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The Impact of Isoprostane Metabolism on the Assessment of Oxidative Stress

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Isoprostanes, a group of 64 prostaglandin-like compounds, are derived primarily by free radical-mediated peroxidation of free or esterified arachidonic acid [1-3]. Based on many comprehensive studies, the level of one representative isoprostane, 15-F_{2t}-isoprostane (formerly denoted 8-iso-prostaglandin $F_{2\alpha}$), in blood or urine is widely regarded as the "gold standard" biomarker for the assessment of oxidative stress [4-7].

Several methods have been developed for the quantification of 15-F_{2t}-isoprostane, including GC/MS [8,9], LC/MS [10,11], RIA [12,13] and ELISA [14-16], and all of these methods are widely employed for the assessment of systemic oxidant stress. However, in

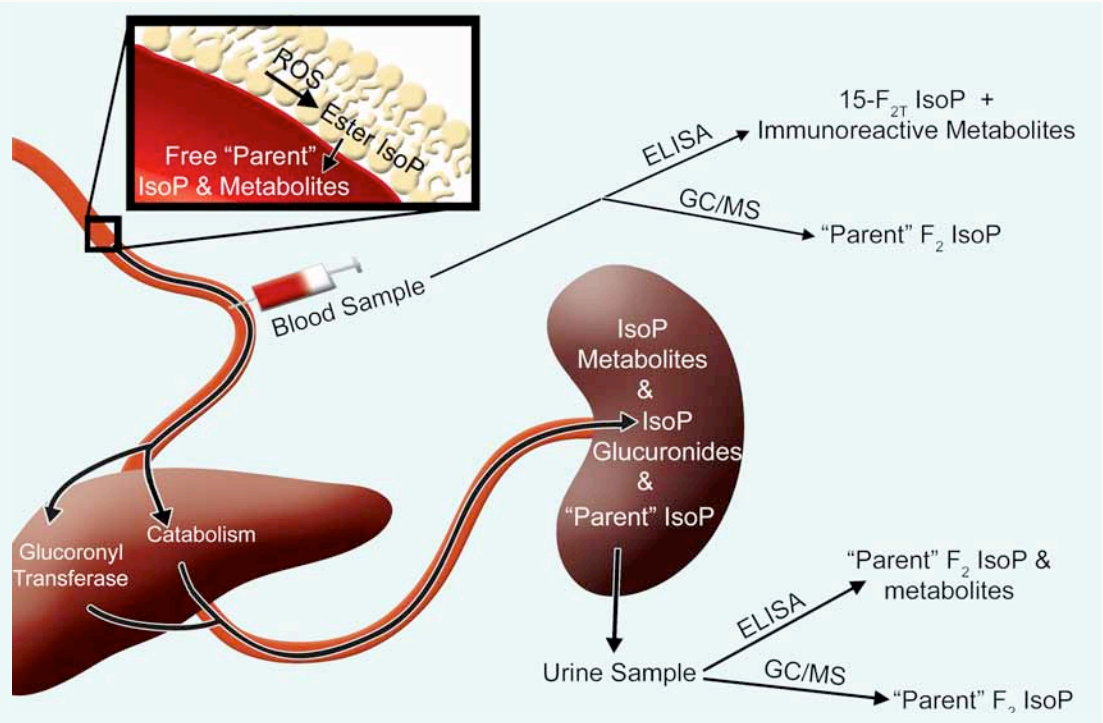

Figure 1. Schematic representation of pathways for the formation, metabolism and excretion of isoprostanes in vertebrates. Multiple pathways for isoprostane metabolism have been identified, including β-oxidation, glucuronidation and the formation of glutathione adducts. Analysis of IsoP-M and/or pretreatment of urine samples with β-glucuronidase can provide insights into variations in metabolism among individuals due to genetics, diet, lifestyle, environment and/or disease.

contrast to enzymatically-derived eicosanoids, 15-F2t-isoprostane concentrations determined by GC/MS or LC/MS do not always correlate well with those obtained using immunoassay methods. Some studies have reported good correlation among these methods [14,15], whereas others have not [17].

Further, there is limited but clear evidence that isoprostanes are rapidly and extensively metabolized in humans [18-20]. Given the need to rapidly clear these potentially toxic substances from the body, this is not unexpected. Although one metabolite has been identified and can be independently quantified [21], there appear to be multiple metabolic mechanisms for isoprostane metabolism. Since even relatively small elevations in isoprostane levels have been reported to be a significant risk indicator for cardiovascular disease [22], consideration of the impact of isoprostane metabolism on results obtained by various assay methods is important (Fig. 1).

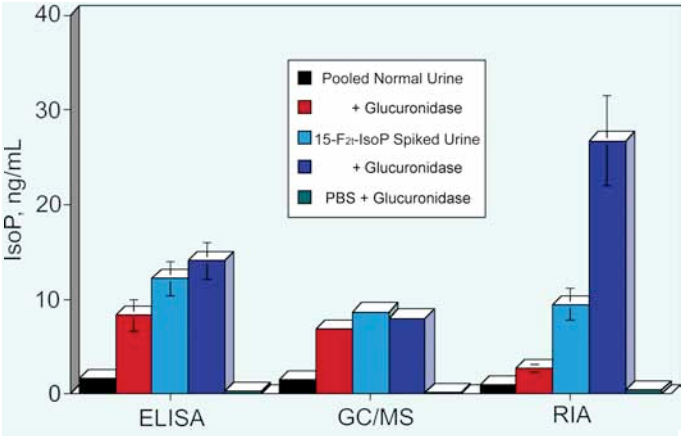

Figure 2. The effect of pretreatment with β-glucuronidase on the concentration of F2-isoprostanes in pooled human urine samples. Human urine collected from 6 normal individuals was separated into two pools. One pool was spiked with 10 ng/mL of synthetic 15-F2t-IsoP. Samples were analyzed using standard published protocols with (+G) or without pretreatment with β-glucuronidase. PBS represents values obtained for phosphate buffered saline preincubated with the same quantity of β-glucuronidase.

Key points to consider:

1. GC/MS and LC/MS methods typically quantify 15-F2t-isoprostane, but not isoprostane metabolites. However, the values obtained can be dependent on the sample preparation protocol [23]. For example, four separate isoprostanes contribute to the GC/MS peak if solid phase extraction and TLC are employed for sample preparation. However, if immunoaffinity chromatography is employed to remove interfering substances, then only 15-F2t-isoprostane is present in the GC/MS peak. A simple one-step method for immunoaffinity purification of 15-F2t-isoprostane, which can then be analyzed by GC/MS, LC/MS or ELISA has recently been published [10]. Convenient and affordable columns for this application are now available from Oxford Biomedical Research (see Table on Page 5).

2. Given the large number of isoprostanes generated from the action of reactive oxygen species on arachidonic acid *in vivo*, and the likelihood that multiple 15-F2t-isoprostanes and isoprostane metabolites undoubtedly contribute to "15-F2t-isoprostane" values determined by immunoassays, with differences among immunoassays expected based on the relative specificity of the antibodies employed [20]. The antibody employed in our ELISA kit has been extensively characterized so that 15-F2t-isoprostane results obtained by ELISA for serum samples following established solid phase extraction protocols – or following immunoaffinity isolation of 15-F2t-isoprostane - correlate very well with those results obtained by GC/MS [14,15].

3. Indeed, given the rapid and extensive metabolism of isoprostanes *in vivo*, including β-oxidation, glucuronidation and other pathways [18,24], and the well documented inter-individual differences that have been reported for at least some of these pathways [27, 28], it is actually pretty amazing that isoprostane assays have emerged as a "gold standard" for oxidative stress. Further complicating comparison of isoprostane values obtained by different analytical techniques are the significant differences among the methods for sample preparation (e.g. multiple Sep-Pak + TLC versus immunoaffinity).

4. Assays have been developed to quantify one major 15-F2t-isoprostane metabolite, 2,3-dinor-8-iso-PGF2α [15, 18], affording the opportunity to evaluate and factor in inter-individual differences in metabolism by one pathway of this isoprostane. To facilitiate these efforts, immunoaffinity columns specific for this metabolite (2,3-dinor-8-iso-PGF2α = IsoP-M) are now available from Oxford Biomedical Research (see Page 5).

5. In addition, the rapid and extensive metabolism of 15-F2t-isoprostane, suggests that elevated isoprostane levels best serve as biomarkers for acute oxidative

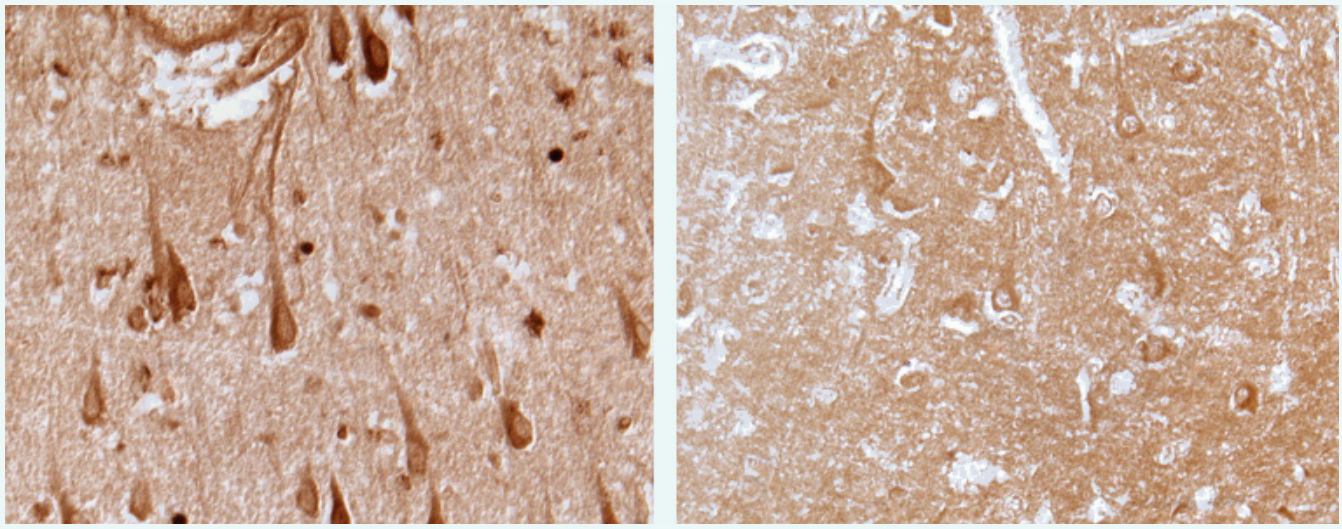

Figure 3. Immunohistochemical localization of isoprostanes in the brain of Alzheimer's Disease (AD) patients (left). Neurons in the hippocampus of AD patients stain intensely in fixed sections treated with anti-15-F2t-Isoprostane. Results obtained for an age-matched control are shown in the right figure. Courtesy of S. Basu. See ref. 26 for additional data.

stress. In animal models, the pronounced elevation of 15-F_{2t}-isoprostane in response to oxidative stress returns to baseline values within 24 hours [2,9]. The strong correlations reported between 15-F_{2t}-isoprostane levels and conditions such as cardiovascular disease in humans is presumably due to chronic oxidative stress to replenish the rapidly metabolized 15-F_{2t}-isoprostane.

6. Although GC/MS or LC/MS may more reliably quantify levels of a specific isoprostane, e.g. 15-F_{2t}-isoprostane, the ability of immunoassays to detect isoprostane metabolites [19], and the more robust assessment of isoprostane production that can be obtained by pretreatment of urine with β-glucuronidase may further improve the utility of isoprostanes as a biomarker for oxidative stress by making the measurements more independent of variations in metabolism.

7. Glucuronidation is a major pathway for isoprostane metabolism: With the exception of 20-HETE [25], glucuronidation is not an important pathway for the excretion of enzymatically-derived eicosanoids. Based on the significant differences between the stereochemistry of isoprostanes and prostaglandins, and on the observation that approximately 50% of radiolabeled 15-F2t-Isoprostane elutes with the aqueous fraction during solid phase extraction of human urine [18], we investigated the extent to which isoprostanes are excreted as glucuronic acid conju-gates in humans. Whether quantified by GC/MS, RIA or ELISA [24,26], pretreatment of human urine samples with β-glucuronidase increased the 15-F2t-Isoprostane levels by an average of ~100%, indicating that glucuronidation is an important pathway for 15-F_{2t}-isoprostane elimination (Fig.2). Moreover, the extent of glucuronidation ranged from 28% to 80% for the human urine specimens examined. This is not surprising based on well-known inter-individual differences in the expression of UDP-glucuronyl transferases and the impact of diet, lifestyle and other factors on the expression of these enzymes [27,28]. Given the extent of and the wide variations observed for 15-F_{2t}-Isoprostane glucuronidation, it is strongly recommended that urine specimens be pretreated with β-glucuronidase prior to isoprostane analysis to provide more accurate assessment of oxidative stress.

Additional Developments: Our new immunoaffinity columns specific for 15-F_{2t}-Isoprostane (IsoP) and 2,3-dinor-8-iso-$PGF_{2\alpha}$ (IsoP-M) are recommended for the preparation of serum or urine samples for analysis – whether by GC/MS, LC/MS or ELISA.

Our purified antibodies to isoprostanes and IsoP-M can also be used for immunohistochemical localization of these biomartkers for oxidative damage [26]. Indeed, 15-F_{2t}-Isoprostane was recently used to visualize the high concentrations of IsoP in the brain of Alzheimer's patients (Fig. 3). Since, as detailed above, the values obtained for the concentration of IsoP in serum or urine samples depend on (a) the method for sample preparation, (b)

the analytic method, and (c) the rate of isoprostane metabolism in the subjects. In order to assist investigators who wish to compare results obtained using different sample preparation and analytical methods, we have prepared a large pool of normal human urine and have obtained IsoP concentrations using SepPak isolation and GC/MS as well as by ELISA using a proprietary extraction-free method. Values were obtained ± β-glucuronidase pretreatment. A second pool of urine has been prepared by spiking with authentic 15-F2t-isoprostane to provide an elevated calibrator. Additional efforts to further standardize and improve isoprostanes as biomarkers for oxidative stress are ongoing. However, it is critical that investigators in this field be cognizant of the impact of experimental methods and IsoP metabolism as they design and execute their studies.

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Products for Isoprostane Quantification

Product	Description	Price
EA 84	Isoprostane ELISA kit for serum, cell extracts, etc	$289.00
EA 85	Extraction-free Isoprostane ELISA kit for urine	$289.00
IS 30	15-F2t-Isoprostane (IsoP) immunoaffinity spin columns	$100.00
IS 40	2,3-dinor-8-iso-PGF2α (IsoP-M) immunoaffinity spin columns	$125.00
IS 20	Goat anti-15-F2t-Isoprostane (anti-IsoP) purified IgG	$131.00
GL 85	β-Glucuronidase pretreatment kit for IsoP analyses	$85.00
IS 50	Normal + elevated human urine IsoP calibrators	$55.00
CR 01	Urinary Creatinine Assay Kit (to normalize IsoP values)	$89.00

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