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Reinterpreting the Best Biomarker of Oxidative Stress: The 8-iso-PGF_{2α} / PGF_{2α} Ratio Distinguishes Chemical from Enzymatic Lipid Peroxidation

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Abstract

The biomarker 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}) is regarded as the gold standard for detection of excessive chemical lipid peroxidation in humans. However, biosynthesis of 8-iso-PGF_{2α} *via* enzymatic lipid peroxidation by prostaglandin-endoperoxide synthases (PGHSs), which are significantly induced in inflammation, could lead to incorrect biomarker interpretation. To resolve the ambiguity with this biomarker, the **ratio** of 8-iso-PGF_{2α} to prostaglandin F_{2α} (PGF_{2α}) is established as a quantitative measure to distinguish enzymatic from chemical lipid peroxidation *in vitro*, in animal models, and in humans. Using this method, we find that chemical lipid peroxidation contributes only 3 % to the total 8-iso-PGF_{2α} in the plasma of rats. In contrast, the 8-iso-PGF_{2α} levels in plasma of human males is generated >99% by chemical lipid peroxidation. This establishes the potential for an alternate pathway of biomarker synthesis, and draws into question the source of increases in 8-iso-PGF_{2α} seen in many human diseases. In conclusion, solely measuring increases in 8-iso-PGF_{2α} do not necessarily reflect increases in oxidative stress; therefore, past studies using 8-iso-PGF_{2α} as a marker of oxidative stress may have been misinterpreted. The 8-iso-PGF_{2α} / PGF_{2α} ratio can be used to distinguish biomarker synthesis pathways and thus confirms the potential change in oxidative stress in the myriad of disease and chemical exposures known to induce 8-iso-PGF_{2α}.

Keywords

F₂-isoprostanes; oxidative stress; inflammation; biomarkers; lipid peroxidation

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Introduction

An increase in the oxidation of biomolecules, or “oxidative stress”, is believed to be involved in the development of various pathologies, *e.g.*, heart disease [1], diabetes, cancer, Alzheimer's [2], obesity [3], and many more. Validating and measuring an *in vivo* biomarker that reflects an increase in biomolecule oxidation is a great methodological challenge. Much effort has been undertaken over several decades to establish the best biomarker indicating genuine free radical-mediated, chemical oxidation of biomolecules. The biomolecules showing the most potential are the oxidation products of polyunsaturated fatty acids [4, 5]. A variety of oxidized fatty acids are continuously formed *in vivo*, and they have profound impacts on cellular biology [6]. These fatty acid oxidation products are formed by both enzymes and chemical lipid peroxidation initiated by a free radical formed on another molecule.

Out of all oxidized fatty acids, the best indicators of oxidative stress *in vivo* are currently the F₂-isoprostanes, specifically 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}) [4, 5, 7]. 8-iso-PGF_{2α} has been studied as a biomarker of lipid peroxidation in nearly 500 animal studies and 900 human studies to date and has been found to correlate with a variety of diseases and exposures [8]. 8-iso-PGF_{2α} is distinguished from its enzymatic lipid peroxidation analog (prostaglandin F_{2α}; PGF_{2α}) by the stereochemistry around the carbon at position 8 (Scheme 1) [9]. This distinct stereochemistry is attributed to the lack of stereospecificity associated with the chemical lipid peroxidation of the precursor, arachidonic acid [7, 10-12].

Attributing the formation of 8-iso-PGF_{2α} solely to chemical lipid peroxidation has been challenged by several investigators [5, 13-19]. Numerous experiments have demonstrated the contribution of prostaglandin-endoperoxide synthases (PGHS-1 & -2) to the formation of 8-iso-PGF_{2α} *in vitro*, in cells, and *in vivo* [5, 13-19]. Due to the two formation mechanisms, there is possible ambiguity in the interpretation and validity of measuring 8-iso-PGF_{2α} as a biomarker of oxidative stress. To alleviate the ambiguity associated with this biomarker, we describe and validate a method based on the ratio of 8-iso-PGF_{2α} to PGF_{2α} that can distinguish and quantitate the mechanistic sources of 8-iso-PGF_{2α} *in vitro* and *in vivo*.

Materials and Methods

Materials

Prostaglandin-endoperoxide synthase 1 (Ovine, 90 % purity) and 2 (Human Recombinant, 70 % purity) were purchased from Cayman (Ann Arbor, MI, USA) and used within 2 weeks of delivery. Enzyme activity was confirmed by an oxygen consumption assay. Arachidonic acid, AAPH, PGH₂, 8-iso-PGF_{2α}, PGF_{2α}, PGE₂, PGA₂, 8-iso-PGF_{2α}-d₄, PGF_{2α}-d₄, and PGE₂-d₄ were also purchased from Cayman. Tris-HCl, phenol, porcine hematin, indomethacin, meclofenamic acid, trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], tin (II) chloride dihydrate, diethyl ether, ethanol, and acetic acid were purchased from Sigma Aldrich (St. Louis, MO, USA).

Sample preparation

PGHS-1 and -2 incubations without reducing agent—PGHSs (40 units) were preincubated with buffer (500 mM Tris-HCl, pH 7.4, 1 mM phenol, and 1 μ M hematin) for 1 minute at 37 °C. Reactions were initiated by the addition of arachidonic acid (3 μ M) and allowed to proceed for 3 minutes. Upon completion of the reaction, an internal standard mix was added and mixed thoroughly. A 150 μ L aliquot was removed and extracted twice with 2 mL of diethyl ether. The ether extracts were dried over anhydrous magnesium sulfate. Diethyl ether was evaporated and samples were reconstituted in 100 μ L of 33 % ethanol and 0.5 % acetic acid prior to LC/MS/MS analysis. Samples were analyzed immediately after preparation.

PGH₂ treatment with reducing agent—To the PGHS incubation buffer, 200 ng of PGH₂ was added. Immediately, an internal standard mix and tin (II) chloride (0 – 65 mg/mL of ethanol) was added. A 150 μ L aliquot was extracted twice with 2 mL of diethyl ether. Diethyl ether was evaporated and samples were reconstituted in 100 μ L of 33 % ethanol and 0.5 % acetic acid prior to LC/MS/MS analysis. Samples were analyzed immediately after preparation.

PGHS-1 and -2 incubations treated with reducing agent—PGHSs (0-50 units) were preincubated with buffer (500 mM Tris-HCl, pH 7.4, 1 mM phenol, and 1 μ M hematin) for 1 minute at 37 °C. Reactions were initiated by the addition of arachidonic acid (3 μ M) and allowed to proceed for 30 seconds (after 30 seconds no additional oxygen uptake was observed). Immediately upon completion of the reaction, an internal standard mix and tin (II) chloride (30 mg/mL ethanol) were added and mixed thoroughly. Continued sample preparation was as described in the previous paragraphs.

PGHS inhibitors and trolox—Inhibitor (meclofenamic acid and indomethacin) and trolox stock solutions were prepared in ethanol, DMSO, and ethanol, respectively. Prostaglandin-endoperoxide synthase enzymes (25 units) were preincubated with buffer (500 mM Tris-HCl, pH 7.4, 1 mM phenol, 1 μ M hematin) + inhibitors or trolox. Reactions were initiated by the addition of arachidonic acid (3 μ M) and allowed to proceed for 30 seconds. Immediately upon completion of the reaction, an internal standard mix and tin (II) chloride (30 mg/mL ethanol) were added and mixed thoroughly. Continued sample preparation was as described in the previous paragraphs.

Chemical lipid peroxidation with AAPH—Arachidonic acid in ethanol (10 μ g) was dissolved in 1.5 mL of water. To this solution, 2.5 mg of AAPH was added to make a 10 mM solution. The solution was stirred at 37 °C for up to 24 h with frequent sampling. The tube was not sealed to allow a constant supply of oxygen. 100 μ L aliquots were removed at designated time points, spiked with internal standard, and extracted with diethyl ether as described previously.

Animals and treatment protocol

Male Fisher 344 rats (260-280 g) were obtained from Charles River Laboratories (Raleigh, NC, USA). Rats were group-housed in a temperature-controlled room at 23–25 °C with a

12/24 h light/dark cycle and allowed free access to food and water. The studies adhered to the National Institutes of Health guidelines for the care and handling of experimental animals. All animal studies were approved by the NIEHS Institutional Review Board. Prior to the study, animals were fasted for 12 h prior to injections. On the day of study, animals (n=4) were anesthetized and blood was drawn through the dorsal aorta with a single draw vacutainer needle (21 gauge) into open vacutainer blood collection tubes containing heparin. Containers were kept on ice until processing.

Volunteer recruitment

Healthy adult male volunteers were recruited to the National Institute of Environmental Health Sciences Clinical Research Unit (CRU) in 2014 (n=4). Eligible participants were at least 18 years of age and nonsmokers. Volunteers were asked to donate 10 mL of plasma in one visit. The workup of this plasma was comparable to that described for rats. All study protocols were approved by the NIEHS Institutional Review Board, and all participants gave informed consent prior to providing samples

Plasma sample preparation

Whole blood was centrifuged no more than 30 min after collection (2000 rpm for 10 min at 4 °C) to remove RBCs. Plasma was removed and stored at -80 °C. Prior to analysis, samples were thawed, spiked with internal standards, and kept on ice during sample preparation. 500 µL of rat plasma or 2000 µL of human plasma was subjected to solid phase extraction using 3 mL HLB SPE columns (Waters Corp. Billerica, MA). SPE columns were prewashed with 2 mL of methanol and water. Plasma aliquots were acidified prior to deposition on the column with 0.1 % vol/vol of 1 % acetic acid, 50 % methanol. After loading, samples were washed with 2 mL of a 5 % methanol solution. F₂-isoprostanes and prostaglandins were eluted from the column with 2 mL of methanol. Prior to evaporation 6 µL of glycerol was added to each sample. Samples were evaporated in a centrifugal vacuum evaporator maintained at 40 °C. Samples were reconstituted with 50 µL of 33 % ethanol and 0.5 % acetic acid solution.

LC/MS/MS oxylipid analysis

Oxylipid levels were determined by liquid chromatography/tandem mass spectroscopy (LC/MS/MS) as previously described [20, 21]. Online liquid chromatography of extracted *in vitro* samples was performed with an Agilent 1200 Series capillary HPLC. Separations were achieved using a Halo C18 column (2.7 µm, 100 × 2.1 mm, Advanced Materials Technology, Wilmington, DE, USA), which was held at 50°C. The flow rate was 400 µL/min. Mobile phase A was 0.1% acetic acid in 85:15 water:acetonitrile. Mobile phase B was 0.1% acetic acid in acetonitrile. Gradient elution was used and the mobile phase percent B was varied as follows: 20% B at 0 min, ramp to 40% B from 0 to 5 min, and ramp to 55% B from 5 to 7 min. The injection volume was 10 µL. Samples were analyzed in triplicate. Electrospray ionization tandem mass spectrometry was used for detection with an AB Sciex API 3000 equipped with a TurboIonSpray source. Plasma samples were run with the same instrumentation and conditions as above or with an UltiMate 3000 RS HPLC system and Quantiva mass spectrometer (Thermo Fisher Scientific.) Injection volume was adjusted to

10 μ L. All analytes were monitored simultaneously as negative ions in a multiple reaction monitoring experiment. Analytes were monitored as parent ion-product ion mass/charge pairs with specific retention times and quantified against standard curves of analytes purchased from Cayman Chemical.

Statistics, data analysis and calculation

One-way analysis of variance was used for statistical analysis. Results are expressed as the mean $\pm$ SE. The differences were considered statistically significant when *P* values were less than 0.05. Error in the ratio was determined by the standard error propagation (Equation 1).

$$\sqrt{\left(\frac{\sigma_{8\text{-iso-PGF}_{2\alpha}}}{\mu_{8\text{-iso-PGF}_{2\alpha}}}\right)^2 + \left(\frac{\sigma_{\text{PGF}_{2\alpha}}}{\mu_{\text{PGF}_{2\alpha}}}\right)^2} \quad \text{Equation 1}$$

Calculations were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY).

Peaks identified as 8-iso-PGF_{2 α} , PGF_{2 α} , or PGE₂ were integrated and normalized to their individual deuterated standards: d₄-8-iso-PGF_{2 α} , d₄-PGF_{2 α} , and d₄-PGE_{2 α} . From previously generated quantitation curves, the normalized peak areas were converted to concentrations. The 8-iso-PGF_{2 α} / PGF_{2 α} ratio was calculated by dividing the concentrations of 8-iso-PGF_{2 α} by the concentration of PGF_{2 α} .

Results

To quantitate the formation of 8-iso-PGF_{2 α} by enzymatic lipid peroxidation, *in vitro* experiments were first performed with purified PGHS and arachidonic acid. In initial experiments with incubations of purified PGHS-1 or -2, no detectable levels of 8-iso-PGF_{2 α} were observed (Figure S1A). The undetectable levels of 8-iso-PGF_{2 α} were due to a nearly exclusive generation of PGE₂ and other ketone products by spontaneous rearrangement of the endoperoxide intermediates. This does not reflect the *in vivo* situation where a reducing environment and enzymes increase the levels of F₂-prostaglandins and F₂-isoprostanes. To better simulate conditions reflecting those found *in vivo*, an endoperoxide reducing agent, tin (II) chloride, was added after the reaction [22-24]. Addition of tin (II) chloride reduces spontaneous rearrangement of the endoperoxide intermediate PGH₂ to PGE₂ and increases the reduction of PGH₂ to PGF_{2 α} (Figure S2) in a dose-dependent manner (Figure S1B). Tin (II) chloride is preferred over other reducing agents such as sodium borohydride as it does not reduce ketones of, *e.g.*, PGE₂ (Figure S3).

With this methodology, we investigated the formation of 8-iso-PGF_{2 α} and PGF_{2 α} as well as the 8-iso-PGF_{2 α} / PGF_{2 α} ratio as formed by PGHS-1 and -2. Linear increases in PGF_{2 α} and 8-iso-PGF_{2 α} amounts were observed with dependence on PGHS-2 and -1 activity (slope of activity vs. concentration is 12 nM/unit and 0.05 nM/unit for PGF_{2 α} and 8-iso-PGF_{2 α} , respectively) (Figure 1A & Figure S4A). The 8-iso-PGF_{2 α} / PGF_{2 α} ratio did not significantly change regardless of enzyme activity [0.008 $\pm$ 0.002 for PGHS-1, and 0.003 $\pm$

0.002 for PGHS-2 (Figure 1B & Figure S4B)]. To investigate the robustness of the enzymatic 8-iso-PGF_{2α} / PGF_{2α} ratio, incubations were performed in the presence of classical PGHS inhibitors (meclofenamic acid and indomethacin) and the lipid radical scavenger Trolox (Figure 1C - E & Figure S4C-E). Inhibition of PGHS activities resulted in the expected decrease of the PGF_{2α} and 8-iso-PGF_{2α} amounts. However, the ratio between these two compounds did not change significantly (PGHS-1 = 0.007 ± 0.001 for meclofenamic acid and 0.007 ± 0.002 for indomethacin; PGHS-2 = 0.004 ± 0.002 for meclofenamic acid and 0.004 ± 0.001 for indomethacin) (Figure 1D - F & Figure S4D - F). Trolox (100 μM) did not significantly change the amounts of 8-iso-PGF_{2α} and PGF_{2α} or the ratio (0.006 ± 0.002) when added to PGHS-1 incubations. These results demonstrate that both compounds were formed by PGHS, but formation of PGF_{2α} is favored over 8-iso-PGF_{2α} by >99%. No other F₂-isoprostane or prostaglandin (regio)isomers were detected in significant amounts.

In addition to determining the 8-iso-PGF_{2α} / PGF_{2α} ratio during enzymatic oxidation, the ratio during chemical lipid peroxidation of arachidonic acid was determined. 8-iso-PGF_{2α} and PGF_{2α} were generated in near equal amounts by 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) at 37 °C for 24 h (Figure S5). The 8-iso-PGF_{2α} / PGF_{2α} ratio observed with this water-soluble azo compound that is used as a free radical generator is 0.97 ± 0.06 (Figure 2A). Also, autoxidation of arachidonic acid at 37 °C for 24 and 72 hours showed a similar 8-iso-PGF_{2α} / PGF_{2α} ratio, 0.97 ± 0.09 (Figure S6). The approximate equal production of 8-iso-PGF_{2α} and PGF_{2α} is in contrast to the enzymatic lipid peroxidation, which is >99 % selective for PGF_{2α}. This means that the 8-iso-PGF_{2α} / PGF_{2α} ratio is highly sensitive to chemical lipid peroxidation and could be used to quantitate the relative contributions of both pathways.

From the ratios determined for PGHS and chemical lipid peroxidation, a mathematical model was constructed to distinguish the relative contribution of both pathways to the total 8-iso-PGF_{2α} pool, Figure 2B. To construct the model, the following parameters were used: the 8-iso-PGF_{2α} / PGF_{2α} ratio as generated by PGHS-1 is 0.008 and the expression of PGHS-1 is assumed constant; the 8-iso-PGF_{2α} / PGF_{2α} ratio as generated by PGHS-2 is 0.004; the 8-iso-PGF_{2α} / PGF_{2α} ratio as generated by CLP is 0.97; and the rate of CLP with respect to the rate of enzymatic lipid peroxidation was varied over 2-fold. From the model, it is predicted that the 8-iso-PGF_{2α} / PGF_{2α} ratio will be highly selective for changes in chemical lipid peroxidation. The relative contribution of each pathway to the total 8-iso-PGF_{2α} levels can be calculated by Equation 2 where y = 8-iso-PGF_{2α} / PGF_{2α} ratio and x = CLP/PGHS ratio.

$$y = \frac{0.966 * x}{0.992 + x} - 2.15^7 x + 0.008 \quad \text{Equation 2}$$

Next, the relative contribution of both enzymatic and chemical lipid peroxidation to the formation of 8-iso-PGF_{2α} was determined *in vivo*. The 8-iso-PGF_{2α} / PGF_{2α} ratio was measured in plasma of untreated male rats and nonsmoking healthy human males, (Figure 3A, 3C, and 3D). From the mathematical model, the relative contribution of both pathways

to the total measured amount of 8-iso-PGF_{2α} can be separated (Figure 3B and 3E). In untreated rats, the contribution of chemical lipid peroxidation to the 8-iso-PGF_{2α} pool is 3.0 ± 1.5 % with and enzymatic contribution of 96.7 ± 1.5 %. Absolute values are compatible to previously published concentrations [5]. In nonsmoking healthy human males, chemical lipid peroxidation is the major contributor to the 8-iso-PGF_{2α} pool, 99.5 ± 0.5 %. Absolute values are compatible to previously published numbers for 8-iso-PGF_{2α} and PGF_{2α} [25]. The mostly enzymatic formation of 8-iso-PGF_{2α} in rats and mostly chemical formation in humans is supported by published reports on the inhibition of 8-iso-PGF_{2α} formation in rats but not in humans by treatment with PGHS inhibitors [5, 18].

Discussion

Here we have demonstrated a strategy to quantitate and distinguish the source of 8-iso-PGF_{2α} as being from PGHSs or chemical lipid peroxidation. Given the massive induction of PGHS-2 observed in various pathologies (Table S1), we argue that increased expression of PGHS-2 could be a plausible alternate source of increases in 8-iso-PGF_{2α} levels seen *in vivo* (Table S2). This necessitates reexamination of previous *in vivo* and human studies concluding increases in oxidative stress from sole measurements of 8-iso-PGF_{2α}.

Arguments raised against the significant contribution of PGHS to 8-iso-PGF_{2α} *in vivo* levels include the relatively low amount of product formed and the inconsistent and incomplete decreases in 8-iso-PGF_{2α} levels when PGHS inhibitors are used [12, 26]. We have shown here that the absolute amounts of 8-iso-PGF_{2α} and PGF_{2α} generated by both PGHS-1 and -2 are enzyme dependent, but the 8-iso-PGF_{2α} / PGF_{2α} ratio is enzyme independent. Since the total enzymatic activity of PGHSs *in vivo* is highly variable between organs and disease states (Table S1), it is virtually impossible to determine whether PGHSs contributed significantly to 8-iso-PGF_{2α} levels *in vivo* without measuring the total enzyme activity [27]. Also, the incomplete inhibition of PGHS-1 and -2 is a highly controversial argument as it is impossible to determine whether 100 % inhibition of PGHS has occurred throughout the entire organism.

It is well recognized that both inflammation and oxidative stress are interconnected, and changes in one can lead to changes in the other [28, 29]. Calculating the 8-iso-PGF_{2α} / PGF_{2α} ratio followed by distinction of the two formation pathways using Equation 2 will allow for both pathways to be measured simultaneously in complex diseases and exposures. In cases where both pathways are altered simultaneously, it will be important to investigate how the pathways are linked and in what order they were affected. The 8-iso-PGF_{2α} / PGF_{2α} ratio should be a highly informative measure during these types of studies.

Several investigators have made observations of the possible contribution of enzymatic lipid peroxidation to the 8-iso-PGF_{2α} pool in various biological systems [5, 13-19]. However, in interpreting the levels of 8-iso-PGF_{2α} as a biomarker of oxidative stress, this “alternate pathway” has been mostly ignored or forgotten in major clinical studies. Our conclusion of chemical lipid peroxidation being the major contributor in healthy human males is somewhat supportive of the myriad of studies already performed with this biomarker. However, the true power of a biomarker is found in its ability to reflect a change between

basal/healthy and affected systems. We believe that there will be instances where enzymatic overexpression due to inflammation is the major source 8-iso-PGF_{2α}, whereas in others, the original conclusion of increased oxidative stress due to chemical lipid peroxidation will hold true. Therefore, it is now imperative to reexamine the sources of 8-iso-PGF_{2α} in multiple *in vivo* models and eventually humans disease that have been shown to increase 8-iso-PGF_{2α} in order to definitively confirm the conclusion of increases in oxidative stress.

Previous studies have identified the major metabolite 15-keto-dihydro-PGF_{2α} (15-K-DH-PGF_{2α}) as a more representative measure of PGF_{2α} formation *in vivo* [30, 31]. This is mostly due to *ex vivo* formation of PGF_{2α} during sample acquisition and processing. No artifactual generation of the 15-K-DH-PGF_{2α} has been reported. Given this fact, in future *in vivo* studies an alternative measure could be the ratio of the metabolites of 8-iso-PGF_{2α} and PGF_{2α} such as the 8-iso-15-keto-dihydro-PGF_{2α}/ 15-keto-dihydro-PGF_{2α} ratio.

In conclusion, regardless of the arguments raised for or against the contribution of enzymes, *e.g.* PGHSs, to 8-iso-PGF_{2α} levels, there is potential ambiguity in the solely measuring 8-iso-PGF_{2α} and it should therefore be considered inadequate as an index of oxidative stress. Continued disregarding of the potential ambiguity will be of great detriment to the power and legacy of this biomarker. To relieve this ambiguity, the two sources of 8-iso-PGF_{2α} should be quantitatively distinguished. In our opinion, the recommended approach for distinguishing the source of 8-iso-PGF_{2α} is to determine the 8-iso-PGF_{2α} / PGF_{2α} ratio. Determining this ratio requires little added work; in fact, modern mass spectrometric approaches used to measuring 8-iso-PGF_{2α} already detect PGF_{2α}. To apply our method, investigators need only quantify this already detected substance. Determining the 8-iso-PGF_{2α} / PGF_{2α} ratio in conjunction with the calculations described here should unequivocally separate the contributions of both formation mechanisms to the levels of 8-iso-PGF_{2α} *in vitro* and *in vivo*. We believe that this new measurement strategy will significantly improve the accuracy and confidence with which oxidative stress is determined in diseases and environmental exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

8-iso-PGF_{2α}	8-iso-prostaglandin F _{2α}
PGF_{2α}	prostaglandin F _{2α}
PGHS	prostaglandin-endoperoxide synthase, cyclooxygenase

Highlights

The gold standard biomarker 8-iso-PGF_{2α} can be formed by two distinct mechanisms.

These two mechanisms should be distinguished prior to concluding an increase in oxidative stress.

A method to make this distinction is measuring the 8-iso-PGF_{2α}/PGF_{2α} ratio.

Fig. 1. PGHS-2 produces 8-iso-PGF_{2α} at a ratio of 0.004 in relation to PGF_{2α}; this ratio is constant regardless of enzyme activity

A: The absolute amounts of 8-iso-PGF_{2α} and PGF_{2α} increase linearly with induction of PGHS-2 activity. **B:** The ratio between the two products does not change significantly. **C:** The absolute amounts of 8-iso-PGF_{2α} and PGF_{2α} are decreased with inhibition of PGHS-2 enzyme activity by meclofenamic acid (66 and 75% inhibition for PGF_{2α} and 8-iso-PGF_{2α}, respectively). **D:** The 8-iso-PGF_{2α} / PGF_{2α} ratio is not altered significantly from control with inhibition of PGHS-2 enzyme activity with meclofenamic acid. **E:** The absolute amounts of 8-iso-PGF_{2α} and PGF_{2α} are decreased with inhibition of PGHS-2 enzyme activity by indomethacin (72% inhibition for both PGF_{2α} and 8-iso-PGF_{2α}). **F:** The ratio between PGF_{2α} and 8-iso-PGF_{2α} is not altered significantly with inhibition of PGHS-2 enzyme activity with indomethacin.

Fig. 2. The ratio of 8-iso-PGF_{2α} to PGF_{2α} is a highly sensitive and selective measure of increased chemical lipid peroxidation

A: Arachidonic acid incubated with the water-soluble free radical generator AAPH produces near equal amounts of 8-iso-PGF_{2α} and PGF_{2α}. **B:** A mathematical simulation of the 8-iso-PGF_{2α} / PGF_{2α} ratio when chemical lipid peroxidation (CLP) is varied relative to the prostaglandin-endoperoxide synthases (PGHS-1 & PGHS-2). The expression of PGHS-2 is varied in three distinct ratios with respect to PGHS-1 to simulate the effect of enzyme induction on the 8-iso-PGF_{2α} / PGF_{2α} ratio. The equation of this model (Equation 2) can be used to determine the relative contributions of both pathways to the measured 8-iso-PGF_{2α} levels.

Fig 3. 8-iso-PGF_{2α} is mainly a product of enzymatic lipid peroxidation in male fisher 344 rats and mostly of chemical lipid peroxidation in healthy human males

A: 8-iso-PGF_{2α} and PGF_{2α} were measured in the plasma of 4 untreated rats, and the ratio between the two compounds calculated ($\text{Ratio}_{\text{rat plasma}} = 0.06 \pm 0.02$; mean $\pm$ SE). **B:** Using Equation 2, at baseline, the source of the measured 8-iso-PGF_{2α} in rat plasma is mainly from PGHS (96.7 ± 1.5 % of total); 3.3 ± 1.5 % comes from chemical lipid peroxidation (CLP). **C:** 8-iso-PGF_{2α} and PGF_{2α} were measured in the plasma of 4 healthy nonsmoking human males. The levels for each individual are reported as mean $\pm$ SE. **D:** The ratio between 8-iso-PGF_{2α} and PGF_{2α} in human plasma is 0.88 ± 0.26 (mean $\pm$ SE). **E:** Using Equation 2, at baseline, the source of the measured 8-iso-PGF_{2α} in human plasma is mainly from CLP (99.5 ± 0.5 % of total); 0.5 ± 0.5 % comes from PGHSs.

Scheme 1.

The peroxidation of arachidonic acid with the chemical structures of intermediates and products integral to this study.