Assessment of Urinary F₂-Isoprostanes in Experimental and Clinical Studies: Mass Spectrometry Versus ELISA

To the Editor:

Ojeda and colleagues¹ reported that oxidative stress renal markers contribute to sex differences in blood pressure in adult growth-restricted offspring rats. The antibody used in the ELISA kit used by Ojeda et al¹ is specific for 15(S)-8-iso–PGF₂α,² (http://www.oxfordbiomed.com/sites/default/files/spec_sheet/EA85.120426.pdf) one of 64 possible F₂-isoprostanes. At first glance, ELISA and gas chromatography-mass spectrometry (GC-MS) seem to correlate (Figure, A); however, the Bland-Altman method reveals enormous differences and systematic errors in the ELISA method (Figure, B). Lack of analytically satisfactory agreement applies to another commercially available 15(S)-8-iso–PGF₂α ELISA kit (http://www.caymanchem.com/pdfs/516351.pdf).

15(S)-8-iso–PGF₂α is excreted in the urine in free and conjugated forms³ (Figure C). The lack of appreciable biological variation in urinary excretion in humans² and rats (58±16 pg/mg creatinine; 1 female, 4 male) and the manifold higher reported 15(S)-8-iso–PGF₂α levels¹ reveal serious analytic shortcomings with the use of 15(S)-8-iso–PGF₂α ELISA kits. This may compromise the scientific outcome. Inclusion of clean-up procedures is likely to improve the analytic performance of ELISA kits.⁴ Yet, reliable assessment of 15(S)-8-iso–PGF₂α in experimental and clinical study samples is best accomplished by tandem mass spectrometry-based methods.²,⁴

![Figure](image)

**Figure.** Comparison between the commercially available ELISA assay (Urinary Isoprostane ELISA Kit, product number EA85; Oxford Biomedical Research) used by Ojeda et al¹ for 15(S)-8-iso–PGF₂α, and a GC-MS assay for F₂-isoprostanes by (A) linear regression and (B) the Bland-Altman method. The straight line theoretical in (A) indicates complete agreement.² C, 15(S)-8-iso–PGF₂α (mean±SD, n=3) measured by GC-tandem MS² in fresh urine of a healthy female subject before (CONTROL) and after treatment with β-glucuronidase (ENZYME; Sigma; 2 hours, 37°C) or 1 mol/L KOH in methanol (KOH; 1 hour, 22°C).²

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**Disclosures**

None.

Dimitrios Tsikas  
Maria-Theresia Suchy  
Institute of Clinical Pharmacology  
Hannover Medical School  
Germany

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Dimitrios Tsikas and Maria-Theresia Suchy

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