified precursors (104). Once liberated, free F_2-IsoPs seem to turn over rapidly, both by metabolism and by excretion (Fig. 1). Indeed, in rabbits after intravenous infusion of 8-iso-$PGF_{2\alpha}$, the plasma level was maximum at 1.5 min after injection and then fell rapidly. Urinary excretion of 8-iso-$PGF_{2\alpha}$ peaked at 20 min and took 60 min or more to normalize (9). In humans, when tritiated [^{3}H]8-iso-$PGF_{2\alpha}$ was infused into the antecubital vein, 75% of the radioactivity was recovered in urine after 4.5 h (the major compound being 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$), and similarly in monkey 95% was recovered in urine after 4 h (88).

A key issue to consider is the rate of hydrolysis of F_2-IsoPs esterified to phospholipids. It often appears to be assumed that this is very rapid and that F_2-IsoPs in either urine or plasma can be measured interchangeably as an index of oxidative stress in disease, toxicology, or nutritional studies. However, a consideration of the available data suggests otherwise. For example, oral administration of CCl_4, a powerful inducer of lipid peroxidation (7, 33, 40, 41, 69, 98) to rodents raised levels of esterified F_2-IsoPs in liver with a peak at 2 h (7, 69) and levels then decreased (Fig. 2). Plasma free F_2-IsoPs also increased, but over a longer time scale, peaking at 8 h (Fig. 2). In another study, plasma free F_2-IsoPs maximized at 4 h, whereas urinary F_2-IsoPs continued to rise up to at least

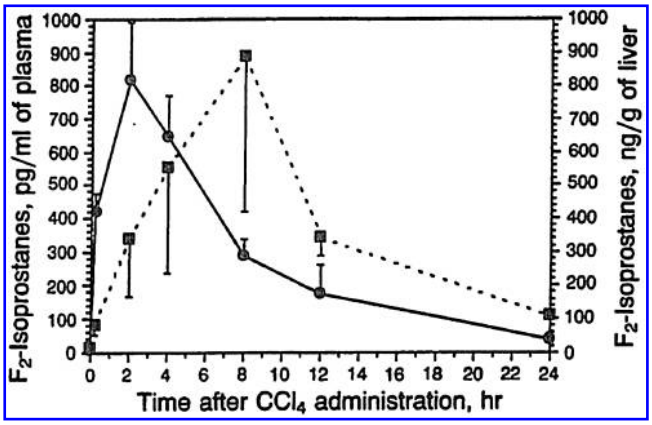

FIG. 2. Time course of appearance of esterified F_2-isoprostanes in liver (●) in comparison with the appearance of free F_2-IsoPs in the circulation (■) of rats after intragastric administration of CCl_4 (1 ml/kg). Each time point represents the mean ± SD of levels measured in at least four animals. [Reproduced with permission from Morrow *et al.* (69)]

6 h (7). In a more recent report (41), CCl_4 administered to rats raised plasma total (free + esterified) F_2-IsoPs substantially at 2 h. Levels were lower at 7 h and close to normal at 16 h. However plasma free F_2-IsoPs were still high at 7 h and urinary F_2-IsoPs were also high then, and remained elevated even at 16 h after a larger CCl_4 dose (41). Thus a "spot" sampling of plasma or urine at a single time point to measure F_2-IsoPs could clearly give different impressions if the data were then used to assess the extent of lipid peroxidation (Figs. 2–6). These studies show that hydrolysis can take some hours. Indeed, mice overexpressing PAF-AH showed higher levels of free F_2-IsoPs in bronchoalveolar lavage fluid (104), suggesting that hydrolysis rates can be affected by PAF-AH activity and that this enzyme activity could affect the rate of generation of plasma (free and hence of urinary) F_2-IsoPs.

PAF-AH activity is associated mostly (~70%) with low density lipoprotein (LDL) particles, but there is some in high density lipoproteins (HDL) (43, 46, 103). The distribution of F_2-IsoPs is also lipoprotein specific (see below). The total plasma activity of PAF-AH could thus be influenced by changes in the ratio of the various plasma lipoproteins that are often seen in disease (103). For example, plasma PAF-AH activity increases in dengue fever (97) and renal disease (80). However, PAF-AH has also been suggested to become inhibited in presence of severe oxidative stress (55, 103, 104) and this may occur in coronary artery disease, hypercholesterolemia, kidney disease, and Parkinson's disease (50, 55, 68, 73, 80, 103, our unpublished observations). If oxidative stress were to increase but PAF-AH decrease (by oxidative stress or otherwise), one can imagine a scenario in which levels of esterified F_2-IsoPs could rise but levels of free F_2-IsoPs could fall, and hence urinary levels might also fall (Fig. 6).

Possible Scenarios

As indicated earlier, for induction of acute oxidative stress, the time course of the levels of F_2-IsoPs will not be the same in tissues, plasma, and urine and spot measurements may be misleading (Fig. 4). We may have recently seen a clinical example of this. In dengue fever, an acute febrile disease of short duration, we found no changes in F_2-IsoPs levels in plasma but there were rises in urine levels. We hypothesize that by the time the patients had been tested (after admission to hospital in an acute febrile state) the elevation of plasma F_2-IsoPs had already passed but urinary levels were still high (97). Some other human diseases represent a similar "acute" oxidative stress, such as ischemia/reperfusion injury in stroke or myocardial infarction, and certain other infectious diseases (reviewed in Ref. 33). Therefore, multiple sequential sampling of blood and/or urine is required to assess the changes in F_2-IsoPs levels and it must be appreciated that plasma and urinary measurements may follow a different time-course (Figs. 2–6). Some authors have also suggested that interpretation of urinary levels of nonmetabolized F_2-IsoPs as an index of total endogenous F_2-IsoPs production can be confounded by the potential contribution of local F_2-IsoPs production in the kidney (112). In addition, it is not impossible that disease or toxins (e.g., cigarette smoke) can alter the rate of metabolism (Fig. 1) of free F_2-IsoPs. For example, if β-oxidation rates were decreased, urinary F_2-IsoPs might rise, with no change in the overall rate of lipid peroxidation. Hence for urine, the ideal is to measure all three products (Fig. 1), so that all these factors can be taken into account, but this is rarely done.

Of course, some diseases produce chronic oxidative stress, such as atherosclerosis and chronic inflammatory diseases (reviewed in Ref. 33), so one or other of the scenarios in Figures 3–6 will apply. Yet others are acute-to-chronic, for example, in stroke patients there is an acute oxidative stress (as measured by plasma F_2-IsoPs) present at the time of admission to hospital (50). Levels of F_2-IsoPs then decline and give way to a longer-lasting elevation of F_2-IsoPs levels, which may be due to inflammatory events (50, and unpublished data). In the reverse sense, if administration of an antioxidant decreases ROS levels and diminishes tissue and plasma esterified F_2-IsoPs levels, it will take a while before such events are reflected in urinary F_2-IsoPs (Fig. 3), and so again timing of measurement is crucial and single spot measurements can be misleading. One cannot safely conclude that an antioxidant is ineffective *in vivo* on the basis of a single F_2-IsoP measurement. It should also be realized that animals are often more responsive to dietary antioxidants than are humans, which raises questions about the validity of some animal models of human disease, particularly neurodegenerative disease (31, 32) and that F_2-IsoPs may sometimes arise by different mechanisms in humans and other animals (discussed in Ref. 19).

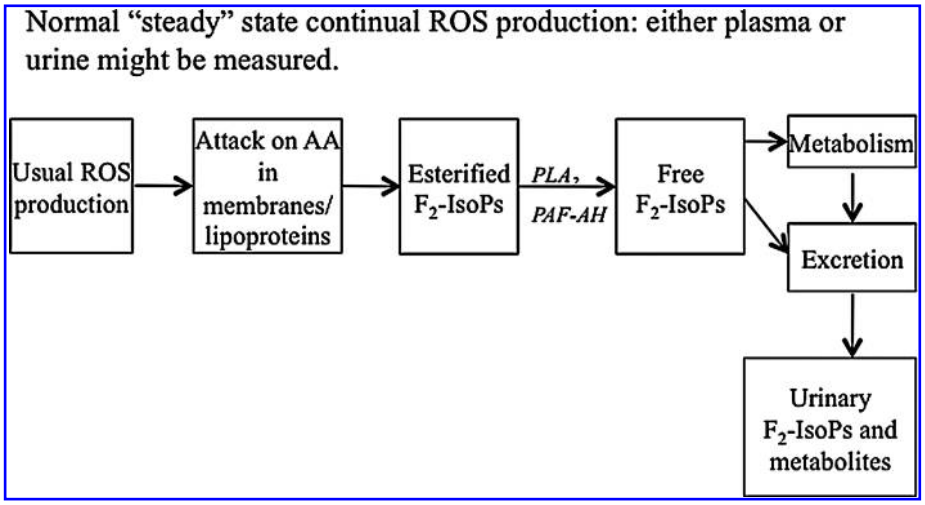

FIG. 3. Formation and turnover of F_2-isoprostanes in normal "steady state" continual production of reactive oxygen species *in vivo*.

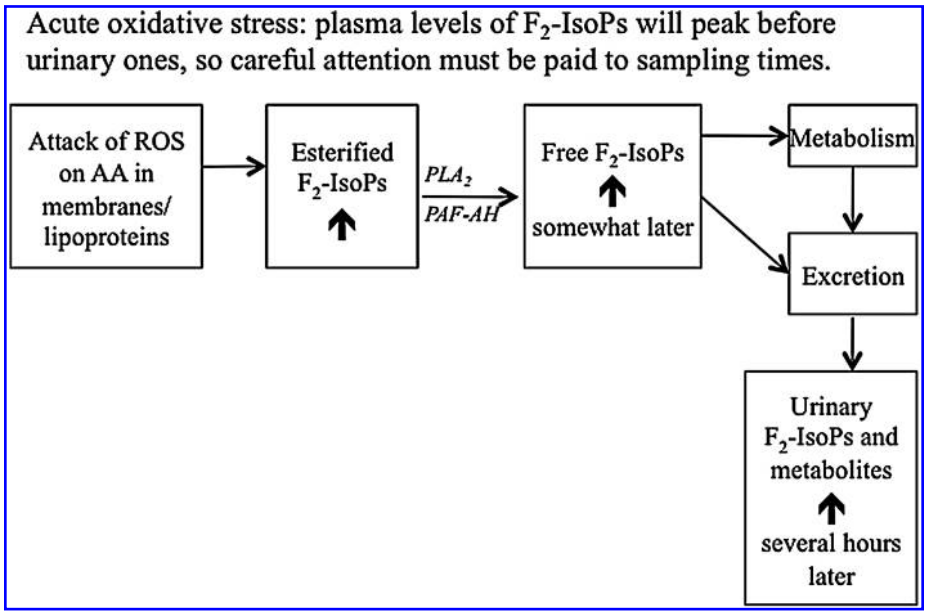

FIG. 4. Formation and turnover of F_2-isoprostanes in acute oxidative stress (e.g., acute onset of disease leading to ROS production, or administration of CCl_4 or other toxins that involve ROS production.)

What Do the Published Data Show?

Over 2000 articles are available in PubMed on F_2-IsoPs but few of them measured F_2-IsoPs in both plasma and urine (indicative of the common view that measurements in either are equivalent as an oxidative stress biomarker). Some of the studies used serum instead. In one early paper, Morrow *et al.* (70) reported a strong correlation between plasma and urinary F_2-IsoPs levels in a mixed group of smokers and nonsmokers. This is consistent with a majority of data indicative of increased oxidative stress in smokers (reviewed in Ref. 84), although others have found correlations to be less good (106). Taking this strong correlation in Ref. 70 into account, many subsequent studies assumed, as stated earlier, that measuring F_2-IsoPs in either plasma or urine is sufficient information to determine the oxidative stress status of the subjects investigated. Smoking creates a chronic oxidative stress (33, 70, 84), so this may be true in smokers. However, our studies in nonsmokers (50) have shown a more complex scenario: correlations between plasma and urinary F_2-IsoPs levels are not always good and are affected by both age and disease.

Tables 1 and 2 summarize observational and interventional studies reported in the literature, that measured both urinary and plasma F_2-IsoPs. Did they always show the same thing? Often yes, but these include multiple cases of observational or intervention studies (with antioxidants or other agents) where neither plasma nor urinary F_2-IsoPs levels changed at all (marked * on Tables 1 and 2). As explained earlier, some of these may need to be readdressed, since "spot" studies can miss things, plus of course the obvious need to consider whether an appropriate dose of antioxidant was used and the issue of inter-individual variability. However, sometimes different results were observed from plasma and urinary measurements (Table 3). For these, it is interesting to speculate

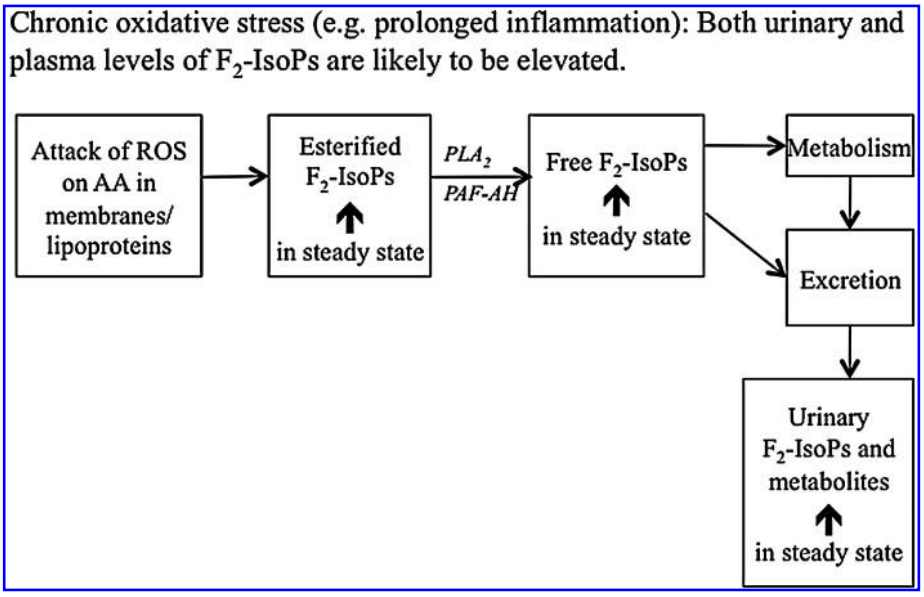

FIG. 5. Formation and turnover of F_2-isoprostanes in chronic oxidative stress (e.g., prolonged inflammation).

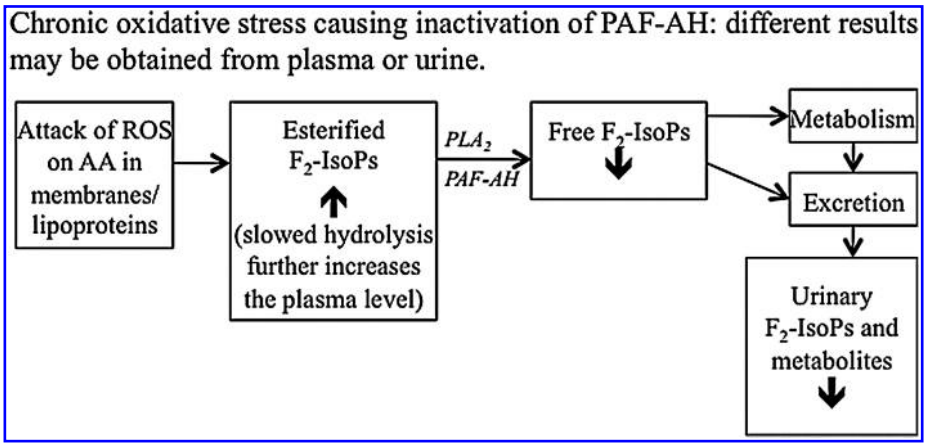

FIG. 6. Formation and turnover of F_2-isoprostanes in chronic oxidative stress associated with inactivation of PAF-AH.

which of the scenarios in Figures 4–6 is responsible (please see the last column of Table 3). Given the methodological issues discussed briefly earlier, we have also indicated in the Tables which methods were employed to measure F_2-IsoPs.

Expressing the Results

A second important but rarely considered issue is to how to express results of F_2-IsoPs measurements. F_2-IsoPs levels in plasma are usually expressed per unit volume (ml of plasma), and in urine per unit creatinine (unless 24 h urine collections are carried out, which is not often done). The expression of urinary levels relative to creatinine seems to work well (discussed in Refs. 49 and 106) unless there are major changes in creatinine production and/or excretion, as can happen in renal failure (27).

However, in several diseases and in some intervention studies with antioxidants or foodstuffs, there are changes in plasma (and probably in tissue) total/esterified arachidonic acid (AA) levels. Should we correct F_2-IsoPs levels for this, bearing in mind, for example, that if there is less substrate (esterified AA), then this lipid could possibly be a less important target of ROS? Of course, plasma AA levels are not necessarily representative of levels in the tissue(s) where the oxidative damage is taking place. Nevertheless, in some of our recent work, we have shown that correction for plasma AA levels (which were changed by the conditions examined) can alter the conclusions drawn (47, 50, 97) (e.g., in some cases observing normal F_2-IsoPs level but a higher F_2-IsoPs to AA ratio because of lower AA). There has been very little debate on this issue. Correction in this way sounds plausible, but is it justified? Are changes in AA in plasma indicative of changes in AA in the membrane lipids in cells that are undergoing peroxidation? Not necessarily. Changes in diet can also transiently change plasma AA levels.

The scenario may be even more complex. Plasma F_2-IsoPs seem to be usually present mostly in HDL (85), although this may change in some circumstances, since increased plasma

Table 1. Observational Studies that Measured F_2-Isoprostanes in Both Plasma and Urine

Study	*Subject*	*Method of Measurement*	*Reference*
Aboutwerat *et al.* (2003)	Primary biliary cirrhosis patients	ELISA Kit	1
Barden *et al.* (2001)	Preeclampsia patients	GC/MS	4
Basu *et al.* (2001)	Spinal cord ischaemia in pigs	RIA	10
Calabrese *et al.* (2007)	Nephropathic type 2 diabetes patients	HPLC	14
Cederberg *et al.* (2001)	Diabetic pregnant rats	RIA	15
Dolgoskwa *et al.* (2009)	Hypertensive patients	ELISA Kit	23
Dogra *et al.* (2001)	Nephrotic syndrome patients*	GC/MS	22
Feillet–Coudray *et al.* (2002)	Diabetes mellitus type 2 patients	EIA & ELISA Kit	25
Ishihara *et al.* (2004)	Pre-eclampsia patients*	RIA	36
Lee *et al.* (2009)	Ischemic stroke patients, Parkinson's disease patients*	GC/MS	50
Matayatsuk *et al.* (2007)	Thalassemic patients	GC/MS	56
McKinney *et al.* (2000)	Pre-eclampsia patients	EIA Kit	58
Montine *et al.* (2000)	Huntington's disease patients*, Alzheimer's disease patients*	GC/MS	42
Morrow *et al.* (1995)	Smoker volunteers	GC/MS	70
Oguogho *et al.* (2000)	Hyperlipoproteinemia patients	Immunoassay	79
Pemberton *et al.* (2005)	Alcoholic liver disease patients	ELISA Kit	82
Rodrigo *et al.* (2007)	Hypertensive patients	ELISA Kit	91
Seet *et al.* (2009)	Dengue fever	GC-MS	97
Sinzinger *et al.* (2001)	Heterozygous familial hypercholesterolemia patients	EIA Kit	100
Vessby *et al.* (2002)	Type 1 diabetes patients*	RIA	107
Ward *et al.* (2004)	Treated hypertensive subjects*	GC/MS	110

*No changes were observed as compared with control subjects.

Table 2. Interventional Studies that Measured F_2-Isoprostanes in Both Plasma and Urine

Study	Subject	Intervention	Method of Measurement	Reference
Abu–Amsha Caccetta *et al.* (2001)	Healthy smokers	Wine	GC/MS	2
Barany *et al.* (2001)	Healthy volunteers	Cyclosporin A* Vitamin E*	GC/MS	3
Barden *et al.* (2004)	Pregnant atopic women	Fish oil Olive oil*	GC/MS	6
Barden *et al.* (2007)	Healthy volunteers	Light beer Normal beer*	GC/MS	5
Basu (1999)	Sprague-Dawley rats	CCl_4	RIA	8
Beltowski *et al.* (2003)	Wistar rats	Leptin	EIA Kit	11
Beltowski *et al.* (2005)	Adult Wistar rats	Leptin NAD(P)H oxidase inhibitors (e.g., apocynin)* Tempol*	EIA Kit	12
Chehne *et al.* (2001)	Atherosclerosis patients	Restarted smoking	Immunoassay	16
Chehne *et al.* (2002)	Atherosclerosis patients	Smoking cessation	Immunoassay	17
Dhawan & Jain (2004)	Healthy controls Hypertensive patients	Garlic pearls (*healthy control)	ELISA Kit	53
Dillon *et al.* (2002)	Nonsmokers Smokers	Garlic extract	EIA Kit	21
Janroz–Wisniewska *et al.* (2008)	Wistar rats	Leptin* Apomycin	EIA Kit	39
Kadiiska *et al.* (2005)	Fischer rats	CCl_4	GC/MS	40
Kadiiska *et al.* (2005)	Fischer rats	CCl_4 Indomethacin Meclofenamic acid	GC/MS	41
Kelly *et al.* (2008)	Healthy volunteers	Vitamin C*	GC/MS	44
Lee *et al.* (2006)	Healthy volunteers	Rice Dark soy sauce	GC/MS	48
Lee *et al.* (2009)	Healthy Volunteers	Rice Tomato sauce	GC/MS	47
Levine *et al.* (2001)	Healthy volunteers	Vitamin C*	GC/MS	51
Loke *et al.* (2008)	Healthy volunteers	Quercetin* Epicatechin* Epigallocatechin gallate*	GC/MS	54
McAnulty *et al.* (2007)	Marathon runners	Ibuprofen	GC/MS	57
Meng *et al.* (2002)	Dahl salt sensitive rats	Low sodium diet* High sodium diet	GC/MS	59
Montero *et al.* (2000)	Type 1 diabetic nephropathy	Vitamin E	GC/MS	60
Montine *et al.* (2002)	Sprague-Dawley rats	Kainic acid*	GC/MS	61
Nieman *et al.* (2004)	Triathlon athletes	Vitamin E*	GC/MS	72
Oguogho *et al.* (2000)	Heterozygous familial hypercholesterolemia patients	LDL-apheresis	EIA Kit	78
Pemberton *et al.* (2004)	Autoimmune hepatitis patients	Prednisolone* Azathioprine*	ELISA Kit	81
Pilz *et al.* (2000)	Atherosclerotic patients	Smoking cessation	EIA Kit	83
Rodrigo *et al.* (2004)	Wistar rats	Wine	EIA	90
Rogers *et al.* (2006)	Pre-eclampsia	Oral glucose	GC/MS	92
Sinzinger *et al.* (2002)	Heterozygous familial hypercholesterolemia patients	Statin	Immunoassay	99
Sodergren *et al.* (2000)	Sprague-Dawley rats	Vitamin E*	RIA	101
Sodergren *et al.* (2001)	Sprague-Dawley rats	Vitamin E* Vitamin E + CCl_4	RIA	102
Stojiljkovic *et al.* (2002)	Obese hypertensive patients	Intralipid Heparin	GC/MS	105
Ward *et al.* (2005)	Treated hypertensive patients	Vitamin C* Polyphenols*	GC/MS	109
Wolfram *et al.* (2002)	Patients	Radioiodine	Immunoassay	111
Wu *et al.* (2007)	Type 2 diabetes mellitus	Tocopherol	GC/MS	112

*The intervention did not change F_2-IsoPs levels in either plasma or urine. Note the common failure of administration of antioxidants (vitamins C and E, polyphenols) to humans to decrease oxidative damage, at least as measured by F_2-IsoPs level.

Table 3. Interventional Studies that Measured F_2-Isoprostanes in Both Plasma and Urine and Where Discrepancy was Found

Study	*Subject*	*Intervention*	*Method of Measurement*	*F_2-Isoprostanes (↑↓↔)*		*Speculative explanation of difference (based on Fig. 3)*
				Plasma	*Urine*	
Abu–Amsha Caccetta *et al.* (2001) (2)	Healthy smokers	1) Red wine 2) Red wine with alcohol removed 3) White wine	ELISA Kit	1) ↔ 2) ↓ 3) ↔	1) ↔ 2) ↔ 3) ↔	Possible changes in plasma lipoprotein levels affecting plasma levels of F_2-IsoPs or rate of hydrolysis of esterified F_2-IsoPs?
Barden *et al.* (2007) (5)	Healthy volunteers	1) Light beer 2) Normal beer	GC/MS	1) ↓ 2) ↔	1) ↔ 2) ↔	Possible changes in plasma lipoprotein levels affecting plasma levels or rate of hydrolysis?
Lee *et al.* (2009) (47)	Healthy Volunteers	1) Rice + olive oil 2) Rice + olive oil + tomato sauce	GC/MS	1)↔ 2)↔	1)↔ 2)↓	Tomato sauce perhaps changed plasma lipoproteins or PAF-AH activity, or the metabolism of free F_2-IsoPs.
Rogers *et al.* (2006) (92)	1) Gestational hypertension/ pre-eclampsia 24–32 weeks 2) Gestational hypertension/ pre-eclampsia 34–37 weeks	Oral glucose	GC/MS	1) ↑ 2) ↑	1) ↑ 2) ↔	ROS levels rise in preeclampsia when glucose is given thus plasma F_2-IsoPs are increased. Subsequently hydrolysis releases urinary F_2-IsoPs. However chronic ROS could perhaps have inactivated PAF-AH somewhat so the rise was not seen in urine, or there were changes in plasma lipoproteins enzyme activity or in metabolism of free F_2-IsoPs.
Sinzinger *et al.* (2002) (99)	1) Nonsmokers with heterozygous familial hypercholesterolemia patients 2) Smokers with heterozygous familial hypercholesterolemia patients	Statin	Immunoassay	1) ↔ 2) ↔	1) ↔ 2) ↓	Statins alter cholesterol metabolism and may have lowered LDL and HDL, carriers of the hydrolytic enzymes needed to release free F_2-IsoPs for excretion from esterified F_2-IsoPs in the phospholipid moiety.
Wu *et al.* (2007) (112)	Type 2 diabetes mellitus	Tocopherol	GC/MS	↓	↔	Authors suggested that some urinary F_2-IsoPs originated from the kidney and were thus not representative of systemic oxidative stress.

↑ Increase; ↓ Decrease; ↔ No change.

F_2-IsoPs were also reported in low HDL subjects (45) and type 2 diabetic patients with low HDL (74). A close association was found in the HDL3c fraction between cholesterol, high PAF activity, and F_2-IsoPs (74). F_2-IsoPs are also present in LDL. Some reports showed levels of blood LDL or oxidized LDL to be correlated with plasma F_2-IsoPs (42, 52). So if we decide to adjust, which do we choose for F_2-IsoPs adjustment: HDL, LDL, the precursor arachidonate, total blood lipids, or none of these? For example, if in a disease or therapeutic intervention there is a large drop in plasma HDL, that may mask an overall rise in HDL F_2-IsoPs content if the F_2-IsoPs data are simply expressed per ml of plasma. We have no simple answer to any of these questions and the issues need to be explored further experimentally. At the moment we simply recommend that investigators record such changes in lipids and lipoproteins, as they frequently happen in disease and sometimes in nutritional studies, and that could influence the interpretation of F_2-IsoPs measurement.

Another factor to consider in nutritional intervention studies is the impact of diet. As far as we know, F_2-IsoPs, although present in foods, are not absorbed through the gut (29, 86). However, we and others have observed a trend for rises in plasma F_2-IsoPs upon 24 h fasting of humans (49, 86), which might be due to changes in plasma lipoprotein patterns and/or rates of hydrolysis of esterified F_2-IsoPs. Refeeding, even with diets containing no antioxidants, can alter these parameters. For example, feeding subjects with rice (poor in antioxidants) altered F_2-IsoPs level, apparently causing transient drops in plasma total F_2-IsoPs and a transient rise in urinary levels (48). One should therefore be careful to use feeding controls when testing the effects of antioxidant-rich foods, to check that the mere act of eating was not changing F_2-IsoPs levels and leading to a false conclusion of an antioxidant effect.

One final factor to consider is the potential impact of the metabolism of free F_2-IsoPs: do ratios of F_2-IsoPs to their metabolites vary in disease or with nutritional or other interventions (Fig. 1)? Disease or toxins (including cigarette smoking) can often change metabolism. If so, measuring urinary F_2-IsoPs alone could again be misleading.

Conclusion

The views that spot measurements of F_2-IsoPs in plasma or urine are adequate to detect oxidative stress *in vivo* and how it is affected by diet or disease are obviously simplistic. Even more so is the view that plasma or urine measurements are interchangeable (Fig. 3, Table 3). Preferably, both should be determined, plus the urinary metabolites (Fig. 1). Although some studies have shown that levels of 8-iso-$PGF_{2\alpha}$ and its metabolites correlate well (94, 96), implying that only one need be measured, this is not always true (e.g., Ref. 106). For example, Dai *et al.* (18) found that urinary F_2-IsoPs level and 2,3,dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ showed complex and different relations to breast cancer risk. If one has to make a choice between plasma and urinary measurements, we prefer plasma total F_2-IsoPs level determination. Bearing in mind the limitations in studying effects of dietary interventions and disease, we recommend tracking changes over a period of time, ideally in both urine and plasma.

Because of the rigorous MS-based methods of chemical analysis usually employed, F_2-IsoPs remain a "gold standard" of lipid peroxidation measurements. As we learn more about them, we need to consider how to refine and interpret F_2-IsoPs measurement (e.g., in the light of blood and tissue lipid changes and their exact origin), so that the gold shines brighter and gives a better reflection of (patho)physiological relevance. In addition, the growing tendency to measure F_2-IsoPs and their metabolites by commercial kits needs proper validation of such "kits" against appropriate mass spectrometric methods (e.g., Ref. 106) in each experimental situation in which such "kits" are employed, as serious reservations have been expressed about the validity of "kit" measurements (28, 34, 53). Differences are nevertheless to be expected, since even GC/MS, GC/MS/MS, and LC/MS/MS may measure somewhat different entities, whereas an antibody might be specific for a single IsoPs isomer (106). Always bear in mind the chemistry behind what you are measuring.

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Abbreviations Used

8-Iso-$PGF_{2\alpha}$ = 8-iso-prostaglandin $F_{2\alpha}$
AA = arachidonic acid
BHT = butylated hydroxytoluene
CCl_4 = carbon tetrachloride
EIA = enzyme immunoassay
ELISA = enzyme linked immunoabsorbent assay
F_2-IsoPs = F_2-isoprostanes
GC/MS = gas chromatography/mass spectrometry
GC/MS/MS = gas chromatography tandem mass spectrometry
HDL = high density lipoproteins
HPLC = high performance liquid chromatography
IsoPs = isoprostanes
LC/MS = liquid chromatography/mass spectrometry
LC/MS/MS = liquid chromatography tandem mass spectrometry
LDL = low density lipoproteins
MS = mass spectrometry
PAF = platelet activating factor
PAF-AH = platelet activating factor acetylhydrolase
PLA_2 = phospholipase A_2
RIA = radioimmunoassay
ROS = reactive oxygen species

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