Supplemental Appendix for

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Supplemental Methods

LC-MS Analysis of Eicosanoid Metabolites in Urine.

Unlabeled and deuterated analogs of 5-HETE (5-HETE-d8); LTE_4 (LTE_4-d5); PGEM (PGEM-d6); PGDM (PGDM-d6); TxM (11-dehydro-TxB2-d4); PGIM(2,3-dinor-6-keto-PGF1α-d3) were purchased from Cayman Chemical.

Systemic urinary production of PGE_2, PGD_2, TxA_2, and PGI_2, was determined by quantification of their major urinary metabolites, PGEM, PGDM, TxM, and PGIM. Stable isotope-labeled internal standards (PGEM-d6 25ng, PGDM-d6 25ng, 2,3-dinor-6-keto-$PGF_{1\alpha}$-d3 5ng, 11-dehydro-TxB2-d4 5ng in 50 µL of acetonitrile) were added to 0.5 ml of urine. Methoxyamine (MO) HCl solution (250 µL of 100 g MO HCl solid in 100 mL water) was added and allowed to equilibrate for 30 min. Sample solution was brought to a total volume of 1 mL by adding 200 µL of water. The samples were purified by solid-phase extraction (SPE) using Strata-X 33µm polymeric reversed phase, 30 mg/ml cartridges (Phenomenex, 8B-S100-TAK). The SPE cartridge was conditioned with 1 ml of acetonitrile and equilibrated with 0.25 ml of water. The sample was applied to the cartridge, washed with 1 ml of 5% acetonitrile in water and dried with a vacuum for 15 min. The analyte and internal standards were eluted from the cartridge using 1 ml of 5% acetonitrile in ethyl acetate. The eluate was collected and dried in Eppendorf vacufuge. The resulting residue was then reconstituted in 180 µl of 5% acetonitrile in water, transferred into an autosampler vial and injected 60 µl into the HPLC-MS/MS system. A Waters ACQUITY UPLC system was used for chromatography with a UPLC column, 2.1 x 150 mm with 1.7 µm particles (Waters ACQUITY UPLC CSH C18). The mobile phase A was prepared from water: mobile phase B, 95:5 (v/v) containing 0.5% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The mobile phase B was prepared with acetonitrile:methanol, 95:5 (v/v). The flow rate was 350 µL/min. Separations was carried out with various linear solvent gradients. The Waters Xevo TQS instrument (Waters Corporation) equipped with a triple quadrupole analyzer will be operated in negative-mode ESI and the analyzer will be set in the MRM mode for the analysis of the urinary metabolites.

Urinary production of 5-HETE and LTE_4 was measured in a separate assay with similar procedure. Internal standard solution (5ng 5-HETE-d8 and 5ng LTE_4-d5 in 50 µL acetonitrile) was added to 0.5 mL sample. Sample solution was then brought to a total volume of 1 mL by adding 450 µL of water. The samples were purified by SPE. The analyte and internal standards were eluted from the cartridge using 1 ml of methanol, containing 0.1% formic acid. The eluate was collected and

dried in Eppendorf vacufuge. The resulting residue was then reconstituted in 100 µl of 50% methanol in water, transferred into an autosampler vial and injected 30 µl into the HPLC-MS/MS system. A Waters ACQUITY UPLC system was used for chromatography with a UPLC column, 2.1 x 150 mm with 1.7 µm particles (Waters ACQUITY UPLC BEH C18). The mobile phase A was prepared from water: mobile phase B, 95:5 (v/v) containing 0.1% formic acid. The mobile phase B was prepared with acetonitrile:methanol, 95:5 (v/v) with 0.1% formic acid. The flow rate was 350 µL/min. Separations was carried out with various linear solvent gradients. The Waters Xevo TQS instrument (Waters Corporation) equipped with a triple quadrupole analyzer will be operated in negative-mode ESI and the analyzer will be set in the MRM mode for the analysis of the urinary metabolites.

Quantitation was done by peak area ratio of unlabeled compounds and their corresponding deuterated analogs. Daily production of eicosanoid metabolites was calculated by multiplying concentration by 24-hour urine volume. Changes were analyzed using analysis of variance (ANOVA) with a two-tailed significance of $p \leq 0.05$.

Levels and changes in PGEM, PGDM, PGIM, 5-HETE, and LTE_4 are presented in the main manuscript (see Figure 2 A-F). No TxM signal was detected in either patients' urine samples at any timepoint. Daily productions of TxM in adult and pediatric healthy controls were 504±1 and 107±3 ng/Day, respectively. No LTB_4 signal was detected in any sample, including healthy controls.

The following abbreviations were used in the text:

PGEM: tetranor-Prostaglandin E Metabolite (9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid)

PGDM: tetranor-Prostaglandin D Metabolite (9α-hydroxy-11,15-dioxo-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid)

PGIM: 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$ (6-oxo-9α,11α,15S-trihydroxy-2,3-dinor-prost-13E-en-1-oic acid)

TxM: 11-dehydro Thromboxane B_2 (9α,15S-dihydroxy-11-oxothromba-5Z,13E-dien-1-oic acid)

5-HETE: 5-Hydroxyeicosatetraenoic Acid (5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid)

LTB_4: Leukotriene B_4 (5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid)

LTE_4: Leukotriene E_4 (5S-hydroxy-6R-(S-cysteinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid)